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Simplified Procedures for Water Examination
AWWA Manual M12

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AWWA MANUAL M12
Including Supplement on
Instrumental Methods

**Simplified Procedures
for Water
Examination—**

Laboratory Manual

**AMERICAN WATER WORKS ASSOCIATION
MANUAL OF WATER SUPPLY PRACTICES**

OBJECTIVES

Our overall objective in this study is to determine the feasibility of several alternative starting mechanisms for vertical axis wind machines. After comparing the alternatives we will select the most promising approach to creating a total system with the highest overall efficiency. We will prepare a report discussing the various alternatives and their advantages and drawbacks.

Simplified Procedures for Water Examination

**Revised 1975
Supplement Added 1978**

**AWWA MANUAL M12
Including Supplement on
Instrumental Methods**

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**American Water Works Association
6666 W. Quincy Ave., Denver, Colo. 80235**

Foreword

This revised manual is meant to fill a need for a volume suited to the capabilities of those workers in the field who find *Standard Methods* too advanced. It does so by presenting plant-control methods that can be used to check day-to-day variations in water quality and to adjust treatment chemical dosages.

This manual is not designed to replace *Standard Methods*. Its purpose is to help the beginner—through experience with simpler methods—acquire the laboratory skills that will eventually enable him to employ the more involved procedures described in *Standard Methods*. The manual, therefore, may be considered an introduction to *Standard Methods*.

The manual is a primer of common methods that rely on inexpensive, readily available apparatus and that depend on simple, easy-to-follow techniques. The methods described are suitable for many drinking waters. However, the very simplicity of the methods limits their use to high-quality waters of relatively known and constant composition. Considerable prominence is given in each method to the section entitled "Warning," which tries to mark off the areas of applicability.

Most state departments of health conduct periodic bacteriologic and chemical analyses of potable waters within their jurisdiction. The operator is urged to obtain a copy of these results and also to get advice on whether the methods herein described are suitable for his particular situation. For some water supplies, only the more complicated procedures offered in the latest edition of *Standard Methods* will yield the proper results.

The manual has been revised by R. Freeman (Water and Wastewater Technical School) and Michael Taras (AWWA Research Foundation) to reflect the most recent methods for accurately assessing a water's chlorine content. Details also have been changed in those sections covering alkalinity, calcium chloride, and dissolved oxygen. And errors that have been caught in the original edition have been eliminated.

Acknowledgement

Grateful acknowledgement is made to the personnel of the Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio, for outstanding contributions to the preparation of this manual. In particular, Dr. Herbert W. Jackson, with the aid of Michael E. Bender and other colleagues, prepared the section on biologic examination; Harold L. Jeter, assisted by center specialists, prepared the section on bacteriologic examination. Thanks are also due the American Microscopical Society for permission to reproduce certain of the illustrations in the biologic-examination section.

Committee 8940 P Personnel, 1964

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General Introduction

Some guidance in quantitative analysis is desirable before any water testing is undertaken. A beginner is strongly urged to consult a competent teacher and attend at least one, and preferably more, short courses which are periodically offered in his area by the state department of health. Such classroom instruction, demonstrations, and supervised laboratory work provide a valuable adjunct to independent reading and practice.

The water plant laboratory is a place for precision work and, therefore, must be maintained in a clean and orderly manner. As a first step in this direction, each piece of equipment should be allocated a particular space in the laboratory. Laboratory cabinets, drawers, and shelves should not be used as general storage places for tools, supplies, and miscellaneous equipment parts; nor should a laboratory be used as a kitchen or lunchroom. Figures 1 and 2 illustrate some of the apparatus and equipment commonly found in the normal laboratory. (Equipment required for bacteriologic and plankton analysis is illustrated in those portions of the Manual.)

All chemicals should be kept in suitable containers and should be clearly labeled. Equipment and chemicals should be protected from dust, moisture, and harmful fumes. Adequate lighting should be available where color comparisons are to be made. Ample facilities for cleaning, washing, drying, lubricating, and general work must be provided. The proper regard should be developed for the analytical balance, volumetric glassware, distilled water, standard solutions, colorimetric equipment, records, and samples.

1. Weighing Equipment

Two types of balances are in common use. The "rough" balance weighs loads up to the kilogram range with an accuracy of 0.1 gram (abbreviated "g") and is adequate for most routine tests.

The ordinary analytical balance weighs substances up to 200 g with an accuracy of 0.0001 g (0.1 milligram, abbreviated "mg"). Such a balance is essential for the preparation of standard solutions. The analytical balance is a delicate instrument and should be treated with respect. If an instruction manual is available, its directions should be followed carefully in order to insure correct weights and to

use the sensitivity of the balance to the fullest extent of manufacturer's specifications.

When the balance is idle, the balance beam and pans should be removed from their knife-edge supports by means of the knob for that purpose, and the balance case door should be closed. This door should also be closed when the final weighing, by means of the beam "rider," is being done; otherwise air currents may cause the pointer to wobble. The balance should be zero-adjusted regularly by means of the adjusting screws. Small ivory-tipped forceps should be used for placing counterweights on the balance pan; the weights should be added one at a time, beginning with the heaviest and continuing down to the next consecutive lighter weight. When adding weights of 1 g or more, it is advisable to lift the beam from its knife edges; when adding weights smaller than 1 g, the pan rests may be used. The weights should always be returned to their proper places in the weight box. It is good housekeeping to clean the balance pans and the floor of the case with a camel's-hair brush, both before and after using the balance.

A set of glass counterpoise pans is desirable for weighing most of the solid chemicals which are needed for the preparation of standard solutions. These glass pans save the metal pans of the balance from being attacked by corrosive chemicals. In practice, one glass pan is placed on each metal pan of the balance and the weighing mechanism is adjusted to exact zero. Then the required analytical weights are transferred with ivory-tipped forceps from the weight box to the glass pan on the right side, and the required amount of chemical is added with a spatula to the glass pan on the left side until the two pans come to a true balance.

2. Glassware

Laboratory glassware should generally be of the heat-resistant type called "pyrex" (sold under the trade names "Pyrex" and "Kimax"; either may be used whenever pyrex is specified in this Manual).

Bottles, beakers, and stirring rods made of polyethylene plastic are suitable for many laboratory operations. However, polyethylene ware fails in the presence of heat and strong oxidizing solutions, and should never be used for such injurious applications.

Volumetric flasks. Volumetric flasks are squat bottles with long, narrow necks. Their capacity ranges from 25 to 2,000 milliliters (abbreviated "ml"), and the level at which this capacity is reached is indicated by an etched ring around the neck.

A liquid contained in a glass tube shows a curvature in its upper surface; the curved surface of the liquid is known as the meniscus. A filled volumetric flask should be viewed at eye level so that the front and back sections of the ring around the neck merge into a straight line, and the bottom of the water level (the meniscus) touches this line. In using volumetric glassware, such as flasks, graduates, burets, and pipets, the reading should always be made at the bottom of the curve (the meniscus), as illustrated in Fig. 3.

Volumetric flasks are used for the preparation and dilution of standard solutions. For example, 0.02*N* acid (see Sec. 5) can be prepared by measuring with a transfer pipet (see Fig. 1) 50 ml of stock 0.1*N* acid solution, and placing in a 250 ml volumetric flask. After filling to the mark with distilled water, the contents are mixed thoroughly by inverting the flask 15 times or more. Since the flask is

designed for measuring purposes, the contents should be promptly poured into a clean storage bottle.

Graduates. The workhorse of the laboratory is the graduated cylinder, popularly called the "graduate." Available in sizes up to several liters, graduates are marked in milliliters, except for the 10 ml size, which is subdivided into fractions of a milliliter, and sizes of 250 ml or larger, which are etched at intervals of 5 or 10 ml.

Many pieces of volumetric glassware are marked "T.C.," meaning "to contain." (Other types of glassware may be marked "T.D.," meaning "to deliver.") Some water always clings to the interior surface of a flask or graduate, resulting in a difference between the amount added to a dry graduate and that later withdrawn. The analyst should be on guard against dirty measuring glassware like pipets, burets, graduates, and volumetric flasks in order to insure the delivery of correct volumes.

Burets. A buret is a glass tube graduated over part of its length. The most commonly favored sizes are 10, 25, and 50 ml. The graduations in tenths of a milliliter enable the estimation of a fraction of a tenth. The buret is clamped to a stand and filled from the top, usually through a funnel. The excess solution above the zero line is allowed to drain to waste through the stopcock before the start of a titration. A buret is always read twice, the difference between the readings representing the volume of titrant dispensed. Precautions should be taken against air bubbles arising from the failure of the titrant to wet the buret uniformly through its entire length. Care should also be taken to avoid a slowly leaking stopcock which allows the titrant to drain as the buret stands idle. A buret stopcock needs frequent lubrication to function satisfactorily. For best results, a lubricant like vaseline should be applied as a thin film on the dried surface of the stopcock. The lubricant should be used sparingly, as an excess on the stopcock can clog the buret tip and contribute to the formation of air bubbles in the tip.

Although a buret is specifically designed for dispensing a titrating solution, individuals who experience difficulty in controlling a pipet may find a buret a convenient substitute for measuring the accurate volumes of standard solutions needed in colorimetric work.

Improvements have been made on the classical buret with a view to speeding up titrations. Units have been devised wherein a self-zeroing buret is mounted directly over a reservoir bottle and a means is provided for quickly transferring the titrant from the bottle to the buret. This type of buret system is available in a number of different forms and shapes. One of the simplest designs is that employing the common polyethylene squeeze bottle as both a reservoir and a pressure pump.

Pipets. Two kinds of pipets are in general use. Those with a single etched ring near the top are called transfer or volumetric pipets. Those with a graduated stem are known as measuring pipets and can measure any volume up to the designated capacity of the pipet.

One end of the pipet is tapered; the other end is fire polished, so that it can be easily closed by the finger. The small tapered end of the pipet is inserted in the bottle, the liquid drawn above the upper etched ring by suction, and the upper opening quickly and tightly closed with the index finger. The pipet's tip is wiped dry with a clean cloth. The finger is then raised just enough to allow the liquid

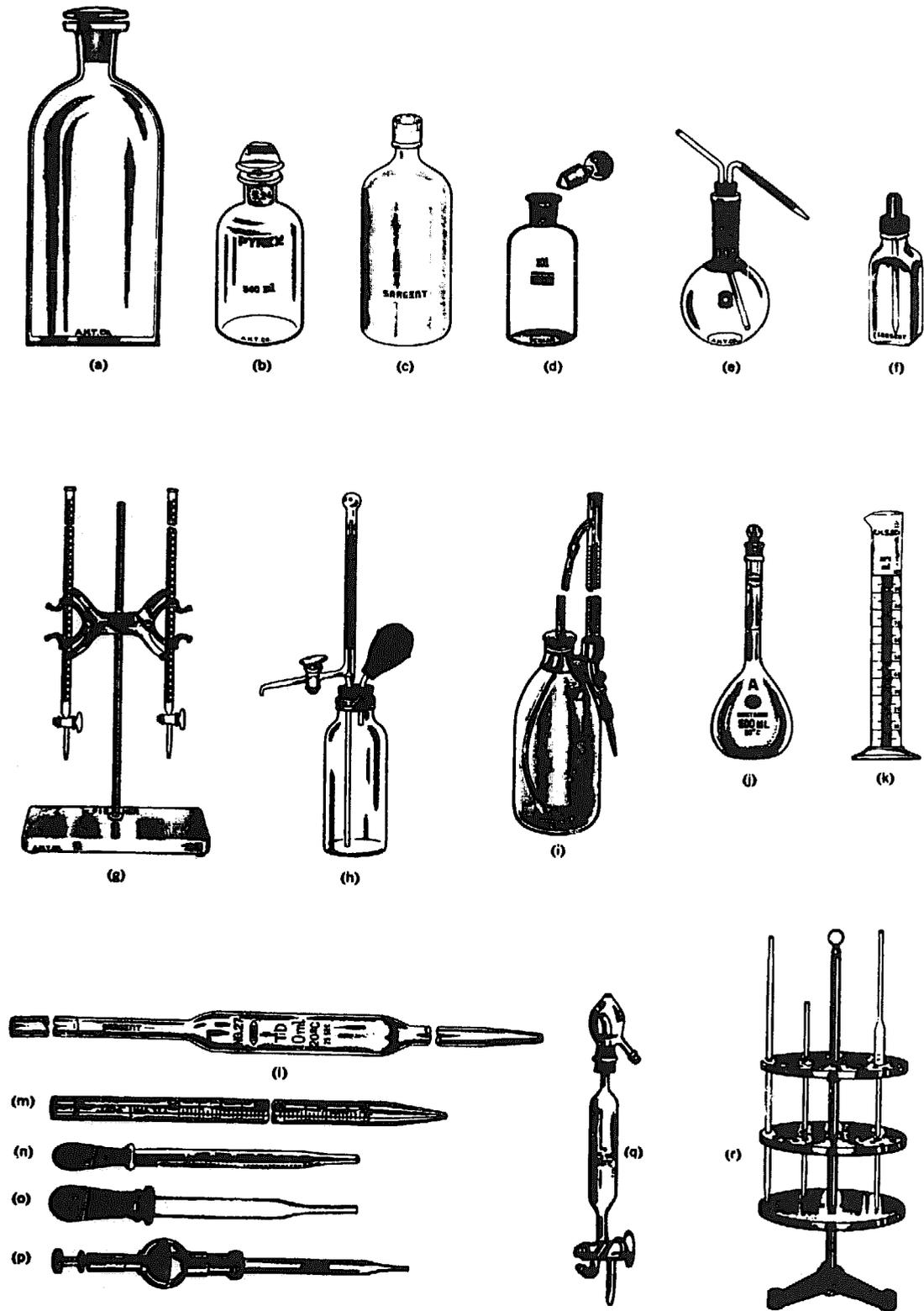


Fig. 1. Laboratory Glassware

gradually to fall to the encircling zero level. The flow is again arrested by restoring full finger pressure, after which the measured liquid is allowed to flow freely into the reaction vessel by entirely removing the finger from the top. After the liquid stops draining, the tip of the pipet is touched to the inner surface of the receiving vessel. The last drop should not be blown out of the pipet.

When drawing liquid into a pipet, always keep the tip submerged as long as suction is being applied. Avoid vigorous suction during the filling operation because bubbles may form and rise to the surface, where they may take some time to break, thus making an exact reading difficult.

Mouth suction should never be used to pipet solutions which give off a harmful

(Continued P. 8)

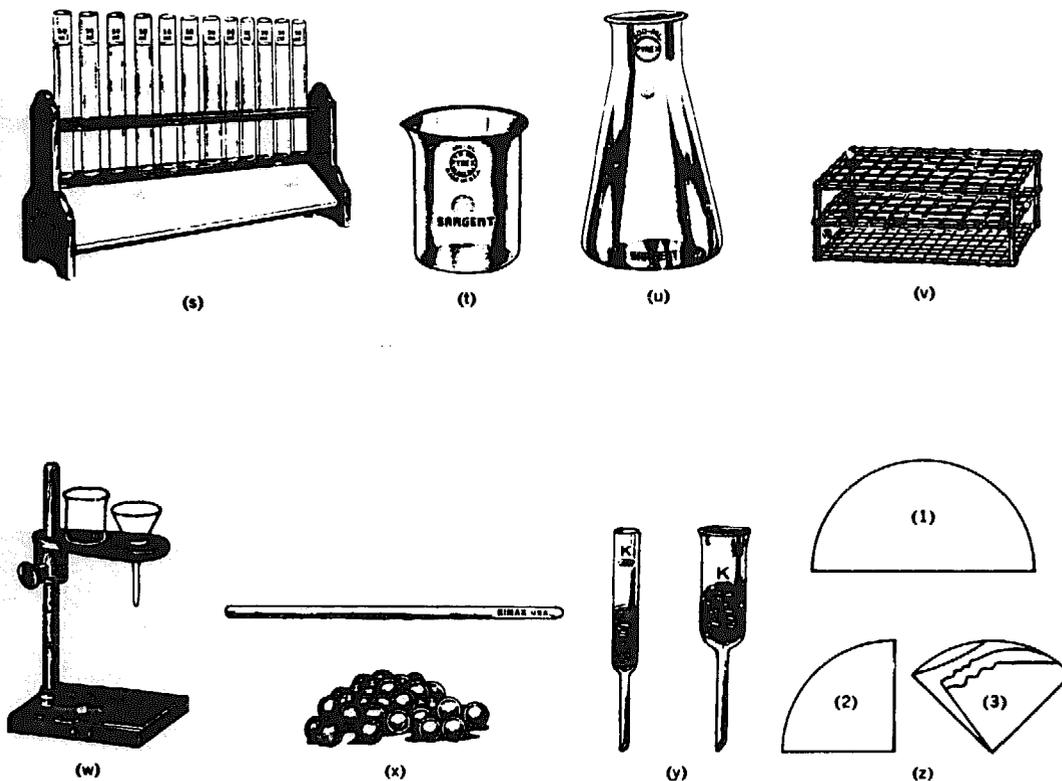


Fig. 1 Laboratory Glassware

Key: (a) water sample bottle; (b) glass laboratory bottle; (c) plastic laboratory bottle; (d) BOD glass-stoppered bottle; (e) wash bottle; (f) bottle with dropping pipet in stopper; (g) burets and support; (h) automatic buret; (i) automatic buret with polyethylene bottle; (j) volumetric flask; (k) graduated cylinder; (l) volumetric transfer pipet; (m) measuring pipet; (n) graduated dropping pipet; (o) medicine dropper pipet; (p) safety pipet; (q) automatic pipet; (r) pipet support; (s) tall-form nessler tubes and support; (t) beaker; (u) erlenmeyer flask with wide neck; (v) test tubes and support; (w) filter funnel and support; (x) glass stirring rod and glass beads; (y) filter tubes with glass wool plugs; (z) filter paper - (1) folded into semicircle, (2) folded again into quarter circle, (3) quarter-folded with one corner torn off to improve contact when inserted (tightly) in filter funnel.



Fig. 2 Other Laboratory Apparatus

Key: (a) analytical balance; (b) pan balance; (c) balance weights and forceps; (d) glass desiccator; (e) aluminum desiccator; (f) counterpoised glass balance pans; (g) drainboard for glassware; (h) porcelain casserole; (i) evaporating dish; (j) mortar and pestle; (k) measuring spoon; (l) bunsen gas burner; (m) Fisher high-temperature gas burner; (n) electric hot plate.

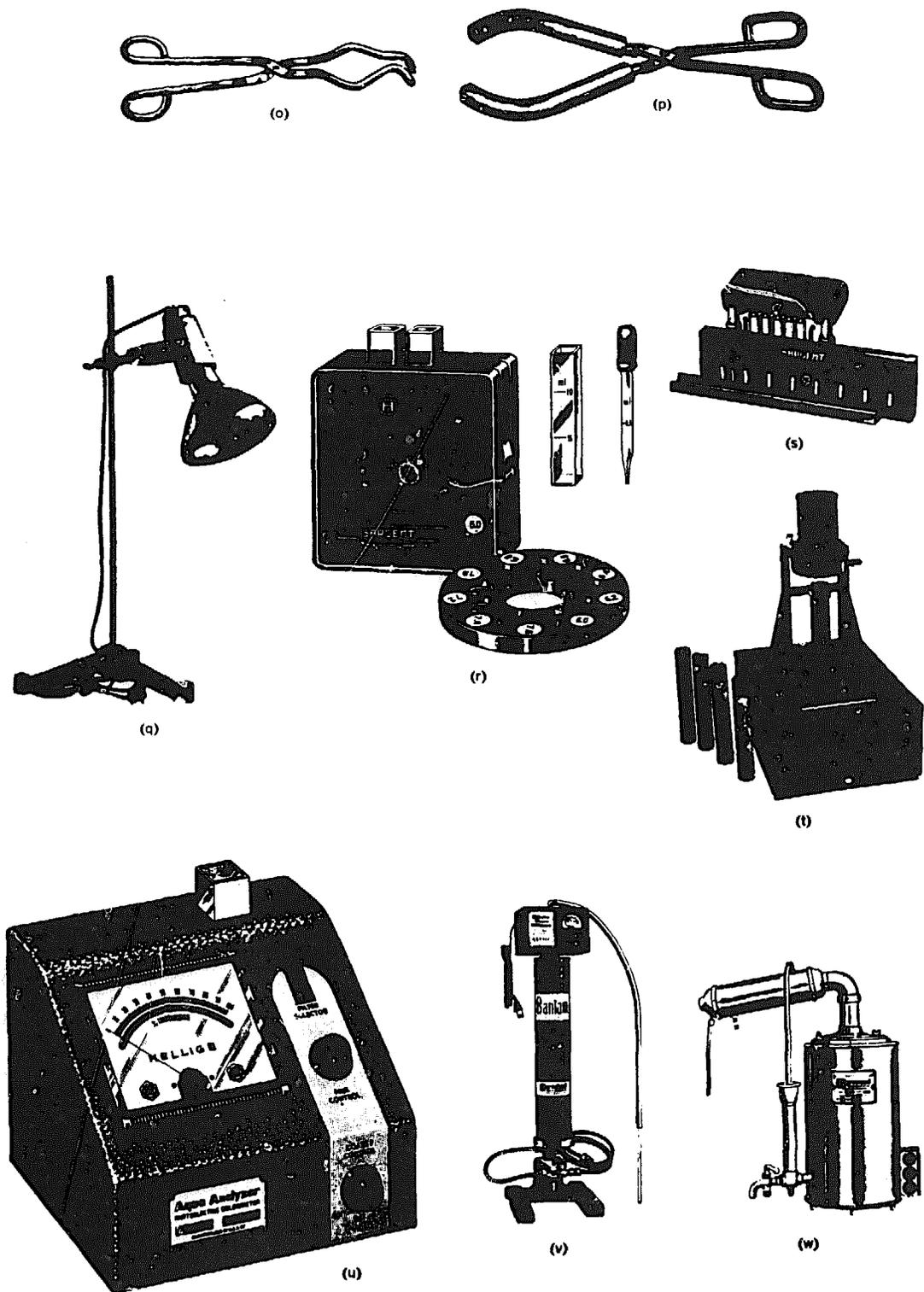


Fig 2 Other Laboratory Apparatus

Key: (o) crucible tongs; (p) beaker tongs; (q) infrared drying lamp; (r) disk-type color comparator; (s) slide-type color comparator; (t) Lumetron nessler tube photometer; (u) Hellige photometer; (v) demineralizer; (w) water distillation unit.

vapor or contain a poison. A safety pipet equipped with a rubber bulb and a check valve should be on hand for such situations. Medicine droppers are convenient when small volumes from a few drops to 1 ml have to be dispensed.

For accurate work, samples should be measured with volumetric pipets. Where speed is desired at a slight sacrifice in accuracy, the careful measurement of sample volumes with 100- or 50 ml graduated cylinders yields results which are acceptable on a routine basis. In the range below 50 ml, however, it is wise to rely on volumetric pipets for the measurement of sample volumes, because measuring errors can play a significant role in the final result.

Care of glassware. The cleaning cabinet should contain an adequate supply of soap, detergent, brushes, and sponges. Many water tests have been spoiled by poor dishwashing. Both measuring and mixing glassware must be clean. All pipets and burets should be rinsed promptly after use - first with tap water, then with distilled water - before the dissolved matter has a chance to air-dry on the glass. A peg board on which to hang bottles upside down, preferably with clasps for pipets, should be near all laboratory sinks. In this way, glassware can be drained immediately after washing. A soiled towel should never be used for wiping the inside of a beaker, flask, or bottle.

A good household detergent is advisable for difficult cleaning problems. Glassware which is covered with dust or has contained a sticky substance like molasses or sewage sludge should be scrubbed well with such a detergent.

Another cleaning mixture which finds extensive laboratory application is made by placing 40 to 50 g potassium dichromate in a glass- or rubber-stoppered bottle, then carefully pouring in 1 liter of concentrated sulfuric acid. Plastic bottles and cork and plastic stoppers should be avoided because they are attacked by this chromic acid cleaning mixture. The absorption of moisture from wet dishes or from the air causes the potassium dichromate crystals to dissolve, with a resultant loss in cleaning power. More potassium dichromate crystals can be added from time to time to restore the cleaning strength. The mixture should be discarded when it turns green.

The chromic acid cleaning solution should be handled with extreme caution and restricted to the most difficult cleaning problems. The cleaner is so powerful that it readily attacks human flesh as well as garments of cotton, wool, and most synthetic fibers.

Where necessary, special cleaning fluids such as alcohol, carbon tetrachloride, and muriatic or hydrochloric acid (especially effective for removing iron and carbonate deposits) should also be on hand.

Desiccators. A desiccator is a container of heavy glass with a removable top and a false bottom above the real base. The space below the false bottom is filled with a drying agent for the purpose of keeping the air dry while hot dishes cool to room temperature. A desiccator which is 12 inches in diameter provides sufficient space for most water plant demands.

3. Miscellaneous Lab Accessories

Appropriate stands must be available to support such apparatus as burets and funnels. These stands must be equipped with clamps which allow a variable height.

Spatulas of stainless steel and plastic are needed for the transfer of solid chemicals.

The ordinary heating chores are performed with a Bunsen or Fisher burner. Many laboratories rely on the electric hot plate. The hot plate eliminates the ring stand necessary with a gas burner. In general, gas heat can be regulated from a low to a high temperature, whereas the cheaper electric heating units are incapable of such fine adjustments.

Tongs and forceps are useful implements for handling heated beakers and erlenmeyer flasks. A wash water bottle for rinsing beakers and erlenmeyer flasks during quantitative transfers is also a necessity.

A laboratory marking pencil is valuable for temporarily identifying sample bottles as well as the glassware in which the subsequent tests are carried out.

4. Distilled Water

Distilled water is needed for the preparation of solutions described in this Manual. An acceptable distilled water should be free of carbon dioxide, ammonia, residual chlorine, and chloride. Boiling distilled water for 15 minutes drives off the carbon dioxide and residual chlorine, but has little effect on the ammonia or chloride. An efficient still or mixed-bed ion exchanger (also called a deionizer) will provide a satisfactory grade of distilled water. In some cases, however, to achieve an acceptable distilled water, ordinary distilled water should be passed through a bed of Amberlite MB-3 resin (a product of Rohm and Haas Co., Philadelphia, Pa.), Bio-Rad AG501-X8(D) resin (a product of Bio-Rad Laboratories, Richmond, Calif.), or similar mixed-bed ion-exchange resin (see Fig. 4).

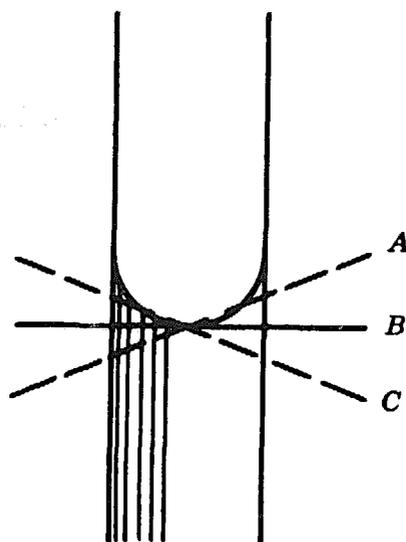


Fig. 3. Reading of Meniscus
Correct reading is along Line B.

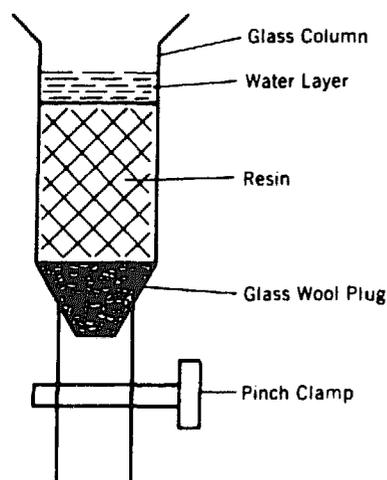


Fig. 4. Ion-Exchange Column

5. Titrant and Standard Solutions

The word "normal" (abbreviated "N") in front of the name of a reagent indicates the concentration or strength. The word "standard" is also used in this connection, but this word means merely that the concentration is exactly known, while normal tells what the concentration is. The concentration of a standard solution is sometimes referred to as its normality. Thus, the normality of the standard acid used in determining the alkalinity of water is 0.02. This can be written 0.02N, N/50, or 1/50N, all of which have the same meaning. The important thing to remember about normal solutions is that, for example, 100 ml of a 0.02N sodium hydroxide solution will exactly neutralize 100 ml of a 0.02 N sulfuric acid solution. A 0.1N sulfuric acid solution, on the other hand, is a different and stronger concentration of the acid.

The ready availability on the market of high-purity reagent chemicals enables the preparation of sufficiently useful titrant solutions by merely dissolving the specified weight of the chemical in the proper volume of distilled water. The standardization of each solution is described in the latest edition of *Standard Methods* (see Sec. 11), should an individual have the time and inclination to prepare his own reagents. Many of the standard solutions, though, can be purchased from reliable supply houses, and the use of these solutions is recommended to those individuals who lack confidence in their understanding or ability to prepare the solutions in question.

All bottles containing standard solutions should be labeled with such data as name, concentration, date of preparation, and, in some cases, the tests for which the solution is intended.

All of the chemicals used for the preparation of solutions should be of the best available quality. Chemicals which are labeled "ACS grade," "primary standard grade," or "analytical reagent grade" - and dyes which are certified by the Biological Stain Commission - will yield the best results, and should be used. In the methods which follow, two names are sometimes given for the same chemical (such as potassium dihydrogen phosphate and potassium monobasic phosphate). These names refer to the same substance, whose formula follows the reference. This formula should be checked with the formula on the container label to be sure the right chemical is used.

Warning: To avoid internal and external injury, all chemical reagents described in this book should be handled with care both in the form purchased and after dilution. Needless to say, extreme caution is required when dealing with chemicals marked Poison, Danger, Caution, or Flammable. Safety, automatic, and bulb-operated pipets are recommended for measuring and transferring chemical solutions which can inflict injury by inhalation, absorption, or contact through the mouth, lungs, and skin.

6. Colorimetric Determinations

Color comparisons. A number of determinations in this Manual specify the use of nessler tubes for making color comparisons. For best results the sample must be placed in a tube identical to those of the color standards. The complete set of tubes should be matched; that is, they should be of the same size and have the same length of viewing path. The color comparison should be made by looking

straight down into the tubes which are resting over a white surface, thereby allowing light to be reflected upward through the columns of liquid. Nessler tube sets are available in tall form or low form. Usually the tall form is preferable.

If color standards like the chromate-dichromate permanent standards for residual chlorine are to be retained for an extended time, the nessler tube can be covered with transparent plastic film secured by rubber bands. This arrangement enables the colors to be viewed through the plastic film.

In many instances, the sample colors are developed directly in the nessler tubes in which the final comparison is made. After the reagents are added, the contents of the nessler tube must be thoroughly mixed. The tubes can be closed with clean, rinsed rubber stoppers and inverted four to six times for an adequate mix. Another way of mixing is by means of a plunger made from a glass rod, one end of which has been flattened in a gas flame.

Comparators and test kits. Like standard solutions, permanent standards for color, turbidity, and many chemical substances are available from reputable suppliers. The colorimetric kits come in two general forms: the disk type, containing a wheel of small colored glasses, and the slide type, containing liquid standards in glass ampules.

The disk comparator consists of a plastic box with an eyepiece in front and a frosted glass in the rear. Behind the eyepiece is a place for attaching the rotating color disk. Between the disk and the frosted glass is a divided compartment for two cells which accommodate both the untreated water sample and the reagent-treated sample. The untreated water sample is positioned on the same side and in line with the rotating permanent colors. The companion side of the compartment is reserved for the sample in which the color has been chemically developed. The concentration is estimated visually by peering through the eyepiece and matching the developed color with the permanent colors on the disk. The color disk can easily be removed from the kit and replaced with a disk for another determination. Thus, one comparator kit can serve for a number of separate determinations.

A good rule is to check all such kits for reliability immediately upon purchase and periodically thereafter. The standard solutions described in this Manual are suitable for checking the calibration of such permanent standards.

Since most kits are designed for use with the manufacturer's own reagents, new batches of reagents must be purchased as the need arises. Strange and questionable results should always prompt a recheck of a newly arrived batch of reagents. The analyst must continually be alert for any deterioration of the purchased reagents after prolonged standing. If the reagents fail to yield reasonable results with the standard solutions, immediate replacement of the purchased reagents is advisable.

When a test is performed at infrequent intervals, a wise policy is to buy the needed solutions and reagents in small amounts, and to date the bottles on delivery so that questionable results can be quickly traced to staleness. Generally, stable reagents are marketed in solution form, while the unstable reagents are put up in the form of a standard-size pill or a powder which can be dispensed with a small measuring spoon. The pill or powder may be added in the dry form or dissolved in a definite volume of water in order to prepare sufficient solution for one test.

Test kits give rapid, fairly acceptable, and consistent results in the hands of individuals with a minimum of training. Their easy portability make them useful for checking operations in the field.

Many test kits are based on a simplified version of a *Standard Methods* test. The reagents come ready for use and normally bear a code number or trade name. Both number and name help in ordering replacements. Among the advantages of a test kit are: freedom from making up solutions; no need for preparing standards on every occasion; an apparatus assembly designed specifically for the determination. On the other side of the picture, the accuracy of the tests run by kits will seldom equal that attainable in a good laboratory. With regard to precision (the ability to reproduce the same result time after time), the kit may approach the results obtained in the laboratory. Test kits should be used for monitoring raw and treated water only after trials have demonstrated that the results on those particular waters equal or closely approximate the values obtained by the recognized and accepted standard methods.

Test kits and reagent solutions can be purchased for the following determinations: aluminum, ammonia nitrogen, residual chlorine, chlorine dioxide, color, copper, fluoride, iron, manganese, pH, phosphate and polyphosphate, silica, turbidity, alkalinity, calcium, calcium carbonate stability test (available under the name of Enslow stability indicator), carbon dioxide, chloride, hardness, and dissolved oxygen. The last seven determinations listed involve titrations rather than colorimetric estimations.

Photometers. These time-saving instruments rely on a photoelectric estimation of the color value. Simpler photometers come equipped with a series of color filters and calibration cards for a number of determinations. Before starting a determination, the correct color filter and calibration card are inserted into the appropriate slots of the instrument. After treatment with the supplied reagents, the colored sample is placed in the beam of the electric light and the concentration read directly from the pointer on the calibration card. Similar precautions must be observed with such photometers as with comparator kits. The manufacturer's reagents should be used and the analyst should be on guard against questionable reagents. The same checking system can also be followed.

A photometer which accepts low-form nessler tubes is also satisfactory for most of the colorimetric determinations in this Manual. The colors are developed as described in the Manual, and the nessler tube is placed in a holder for the purpose of measuring the light absorption produced by the combination of a color filter and the colored sample. Before any water tests are undertaken, a calibration curve must be prepared from a series of standard solutions. Then the treated sample is measured in the same way and the reading referred to the calibration curve for the concentration. The use of this instrument requires some knowledge of photometric methods and therefore will give the best results in the hands of an analyst who is properly qualified.

Other types of comparator kits and photometric and spectrophotometric instruments are available on the market. Mention or description of all the different models is obviously beyond the scope of this Manual, nor can the Manual make recommendations regarding the suitability of any such equipment for a particular lab.

7. Availability of Laboratory Supplies

Many reputable laboratory suppliers are scattered throughout the United States, Canada, and elsewhere. The names and addresses of some suppliers are listed in the Buyers' Guide, part 2, *Journal American Water Works Association*. The advertising columns of each issue of *Journal AWWA* are also fine sources of such information. Many of the large suppliers publish catalogs illustrating the apparatus in stock. The beginner will also find the state department of health most helpful in recommending the nearest supply houses for the satisfaction of these needs.

Where reference is made in this Manual to a product of a specific manufacturer, it should be understood that an equivalent may be substituted, if the user is able to satisfy himself that the substitute will be equally suitable for the purpose described.

8. Records

All important data should be recorded in some form of bound notebook. This permits a ready reference to all previous tests and makes all laboratory results available at any time for checking or for comparing water conditions. Often it is possible to save considerable time in routine analyses by consulting these records. Such frequently used data as standard solution proportions, conversion factors, and the like can be placed in conveniently indexed form in the back of the current record book. Most water plants develop record forms for their own files to suit the individual needs of the plant. These provide for laboratory data, as well as for other vital information relating to plant operations. Such record books can be arranged to cover suitable periods of time — weekly, monthly, or yearly — with provisions for a permanent file for back records. State boards of health generally require plants to submit periodic reports on forms issued for this purpose. Each water plant should check with the board of health in this regard and it is a good idea to make copies of these reports a part of the plant records. Should there ever occur situations in which the water supply might be blamed for disease, corrosion, or other unfortunate events, complete accurate records will provide the best possible protection.

9. Sampling

No laboratory test is of any value unless the sample tested is representative of the water being checked. Haste or carelessness in securing and preparing a sample can completely offset the accuracy of the determinations made in the laboratory. It is seldom sufficient to rely on a single, or "grab," sample. Often it is necessary to use a composite sample made up of a number of individual samples. Good judgment must be used in selecting a sampling method, and the selection of the method is influenced primarily by the laboratory facilities available. A few factors which should be considered in taking a sample are:

- (a) The character of the laboratory examinations to be made.
- (b) The use to be made of the results of the tests or analyses.
- (c) The nature of the water sampled and the variation in characteristics over the period of sampling.
- (d) The variation in the rate of flow over a period of sampling.

For the ordinary chemical or physical analysis of water, the sample should be collected in a clean, glass-stoppered glass bottle or in a plastic bottle. The size of

the sample will depend upon the tests to be made.

As well waters are usually quite constant in composition, composite sampling is seldom necessary. However, the well should be pumped long enough to insure that the sample which is taken will represent the ground water from which well is fed.

Ponds, lakes, and impounded waters are often subject to variable conditions resulting from natural causes such as seasonal turnovers, rains, winds, and internal currents. The variation is usually not so rapid nor over such a wide range as is encountered in flowing waters. In general, a single sample will be representative, but if conditions cause variations, several samples should be taken from different locations. These can be combined into a single composite sample if desired. It should be borne in mind that lakes may be stratified, and the water composition may vary with depth.

Most sampling is done at the water treatment plant itself. This is the logical point for sampling, particularly if raw water is to be treated and stored for distribution. The locations and frequency of sampling will be governed by the type of plant and the treatment applied. For example, raw water from wells may require only one sample a day, while a supply pumped from a stream may require a sample every hour. Also, samples should be taken at appropriate intervals at points where it is necessary to determine the quantity of chemicals required for treatment, and at points where the effectiveness of the treatment can be judged. Many laboratories, especially those in utilities serving large populations, have permanent provisions for taking constant samples at all points in the process.

TABLE 1
Conversion Factors

| | Equivalent | | | | |
|-----------------------------------|--------------------|------------------|------------------------|-------------------|-------------------------|
| | <i>ppm or mg/l</i> | <i>gr/US gal</i> | <i>lb/1,000 US gal</i> | <i>gr/Imp gal</i> | <i>lb/1,000 Imp gal</i> |
| 1 part per million | 1 | 0.0583 | 0.00834 | 0.0700 | 0.100 |
| 1 grain per US gallon | 17.1 | 1 | 0.143 | 1.20 | 0.172 |
| 1 pound per 1,000 US gal | 120 | 7 | 1 | 8.41 | 1.20 |
| 1 grain per Imperial gallon | 14.3 | 0.833 | 0.119 | 1 | 0.143 |
| 1 pound per 1,000 Imperial gallon | 99.8 | 5.83 | 0.833 | 7 | 1 |

A growing practice is to collect samples from the distribution system for both bacteriologic and chemical examination. Clean and externally sanitary areas should be selected for the collection of bacteriologic samples. If possible, public lavatories should be avoided as sampling points for bacteriologic purposes. Whether the sample is intended for bacteriologic or chemical examination, care should be taken to see that it is representative of the distribution flow and that external matter is ex-

cluded from the bottle. The best procedure is to scrub and clean the outer parts of the faucet, and then let the water run freely until its temperature reaches the steady reading known to exist in the distribution mains of the particular geographic area. Only then should the sample collection be undertaken. The following data should accompany the sample: location of the station or site, date of collection, time of collection, temperature of the water, name of collector, and any other information that may be useful in accounting for unusual results.

10. Conversion Factors

In the laboratory, chemicals are weighed in grams and milligrams, and liquids are measured in liters and milliliters. For this reason, standard reports are made in milligrams per liter (abbreviated "mg/l"). Since 1 liter of water usually weighs close to 1,000,000 mg, 1 mg of substance in 1 liter of water represents 1 part per million (abbreviated "ppm"). The unit "ppm" is often used in plant operations for simplicity. It should be understood that, in water analysis, ppm always means ppm *by weight*, never by volume. So 1 ppm equals 1 mg of material per 1,000,000 mg of water or 1 pound of material per 1,000,000 pounds of water, etc., but not 1 gallon of material per 1,000,000 gallons of water. Likewise, 10 ppm hardness as calcium carbonate means 10 mg of calcium carbonate per 1,000,000 mg of water or 10 pounds of calcium carbonate per 1,000,000 pounds of water.

Occasionally it is necessary to convert milligrams per liter to grains per gallon, or to pounds per 1,000 gallons. Table 1 presents a set of conversion factors useful for this purpose.

11. Other Publications

There are a number of excellent publications on water plant laboratory operations, and it is a good idea to have as many of these reference volumes as possible in the laboratory library. Three that should be on every bookshelf are:

Standard Methods for the Examination of Water and Wastewater (prepared jointly by the American Public Health Association, the American Water Works Association, and the Water Pollution Control Federation). *Standard Methods* describes all of the accepted procedures for analyzing water and waterborne wastes.

Safety Practice for Water Utilities. This manual, published by AWWA, describes the proper ways of handling materials and avoiding injuries in the laboratory and elsewhere.

Water Quality and Treatment. Also published by AWWA, this comprehensive volume is an aid in understanding the relationship of laboratory results to plant operation and water quality.

I. Chemical Examination

Alkalinity

1. Purpose of Test

Many of the chemicals used in treating water can cause a change in its alkalinity, but the most pronounced changes are caused by coagulants and by the softening chemicals, lime and soda ash (sodium carbonate). This alkalinity test is intended to provide results for use in calculating the chemical dosages needed in the coagulation and softening processes. Total alkalinity must also be determined in the course of the calcium carbonate stability test (see page 28) and in estimating carbonate hardness (see page 53).

2. Warning

This method is suitable for the titration of waters containing hydroxide, carbonate, or bicarbonate alkalinity. The water should be free of color or turbidity which might obscure and affect the indicator response. The residual chlorine content of the water should not exceed 1.8 mg/l. When a water fails to satisfy any of these conditions, the procedures in the latest edition of *Standard Methods* should be followed.

3. Apparatus

- 3.1. A 25 ml buret and support.
- 3.2. A 100 ml graduated cylinder or appropriate volumetric pipets for measuring the sample.
- 3.3. Two or more 250 ml flasks or porcelain casseroles.
- 3.4. Two or more stirring rods.
- 3.5. Three dropping pipets or medicine droppers of 0.5-1 ml capacity for dispensing sodium thiosulfate, phenolphthalein indicator, and methyl orange indicator solutions.

4. Reagents

- 4.1. Sodium thiosulfate solution, 0.1*N* (not required if the water contains no

residual chlorine). Weigh 2.5 g sodium thiosulfate, $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$, and dissolve in 100 ml distilled water.

4.2. Phenolphthalein indicator solution. Weigh 0.5 g phenolphthalein disodium salt powder and dissolve in 100 ml distilled water.

4.3. Methyl orange indicator solution. Weigh 0.05 g methyl orange indicator powder and dissolve in 100 ml distilled water. The powder may take quite a bit of time and stirring to dissolve at room temperature.

4.4. Sulfuric acid titrant, 0.0200N H_2SO_4 . This solution requires some skill to prepare, standardize, and adjust to exactly 0.0200N (consult the latest edition of *Standard Methods*). The 0.0200N solution can be purchased from reputable supply houses.

5. Procedure

5.1. Fill the buret with sulfuric acid titrant. Record the liquid level in the buret by reading at the bottom of the meniscus. Guard against a leaky stopcock which results in the loss of titrant on standing.

5.2. Measure the appropriate sample volume for the indicated alkalinity ranges:

| Sample Volume ml | Alkalinity Range mg/l as CaCO_3 |
|---------------------|---|
| 100 | 0 - 250 |
| 50 | 251 - 500 |
| 25 | 501 - 1,000 |

Example: If the alkalinity falls within the range of 0-250 mg/l as CaCO_3 (calcium carbonate), take a 100 ml sample.

Place equal volumes of the sample into two 250 ml flasks (or porcelain casseroles), one of which will be used as a blank for color comparison.

5.3. If necessary, remove the residual chlorine by adding 1 drop (0.05 ml) of sodium thiosulfate solution to each flask (or casserole), and mix.

5.4. Add 2 drops of phenolphthalein indicator solution to one flask (or casserole), and mix. If the sample turns pink, carbonate or hydroxide is present; proceed with Step 5.5. If the sample remains colorless, the water contains bicarbonate or is acid; skip Steps 5.5-5.7 and go on to Step 5.8.

5.5. If the sample turns pink, gradually add sulfuric acid titrant from the buret, shaking the flask constantly (or stirring the contents of the porcelain casserole constantly) until the pink just disappears. Use the flask without any phenolphthalein indicator as the color comparison blank.

5.6. Read the new buret level at the bottom of the meniscus, and calculate the volume of acid used by subtracting the initial buret reading (Step 5.1) from the present reading.

TABLE 2

Calculation of Alkalinity From Titration Results*

| Titration Result | Alkalinity - mg/l as CaCO ₃ | | |
|--------------------|--|-----------|-------------|
| | Hydroxide | Carbonate | Bicarbonate |
| $P = 0$ | 0 | 0 | T |
| P less than $1/2T$ | 0 | 2P | T - 2P |
| $P = 1/2T$ | 0 | 2P | 0 |
| P more than $1/2T$ | 2P - T | 2T - 2P | 0 |
| $P = T$ | T | 0 | 0 |

*P = phenolphthalein alkalinity (mg/l); T = total alkalinity (mg/l).

5.7. Calculate the phenolphthalein alkalinity in terms of mg/l as calcium carbonate by multiplying the result found in Step 5.6 by the appropriate factor: For a sample volume of 100 ml, multiply milliliters of H₂SO₄ by 10; for a 50-ml sample, multiply by 20; and for a 25-ml sample, multiply by 40.

5.8. Add 2 drops (0.1 ml) of methyl orange indicator solution to both of the flasks (or casseroles) containing the water sample.

5.9. Again titrate with small volumes of sulfuric acid until the yellow color just begins to change to an orange. Continually compare the color of the sample with that of the blank against a white background. At the endpoint a slight difference should be noticeable between the color of the comparison blank, which remains a constant yellow throughout, and the titrated sample, which has gradually turned a very faint orange. The color change takes place within the span of 2 to 4 drops of sulfuric acid. If you have difficulty recognizing the change when adding 1 drop at a time, add the titrant 2 drops at a time near the endpoint. This will intensify the color change, and the slight loss in accuracy is not significant.

5.10. Again read the buret and calculate the total volume of acid used in both the phenolphthalein titration (Step 5.5, if carried out) and the methyl orange titration (Step 5.9). Multiply this total by the appropriate factor given in Step 5.7. The result is the total alkalinity (also called methyl orange alkalinity) in terms of mg/l as calcium carbonate.

6. Practical Applications of Alkalinity Titrations

6.1. The phenolphthalein alkalinity (abbreviated "P") and the total alkalinity (abbreviated "T") can be used to calculate the hydroxide, carbonate, and bicarbonate in terms of calcium carbonate. The titration to methyl orange (T) measures all of the alkalinity due to bicarbonate, carbonate, and hydroxide. The titration to phenolphthalein (P) on the other hand, measures all of the hydroxide and exactly one-half of the carbonate, but none of the bicarbonate. Therefore:

(a) Bicarbonate is present when the phenolphthalein alkalinity is less than one-half of the total alkalinity.

(b) Carbonate is present when the phenolphthalein alkalinity is not zero but is less than the total alkalinity.

Table 2 shows how to calculate the various types of alkalinity. The calculations are based on the following assumptions. Bicarbonate and carbonate alkalinities may exist in the same sample. Carbonate and hydroxide cannot coexist. Carbon dioxide may be present when phenolphthalein alkalinity is absent. Conversely, the existence of phenolphthalein alkalinity means the absence of carbon dioxide in the water. A pH of 8.3 represents the borderline of carbonate alkalinity. Since mineral acids are not normal constituents of water, their presence indicates pollution.

(c) Hydroxide is present when the phenolphthalein alkalinity is more than one-half of the total alkalinity.

Table 2 shows how to calculate the various types of alkalinity.

6.2. Hydroxide or caustic alkalinity in a water undergoing softening treatment indicates the presence of excess lime, which may be in the range of 10 to 50 mg/l. The alkalinity can be reduced to a suitable level by carbonation.

6.3. Soda ash (sodium carbonate) is added to some soft waters to provide sufficient carbonate alkalinity to improve alum coagulation. The alkalinity titrations afford a means of properly controlling the soda ash dosage.

Aluminum

1. Purpose of Test

This control method is designed for treatment plants where an aluminum salt is used as a coagulant. A concentration of aluminum greater than 0.05 mg/l in the finished water is a good indication that an incorrect coagulant dosage is being used.

2. Warning

This method is suitable only when fluoride and metaphosphate are absent. The water should be free of color and turbidity. The latest edition of *Standard Methods* should be consulted for procedures to remove any of the interfering substances.

3. Apparatus*

- 3.1. Seven or more 50 ml nessler tubes, matched, tall form; and support.
- 3.2. A 25 or 50 ml buret or appropriate pipets for measuring the standard aluminum solution.
- 3.3. 5 ml and 10 ml volumetric pipets.
- 3.4. Several 1 ml, 2 ml, and 10 ml measuring pipets.
- 3.5. Seven or more 250 ml erlenmeyer flasks.
- 3.6. A 25 ml graduated cylinder.
- 3.7. Wash water bottle for rinsing flasks.

4. Reagents

4.1. Stock aluminum solution:

- (a) On an analytical balance weigh 4.3965 g reagent grade aluminum potassium

*All glassware in 3.1 through 3.5 should be rinsed first with hot 1+1 hydrochloric acid, then with distilled water. Prepare 1+1 hydrochloric acid by mixing equal volumes of the concentrated acid with distilled water.

sulfate, $\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$.

(b) Carefully transfer the chemical to a 500 ml beaker and dissolve it in about 300 ml distilled water.

(c) Carefully transfer the solution from the beaker to a 1 l volumetric flask, rinsing the beaker with three 50 ml portions of distilled water. Dilute further to the 1 l mark and mix thoroughly.

4.2. Standard aluminum solution. Using a 10 ml volumetric pipet, transfer 10 ml of the stock aluminum solution to a 1 l volumetric flask, dilute to the mark with distilled water, and mix. This solution should be prepared fresh each day.

4.3. Buffer solution:

(a) Weigh 136 g sodium acetate, $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$.

(b) Carefully transfer the chemical to a 400 ml beaker and dissolve it in about 200 ml distilled water.

(c) Add 40 ml 1*N* acetic acid (prepare by mixing 2.4 glacial acetic acid with 38 ml of distilled water) to the solution in the beaker.

(d) Carefully transfer the solution from the beaker to a 1 l volumetric flask, rinsing the beaker with three 50 ml portions of distilled water. Dilute further to the 1 l mark with distilled water and mix thoroughly.

4.4. Stock dye solution: Use any one of the following available products:

(a) Solochrome cyanine R-200* or eriochrome cyanine[†]. Dissolve 100 mg of the dye in distilled water and dilute to 100 ml in a volumetric flask. This solution should have a pH value of about 2.9.

(b) Eriochrome cyanine R[†]. Dissolve 300 mg of the dye in about 50 ml distilled water. Adjust the pH value, which will be about 9, to about 2.9 with 1 + 1 acetic acid (approximately 3 ml required). Dilute with distilled water to 100 ml.

(c) Eriochrome cyanine R^{**}. Dissolve 150 mg of the dye in about 50 ml distilled water. Adjust the pH value, which will be about 9, to about 2.9 with 1 + 1 acetic acid (approximately 2 ml required). Dilute with distilled water to 100 ml.

Stock solutions prepared as described above have excellent stability, and can be kept for at least a year and perhaps longer.

4.5. Working dye solution: Using a volumetric pipet, dilute 10 ml of any of the stock dye solutions to 100 ml in a volumetric flask with distilled water. Working solutions are stable for a least 6 months.

4.6. Ascorbic acid solution: Dissolve 100 mg ascorbic acid in distilled water and make up to 100 ml in a volumetric flask. Prepare fresh daily.

4.7. Sulfuric acid, 0.02*N*.

5. Procedure

5.1. Prepare the following series of aluminum standards by measuring the indicated volumes of standard aluminum solution (4.2) into separate 250 ml Erlenmeyer flasks:

* A product of Arnold Hoffman and Co., Providence, R.I.

† A product of K & K Labs, Plainview, N.Y.

‡ A product of Pfaltz and Bauer Inc., Flushing, N.Y.

** A product of Hartmen-Leddon Co., Philadelphia, Pa.

| Standard Aluminum Solution <i>ml</i> | Aluminum <i>mg/l</i> |
|---|-------------------------|
| 0 | 0 |
| 0.5 | 0.05 |
| 1.0 | 0.10 |
| 1.5 | 0.15 |
| 2.0 | 0.20 |
| 2.5 | 0.25 |
| 3.0 | 0.30 |

5.2. Using a graduated cylinder, add 25 ml of distilled water to each flask.

5.3. Place 25 ml of sample in another 250 ml erlenmeyer flask. (If aluminum concentration is greater than 0.3 mg/l, take a smaller sample and dilute to 25 ml with distilled water.)

5.4. To each flask add 1 ml 0.02*N* sulfuric acid. Add to the flask containing the sample additional 0.02*N* sulfuric acid in order to neutralize the alkalinity of the original sample. (If necessary, determine this amount by making an alkalinity test on 25 ml of the original sample.)

5.5. Add 1 ml ascorbic acid to the sample and standard and mix.

5.6. Add to each flask 10 ml buffer solution and mix.

5.7. Using a volumetric pipet, add 5 ml of working dye solution (4.5) and mix.

5.8. Immediately pour the solution into marked nessler tubes. Use several small portions of distilled water to rinse out each flask and add the rinsings to the nessler tubes. Make up to a volume of 50 ml with distilled water and mix.

5.9. Let stand for 5-15 min. then compare the color of the sample with the standards and determine from the color the amount of aluminum present in the sample.

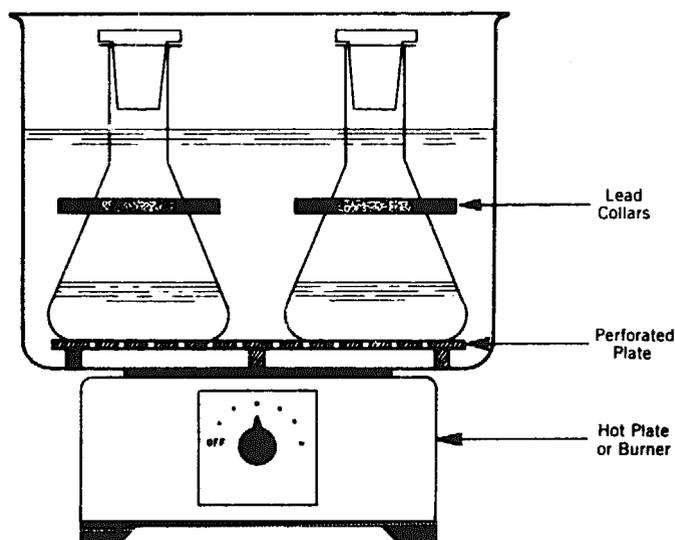


Fig. 5. Water Bath for Aluminum Determination

Ammonia Nitrogen

1. Purpose of Test

Trace amounts of ammonia occur naturally in many water supplies or may be artificially applied along with chlorine to form a combined chlorine residual. Ammonia is important in a surface water because a sudden increase may suggest the entry of sewage or industrial pollution. A rise in the ammonia content is associated with a corresponding rise in the chlorine demand of the water when the free available residual chlorination process is used. Rivers receiving untreated, partially treated, and so-called completely treated sewage may show a large rise in ammonia nitrogen when the river becomes completely or nearly completely ice covered, thereby resulting in a commensurately large chlorine demand. This method is suitable for the estimation of ammonia nitrogen concentrations in the range of 0.1 to 1.2 mg/l.

2. Warning

The results obtained with this method are approximate. The accuracy can be improved by adjusting the color of the permanent standards to an exact match with a set of prepared ammonium chloride standards.

The sample must be clear and colorless after coagulation with zinc sulfate and sodium hydroxide (Step 5.2). The addition of nessler reagent should produce no off-color yellow or green, nor turbidity. The appearance of these conditions usually means the presence of pollution. The latest edition of *Standard Methods* should be consulted on steps for overcoming the off-colors and turbidity, and improving the accuracy of the determination.

A number of commercially available nessler reagents are unsatisfactory for the determination of small amounts of ammonia found in many source waters of potable quality. Only the preparation described in Sec. 4.6 can be recommended for this determination.

3. Apparatus

- 3.1. Nessler tubes, 50 ml matched, tall form; and support.
- 3.2. A 25- or 50 ml buret or appropriate pipets for measuring the platinum chloroplatinate and cobaltous chloride solutions.
- 3.3. Clean rubber stoppers (No. 2 size) for nessler tubes.
- 3.4. Measuring pipets for adding zinc sulfate and sodium hydroxide solutions.
- 3.5. A dropping pipet for dispensing Rochelle salt solution.
- 3.6. A 1-l automatic or safety pipet for dispensing nessler reagent.
- 3.7. A 100-ml graduated cylinder for measuring the sample.
- 3.8. Appropriate volumetric pipets for measuring the sample filtrate.
- 3.9. A filter funnel.
- 3.10. Filter paper of soft, loose texture. Whatman No. 41 or Schleicher & Schull No. 589 are satisfactory.

4. Reagents

- 4.1. Deionized distilled water. Since many distilled waters contain some am-

monia nitrogen, pass ordinary distilled water through a bed of Amberlite MB-3 (a product of Rohm and Haas Co., Philadelphia, Pa.) or Bio-Rad AG501-X8(D) (a product of Bio-Rad Laboratories, Richmond, Calif.) resin for best results. (See Fig. 4 for the construction of the necessary column.) Use this water for the preparation of all reagents except the solutions in Sec. 4.2, for which ordinary distilled water is satisfactory.

4.2. Solutions for permanent color standards:

(a) Potassium chloroplatinate solution:

(1) Weigh 2.0 g potassium chloroplatinate (K_2PtCl_6). Transfer to a 1 l beaker and dissolve in 400 ml distilled water.

(2) Add 100-ml concentrated hydrochloric acid and mix thoroughly.

(3) Transfer the solution to a 1 l volumetric flask, rinsing the beaker with three 100-ml portions of distilled water. Dilute further to the 1 l mark with distilled water. Stopper and mix thoroughly.

(b) Cobaltous chloride solution:

(1) Weigh 12.0 g cobaltous chloride ($CoCl_2 \cdot 6H_2O$). Transfer to a 600 ml beaker and dissolve in 200 ml distilled water.

(2) Add 100 ml concentrated hydrochloric acid and mix thoroughly.

(3) Transfer the solution to a 1 l volumetric flask, rinsing the beaker with three 100 ml portions of distilled water. Dilute further to the 1 l mark with distilled water. Stopper and mix thoroughly.

4.3. Zinc sulfate solution. Weigh 100 g zinc sulfate ($ZnSO_4$). Dissolve in 96 ml deionized distilled water (4.1).

4.4. Sodium hydroxide solution. Weigh 250 g sodium hydroxide (NaOH) pellets. Dissolve in 97 ml deionized distilled water.

4.5. Rochelle salt solution:

(a) Measure 100 ml distilled water into a 250 ml beaker. Mark off a line at the 100 ml water level on the beaker.

(b) Weigh 50 g potassium sodium tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$). Dissolve the chemical in the 100 ml distilled water in the 250 ml beaker.

(c) Remove the ammonia impurities in the Rochelle salt by boiling off the water until the liquid level just reaches the 100 ml line on the 250 ml beaker. Cool the solution to room temperature and transfer to a bottle.

4.6. Nessler reagent:

(a) Weigh separately 100 g mercuric iodide (HgI_2) and 70 g potassium iodide (KI). Transfer to a 400 ml beaker and dissolve in 100 ml deionized distilled water.

(b) Weigh 160 g sodium hydroxide (NaOH) pellets. Transfer to a 1 l beaker and dissolve in 500 ml deionized distilled water.

(c) While stirring with one hand, slowly pour Solution (a) into Solution (b).

(d) Transfer Solution (c) to a 1 l volumetric flask or graduated cylinder and dilute to the mark with deionized distilled water. Mix thoroughly. Store in a tightly stoppered pyrex bottle.

Handle this poison with caution and avoid taking into the mouth.

Dispense the solution with an automatic or safety pipet.

5. Procedure

5.1. Preparation of color standards:

(a) Prepare the following series of color standards by measuring the indicated volumes of the potassium chloroplatinate solution and the cobaltous chloride solution into separate 50 ml nessler tubes:

| Potassium Chloroplatinate Solution <i>ml</i> | Cobaltous Chloride Solution <i>ml</i> | Ammonia Nitrogen Equivalent <i>mg</i> |
|---|--|--|
| 1.2 | 0 | 0 |
| 2.8 | 0 | 0.002 |
| 4.7 | 0.1 | 0.004 |
| 5.9 | 0.2 | 0.007 |
| 7.7 | 0.5 | 0.010 |
| 9.9 | 1.1 | 0.014 |
| 11.4 | 1.7 | 0.017 |
| 12.7 | 2.2 | 0.020 |
| 15.0 | 3.3 | 0.025 |
| 17.3 | 4.5 | 0.030 |
| 19.0 | 5.7 | 0.035 |
| 19.7 | 7.1 | 0.040 |
| 19.9 | 8.7 | 0.045 |
| 20.0 | 10.4 | 0.050 |
| 20.0 | 15.0 | 0.060 |

Select and prepare any appropriate smaller number of standards from this series if the ammonia nitrogen variation at the plant falls within a narrower range.

(b) Add distilled water to each tube to bring the volume to the 50 ml mark, and mix.

(c) Protect these standards by capping the tubes with clean rubber stoppers if the standards are to be used over a period of several months.

5.2. Coagulation and filtration of sample:

(a) With a graduated cylinder measure 100 ml of sample into a 250 ml beaker.

(b) With a measuring pipet add 1 ml zinc sulfate solution. Mix thoroughly.

(c) With a measuring pipet add 0.5 ml sodium hydroxide solution. Mix thoroughly.

(d) Allow the resulting white floc to settle for 5 minutes.

(e) Prepare a filter with a rapid filter paper.

(f) Pour an estimated 25 ml of the clear liquid through the filter. Catch the filtrate in a beaker and discard this filtrate.

(g) Pour the remaining clear liquid through the same filter, but catch and save this filtrate in a 50 ml nessler tube or a clean 100 ml beaker.

5.3. Color development of sample:

(a) Measure the appropriate volume of the filtrate for the indicated ammonia nitrogen range:

| Sample Volume <i>ml</i> | Ammonia Nitrogen Range <i>mg/l</i> |
|----------------------------|---------------------------------------|
| 50 | 0.1 - 1.0 |
| 25 | 1.1 - 2.0 |
| 10 | 2.1 - 5.0 |

Place the filtrate in a 50 ml nessler tube. If necessary, dilute to the 50 ml mark with deionized distilled water.

(b) With a dropping pipet add 2 drops of Rochelle salt solution. Cap the nessler tube with a rubber stopper and mix the contents by inverting the tube four to six times.

(c) With an automatic or safety pipet add 1 ml nessler reagent.

(d) Recap the tube with a rubber stopper and mix the contents by inverting the tube six times.

(e) Allow the yellow or brownish color to develop for 10 minutes.

(f) Compare the sample with the permanent standards and estimate from the color the amount (mg) of ammonia nitrogen in the sample.

(g) Calculate the ammonia nitrogen in terms of mg/l by multiplying the result found in Step (f) by the appropriate factor:

| Sample Volume <i>ml</i> | Multiply mg Ammonia Nitrogen by: |
|----------------------------|---|
| 50 | 20 |
| 25 | 40 |
| 10 | 100 |

Calcium

1. Purpose of Test

Calcium and magnesium are the major hardness components in a water. Calcium in the form of lime or calcium hydroxide may be used to soften water or to control corrosion through pH adjustment.

This method is designed for the routine determination of calcium in potable water supplies.*

2. Warning

The water should be free of color or turbidity which might obscure or affect the indicator response.

Fortunately, substances which cause errors in this titration are seldom present in potable water supplies. Barium, strontium, copper, aluminum, lead, tin, zinc, manganese, and iron can affect the results if present in sufficient quantity. The

*Two United States patents (No. 2,583,890 and 2,583,891) have been issued to G. Schwarzenbach disclosing titration and complexometric methods for quantitative determination of water hardness. Nothing contained in this Manual is to be construed as granting any right, by implication or otherwise, for manufacture, sale, or use in connection with any method, apparatus, or product covered by patent, or as insuring anyone against liability for infringement of patent.

latest edition of *Standard Methods* should be consulted for the concentrations of all these substances which can be tolerated in a sample.

Alkalinity in excess of 300 mg/l as calcium carbonate (CaCO_3) may cause an indistinct endpoint with some hard waters.

The titration should be performed on a sample which is at room temperature and should be completed within 5 minutes to avoid trouble arising from the precipitation of calcium carbonate.

The solid indicator mixture is subject to deterioration and should be stored in a tightly stoppered bottle.

3. Apparatus

- 3.1. A 25 ml buret and support.
- 3.2. A 50 ml graduated cylinder or appropriate pipets for measuring the sample.
- 3.3. Two or more 250 ml porcelain casseroles.
- 3.4. Two or more stirring rods.
- 3.5. A 1 ml measuring pipet for dispensing the sodium hydroxide solution.
- 3.6. A small measuring spoon, 0.2-0.3 ml capacity, for dispensing the dry indicator mixture.

4. Reagents

The following prepared solutions and solid mixture are commercially available from reputable suppliers:

4.1. Sodium hydroxide solution, 1*N*. Weigh 4 g NaOH. Dissolve in 100 ml distilled water.

Be careful not to suck this solution into the mouth because it will attack the inner mouth lining.

4.2. Solid indicator mixture. Either murexide or Calcon indicator mixture is satisfactory. Murexide has been in use longer, but many analysts find the Calcon color changes during titration easier to observe. Murexide changes from red through various purple stages to an orchid purple; Calcon changes from red through purple to a definite blue without any reddish tint. Prepare either as follows:

(a) Weigh separately: 100 g sodium chloride (NaCl); and either 0.2 g murexide (also called ammonium purpurate) or 0.2 g Calcon (also called Erichrome Blue-Black R).

(b) Place the sodium chloride and the indicator in a mortar and grind together with a pestle until the red dye is uniformly distributed throughout the white salt. Store in a tightly stoppered bottle.

4.3. EDTA titrant. Prepare as directed in Hardness, Sec. 4.4.

5. Procedure

5.1. Fill the buret with EDTA titrant. Record the liquid level in the buret by reading at the bottom of the meniscus. Guard against a leaky stopcock which results in the loss of titrant on standing.

5.2. Measure the appropriate sample volume for the indicated calcium range:

| Sample Volume ml | Calcium Range mg/l as $CaCO_3$ |
|------------------|--------------------------------|
| 50 | 0 - 200 |
| 25 | 201 - 400 |
| 10 | 401 - 1,000 |

If a sample of only 25 ml is needed, add 25 ml distilled water to bring the total volume to 50 ml; with a 10 ml sample, add 40 ml distilled water. Measure the additional distilled water with a graduated cylinder. Place the sample (and extra distilled water if needed) in a porcelain casserole. (Go on with Step 5.3 if murexide indicator is used, or skip to Step 5.4 if Calcon is used.)

5.3. If murexide indicator is used, proceed as follows:

(a) Prepare a color comparison blank by placing 50 ml distilled water (measured with a graduated cylinder) in a similar porcelain casserole.

(b) Comparison blank and the sample; and mix. (The resulting pH of 12-13 ties up the magnesium as hydroxide.)

(c) With a small measuring spoon add 1 measure (0.2 g) solid indicator mixture to the color comparison blank and the sample; and mix to dissolve.

(d) To the color comparison blank carefully add from the buret 1 or 2 drops of EDTA titrant. Stir the distilled water containing the sodium hydroxide and murexide indicator mixture until the red color turns to an unchanging orchid purple color. Record the new buret level by reading at the bottom of the meniscus.

(e) If the sample turns a red color, gradually add EDTA titrant from the buret, stirring constantly. Continue adding the titrant until the red color turns to a faint purple color. Stop the titrant addition at this point for 10 seconds but continue the stirring. Resume adding the EDTA titrant drop by drop until the faint purple turns to the same deep orchid purple as the comparison blank. Stir the contents of the porcelain casserole as before throughout the new addition. The color change from a faint purple to orchid purple usually occurs within a span of 6 drops.

(f) Record the new buret level by reading at the bottom of the meniscus.

(g) Calculate the gross volume of titrant used by subtracting the buret reading found in Step 5.3(d) from the last reading, Step 5.3(f).

(h) Calculate the blank correction by subtracting the buret reading in Step 5.1 from the buret reading in Step 5.3(d).

(i) Calculate the net volume of titrant used for the sample by subtracting the result found in Step 5.3(h) from the result found in Step 5.3(g). (Go on to Step 5.5.)

5.4. If Calcon indicator is used, proceed as follows:

(a) With a measuring pipet add 2 ml sodium hydroxide solution to the sample.

(b) With a small measuring spoon add 1 measure (0.2 g) solid indicator mixture; mix to dissolve.

(c) If the sample turns a red color, add EDTA titrant from the buret drop by

drop, stirring constantly, until the color changes through purple to a definite blue at the endpoint.

(d) Record the new buret level by reading at the bottom of the meniscus. Subtract the initial buret reading (Step 5.1).

5.5. Calculate the calcium in terms of mg/l as calcium carbonate by multiplying the result found in Step 5.3(i) or 5.4(d) by the appropriate factor:

| Sample Volume <i>ml</i> | Multiply ml EDTA Titrant by: |
|----------------------------|---------------------------------------|
| 50 | 20 |
| 25 | 40 |
| 10 | 100 |

5.6. If you have difficulty recognizing the color changes when adding 1 drop of titrant at a time, add the titrant 2 drops at a time near the endpoint. This will intensify the color changes, and the slight loss in accuracy is not significant. A good light also aids in detecting the color changes.

Calcium Carbonate Stability Test

1. Purpose of Test

This test has two general uses. It is valuable in checking the stability of a water which has been softened by the lime-soda ash process. It also determines the total alkalinity required by a water to prevent corrosion through the deposition of a protective coating of calcium carbonate in the mains.

2. Warning

The sample should be collected with as little splashing and aeration as possible in order to prevent the loss of dissolved carbon dioxide. For the same reason, the bottle should be stoppered in such a way as to prevent the entrapment of air near the stopper.

3. Apparatus

- 3.1. A glass-stoppered BOD bottle, 300 ml capacity.
- 3.2. A 100 ml pipet.
- 3.3. A filter funnel.
- 3.4. Filter paper. Whatman No. 50 is satisfactory.
- 3.5. Apparatus for determining total alkalinity (see Alkalinity, Sec. 3).

4. Reagents

- 4.1. Calcium carbonate (CaCO_3), precipitated powder, reagent grade.
- 4.2. Reagents for determining total alkalinity (see Alkalinity, Sec. 4).

5. Procedure

- 5.1. Determine the total alkalinity of the water sample as described in Alkalinity, Sec. 5.
- 5.2. Collect another sample without splashing and agitation by filling a 300 ml glass stoppered BOD bottle (see Carbon Dioxide, Sec. 5).
- 5.3. Add approximately 0.3-0.4 g calcium carbonate powder to the bottle.
- 5.4. Carefully replace the stopper so that no air bubbles are left at the top.
- 5.5. Mix the powder and the water by shaking the bottle at frequent intervals (every 10-15 minutes) for at least 3 hours.
- 5.6. Allow the sample to settle overnight. During the first part of the settling, tap the bottle gently and twist the stopper so that the powder which adheres to the walls and to the stopper can be loosened and thereby enabled to settle to the bottom of the bottle.
- 5.7. With a 100 ml pipet, carefully remove two portions of the supernatant (the clear layer above the settled material). If desired, carefully decant the supernatant.
- 5.8. Filter the withdrawn supernatant through filter paper. Discard the first 25 ml of the filtrate and save the rest.
- 5.9. Determine the total alkalinity on the filtrate (see Alkalinity, Sec. 5). Make sure that the calcium carbonate powder is completely removed from the filtrate to avoid any error in the total alkalinity.

6. Practical Application of Test

- 6.1. The water is undersaturated with respect to calcium carbonate and may be corrosive if the second total-alkalinity result (Step 5.9) is greater than the first total-alkalinity result (Step 5.1).
- 6.2. The water is supersaturated with calcium carbonate and may deposit a protective film or coating in the mains if the first total-alkalinity result (Step 5.1) exceeds the second total-alkalinity result (Step 5.9).
- 6.3. The water is stable and in equilibrium with calcium carbonate if the first (Step 5.1) and the second (Step 5.9) total-alkalinity results are similar. Such a water should be non-corrosive if a calcium carbonate film or coating already exists in the mains.
- 6.4. Changes in the total alkalinity of the sample can be brought about by the following amounts of chemicals:
 - (a) Each 1 mg/l dose of pure lime (CaO) or 1.1 mg/l dose of 90 per cent commercial quicklime increases the total alkalinity by 1.79 mg/l or reduces the carbon dioxide by 1.57 mg/l.
 - (b) Each 1 mg/l dose of pure hydrated lime, Ca(OH)_2 , or 1.41 mg/l dose of 93 per cent commercial hydrated lime increases the total alkalinity by 1.35 mg/l or reduces the carbon dioxide by 1.19 mg/l.

(c) Each 1 mg/l dose of pure soda ash (Na_2CO_3) or 1.02 mg/l dose of 98 per cent commercial soda ash increases the total alkalinity by 0.944 mg/l or reduces the carbon dioxide by 0.415 mg/l.

These quantities will not necessarily stabilize every water because stability is interrelated with other important factors. For a more extended discussion of this test and its various applications see the bibliography given under Saturation and Stability With Respect to Calcium Carbonate in the latest edition of *Standard Methods*.

6.5. The scaling of pipes and equipment by water which is supersaturated with calcium carbonate may be minimized by the use of phosphate salts or by stabilizing the softened water by recarbonation, addition of alum, or contact with limestone.

Carbon Dioxide (Free)

1. Purpose of Test

The corrosive properties of dissolved carbon dioxide gas make its elimination from a water desirable. This can be achieved either through physical removal by aeration or through chemical conversion to the less aggressive bicarbonate or carbonate states by the addition of alkaline compounds.

A knowledge of the carbon dioxide content is also important in softening treatment. First, carbon dioxide consumes additional lime and soda ash during its neutralization. Second, carbon dioxide may be applied to the softened water just before filtration in order to dissolve any unsettled calcium carbonate, which might otherwise deposit later on the filter sand or in the distribution mains. As a rule, well waters contain more dissolved carbon dioxide gas than river waters. In the case of most potable water supplies carbon dioxide represents the important acid factor, thereby making the titration for carbon dioxide substantially equal to the acidity titration.

2. Warning

The sample should be collected with as little splashing and aeration as possible in order to prevent the loss of dissolved carbon dioxide. For best results the determination should be performed immediately after sample collection. When the carbon dioxide concentration exceeds 10 mg/l, the titration should be repeated on a fresh sample to check the reliability of the first value. If the determination must be delayed for any reason, the sample should be collected in a 500 ml bottle and stoppered in such a way as to prevent the entrapment of air near the stopper.

3. Apparatus

3.1 A supply of one-hole rubber stoppers for insertion in the water tap.

3.2 Copper, stainless steel or other non-corrosive metal tubing of proper diameter for insertion into the rubber stopper.

3.3 Rubber or plastic tubing for connection to the metal tube.

3.4. Two or more 100 ml graduated cylinders or nessler tubes.

3.5. A dropping pipet or medicine dropper of 0.5- or 1 ml capacity for dispensing phenolphthalein indicator solution.

3.6 A long stirring rod.

4. Reagents

4.1. Boiled distilled water. Since most distilled waters contain some carbon dioxide, place ordinary distilled water in a large unstoppered pyrex flask or bottle and boil the water for at least 15 minutes to expel the carbon dioxide. Then cover the top and neck of the vessel with an oversize inverted beaker, and cool the water to room temperature in a bath of cold running water. Prepare the boiled distilled water immediately before needed in the preparation of standard sodium carbonate titrant (4.3).

4.2. Phenolphthalein indicator solution. Prepare as directed in Alkalinity, Sec. 4.2.

4.3. Sodium carbonate titrant, 0.0454*N*:

(a) On an analytical balance, carefully weigh 2.407 g dry sodium carbonate (Na_2CO_3) of primary standard grade quality.

(b) Carefully transfer to a 250 ml beaker and dissolve in 150 ml boiled distilled water.

(c) Carefully transfer the solution to a 1 l volumetric flask, rinsing the beaker with three 100 ml portions of boiled distilled water.

(d) Dilute further to the 1 l mark with boiled distilled water. Stopper and mix thoroughly.

Note: When storing this titrant, tightly stopper the storage container to prevent entry of atmospheric carbon dioxide. Use a rubber stopper to avoid the "freezing" which occurs with glass stoppers.

5. Procedure

5.1. Construct a special sampling line (see Fig. 6) for collecting water containing dissolved gases:

(a) Select a one-hole rubber stopper that fits snugly into the inside of the water tap from which the sample is to be drawn.

(b) Cut off a length of metal tubing equal to four times the length of the rubber stopper.

(c) Wet the outside surface of the entering end of the metal tube with a lubricating film of glycerol or glycerine and carefully introduce the metal tube into the hole of the rubber stopper. Work the tube into the hole until the end of the tube is flush with the surface of the stopper.

(d) Take a rubber (or plastic) tube of the proper diameter and attach a piece of sufficient length to the projecting end of the metal tube. If necessary, again wet the outside surface of the metal tube with a lubricating film of glycerol to ease the penetration of the rubber tubing.

(e) Thoroughly clean the rubber stopper, metal tube, and rubber tubing of all traces of glycerol by rinsing with the water to be sampled.

5.2. Collection of sample. Use the following procedure for the collection of all samples required in Sec. 5.3 and 5.4:

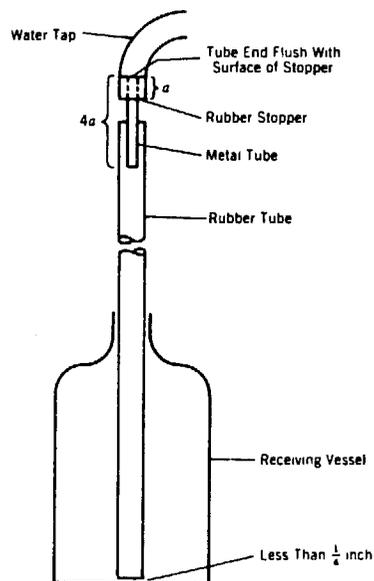


Fig. 6. Apparatus for Collecting Samples with Dissolved Gases

(a) Insert the rubber stopper in the water tap. Make sure that the entire sampling line is airtight and that no atmospheric oxygen can come into contact with the flowing water sample.

(b) Flush out the inside of the metal and rubber tubes with the water to be sampled.

(c) Insert the open end of the rubber tubing to within 1/4 inch of the inside bottom of the receiver (a bottle or a graduated cylinder or nessler tube, as required).

(d) Let the water sample overflow the receiver to the extent of several times the receiver capacity. Two or three minutes of overflow is usually sufficient.

(e) Gently withdraw the rubber tubing from the receiver as the water continues to overflow it.

5.3. Titration of sample:

(a) Collect a sample as described in Sec. 5.2, using a 100 ml graduated cylinder or nessler tube as the receiver. Flick the cylinder or nessler tube to throw off the excess sample above the 100 ml mark.

(b) Set the sample aside as the color comparison blank in the titration to follow.

(c) Fill the buret with sodium carbonate titrant. Record the liquid level in the buret by reading at the bottom of the meniscus.

(d) Collect a second sample as described in Sec. 5.2 and 5.3(a).

(e) Add 10 drops of phenolphthalein indicator solution to the second sample. If the sample turns pink, no carbon dioxide is present.

(f) If the sample remains colorless, add sodium carbonate titrant to the cylinder or nessler tube. Stir gently with a long stirring rod until a definite pink color persists for 30 seconds. Look down the length of the cylinder or nessler tube for evidence of the color change. Use the first sample without any phenolphthalein indicator solution as the color comparison blank.

(g) Read the new buret level at the bottom of the meniscus, and calculate the volume of sodium carbonate used by subtracting the initial buret reading, Step 5.3(c), from the present reading.

(h) Collect a third sample as described in Sec. 5.2 and 5.3(a). Immediately add the full amount of sodium carbonate titrant found in Step 5.3(g). Then add 10 drops of phenolphthalein indicator solution and stir with the long stirring rod. If the sample remains colorless, continue to add sodium carbonate until a definite pink color persists for 30 seconds. Accept the new result as the reliable titration.

(i) Calculate the free carbon dioxide concentration by multiplying the number of milliliters of sodium carbonate used by 10.

5.4. Handling of sample whose analysis must be delayed. When a sample must be transported to the laboratory for analysis, collect the sample as described in Sec. 5.2, using a 500 ml glass-stoppered bottle. Completely fill the bottle and replace the stopper in such a manner as to leave no air space near the top. To minimize the escape of dissolved carbon dioxide from the water, keep the bottle at a temperature lower than that at which the water was collected. Perform the analysis as soon as possible after collection, according to Sec. 5.3. Provide the 100 ml sample by siphoning the water from the 500 ml bottle into a graduated cylinder or nessler tube, allowing overflow to occur and gently withdrawing the rubber tubing.

Chloride

1. Purpose of Test

Chloride is a common constituent of water. In the form of sodium chloride, it is used to regenerate exhausted cation-exchange softeners. A small increase in the chloride concentration may result through the addition of chlorine disinfectant.

This test is designed for the routine determination of chloride in potable water samples.

2. Warning

The water should be free of color or turbidity which might obscure and affect the indicator response. The water sample should be neutral or slightly alkaline before the titration is begun. If the water is alkaline or acid beyond the pH range of 6.5-10.5, or contains bromide, iodide, sulfide, sulfite, thiosulfate, orthophosphate, or high concentrations of iron, the latest edition of *Standard Methods* should be consulted for ways of coping with the situation.

3. Apparatus

- 3.1. A 25 ml buret and support.
- 3.2. A 100 ml graduated cylinder or appropriate volumetric pipets for measuring the sample.
- 3.3. A medicine dropper or 1 ml measuring pipet for dispensing the indicator solution.
- 3.4. Two or more 250 ml porcelain casseroles.
- 3.5. Two or more glass stirring rods.

4. Reagents

- 4.1. Silver nitrate titrant, 0.014*N*:
 - (a) On an analytical balance, weigh 2.396 g dry silver nitrate (AgNO_3) of reagent grade quality.
 - (b) Carefully transfer the chemical to a 250 ml beaker and dissolve in 100 ml distilled water.
 - (c) Transfer the solution to a 1 l volumetric flask, rinsing the beaker with three 100 ml portions of distilled water. Dilute further to the 1 l mark with distilled water.
 - (d) Stopper, mix thoroughly, and store this solution in a brown bottle in a dark cupboard to protect against the destructive effects of light.
- 4.2. Potassium chromate indicator solution:
 - (a) Weigh 50 g potassium chromate (K_2CrO_4). Transfer to a 500 ml beaker and dissolve in 250 ml distilled water.
 - (b) Add silver nitrate titrant (4.1) until a definite red precipitate is formed. Cover the beaker with a watch glass and allow the precipitate to settle overnight.
 - (c) Cleanly separate the settled precipitate from the solution by decanting or by pouring the solution through a filter paper.
 - (d) Dilute the clear solution to 1 l in a 1 l graduated cylinder and mix thoroughly.

5. Procedure

5.1. Fill the buret with silver nitrate titrant. Record the liquid level in the buret by reading at the bottom of the meniscus. Guard against a leaky stopcock, which results in the loss of titrant on standing.

5.2. Measure the appropriate sample volume for the indicated chloride range using the table below on the left.

| Sample Volume <i>ml</i> | Chloride Range <i>mg/l</i> |
|----------------------------|-------------------------------|
| 100 | 1-50 |
| 50 | 51-100 |
| 25 | 101-200 |
| 10 | 201-500 |

| Sample Volume <i>ml</i> | Multiply ml Silver Nitrate Titrant by: |
|----------------------------|--|
| 100 | 5 |
| 50 | 10 |
| 25 | 20 |
| 10 | 50 |

If a sample of only 50 ml is needed, add 50 ml distilled water to bring the total volume to 100 ml; with a 25 ml sample, add 75 ml distilled water; with a 10 ml sample add 90 ml distilled water. Measure the additional distilled water with a graduated cylinder. Place the sample (and extra distilled water if needed) in a porcelain casserole.

5.3. Prepare a color comparison blank by placing 100 ml distilled water (measured with a 100 ml graduated cylinder) into a similar porcelain casserole.

5.4. With a measuring pipet or medicine dropper, add 1.0 ml potassium chromate indicator solution to the color comparison blank and the sample; and mix.

5.5. To the color comparison blank carefully add from the buret 0.30 ml of silver nitrate titrant. With a stirring rod, mix the resulting orange-red color throughout the solution. Record the new buret level by reading at the bottom of the meniscus.

5.6. If the sample turns a yellow color in Step 5.4, gradually add silver nitrate titrant from the buret. Stir the sample constantly. Continue adding the titrant until the sample turns the same orange-red color as in the color comparison blank. If you have difficulty recognizing the change when adding 1 drop at a time, add the titrant 2 drops at a time near the endpoint. This will intensify the color change, and the slight loss in accuracy is not significant.

5.7. Record the new buret level by reading at the bottom of the meniscus.

5.8. Calculate the gross volume of titrant used by subtracting the buret reading in Step 5.5 from the buret reading in Step 5.7.

5.9. Calculate the net volume of titrant used for the sample alone by subtracting 0.3 from the result found in Step 5.8.

5.10. Calculate the mg/l chloride by multiplying the result found in Step 5.9 by the appropriate factor shown in the table above on the right.

Chlorine (Residual) – General

1. Purpose of Test

Chlorine not only is an important disinfectant but also fills several other plant needs. It can react with ammonia, iron, manganese, protein substances, sulfides, and

some taste-and-odor-producing bodies to improve the finished water. Biologic growths also can be minimized and filter runs lengthened when free residual chlorination is practiced prior to filtration.

Two general types of residual are produced in a water from the chlorination process—the free available residual and the combined available residual. The free available residual, which occurs when the water is thoroughly chlorinated, may be of three kinds. Molecular chlorine (Cl_2) exists in the pH range of 1–4. Hypochlorous acid (HOCl) also occurs over the pH range of 1–9, being dominant within the 2–7 range. At pH 7.4 equal proportions of hypochlorous acid and hypochlorite ion (OCl^-) coexist, with hypochlorite gaining complete ascendancy above pH 9.5.

Residual combined chlorine in the form of monochloramine (NH_2Cl), dichloramine (NHCl_2), or trichloramine (also called nitrogen trichloride, NCl_3) is a less active oxidizing agent and slower in its bactericidal action than free chlorine. Residual combined chlorine is formed when the chlorination process is coupled with an ammonium compound, either naturally present or artificially added for the purpose.

Two test modifications are available for plant control of clear and colorless potable waters that are chlorinated to a predominantly free available chlorine residual or a predominantly combined available chlorine residual. The word *predominantly* means that three fourths or more of the residual is either in the free available form or the combined available form. Most drinking waters are chlorinated to a final residual of less than 1 mg/l. Chlorine residual determination can be performed according to individual preference: titrimetrically as in Method A or colorimetrically as in Method B.

2. Warning

Chlorine dioxide responds in the free available chlorine step to the extent of one fifth of its total available chlorine content.

Oxidized manganese, whether present in the natural form or as potassium permanganate added in the course of water treatment, reacts with the N, N-diethyl-p-phenylenediamine (DPD) reagent to yield a pink or red color identical to that produced by chlorine. In such a case a correction must be applied for this interference.

The reaction pH must be maintained within the 6.2 to 6.5 range for accurate results. Too low of a pH in the free chlorine step will enable some chloramine to masquerade as free chlorine. Too high of a pH may cause dissolved oxygen to yield a false pink color.

In all methods for differentiating free chlorine from chloramine, high temperature encourages chloramine to react with the color-forming reagents, thereby leading to an increase in apparent free chlorine after a fixed time interval.

Chlorine (Residual) A – Titrimetric Method

1. Purpose of Test

See Chlorine (Residual) – General.

2. Warning

See Chlorine (Residual) – General.

3. Apparatus

- 3.1. A 10-ml buret and support.
- 3.2. A 100-ml graduated cylinder or appropriate volumetric pipets for measuring the sample.
- 3.3. One or more 250-ml flasks.
- 3.4. Two 5-ml automatic, safety, or bulb-operated pipets for dispensing DPD reagent and phosphate buffer solution.
- 3.5. A small spatula for dispensing potassium iodide crystals.
- 3.6. A dropping pipet or medicine dropper, 0.5 ml, for dispensing sodium arsenite solution.

4. Reagents

- 4.1. Chlorine-free distilled water for the preparation of reagents 4.2, 4.3, and 4.4.

If necessary, prepare chlorine-free distilled water by exposing a tightly stoppered borosilicate glass bottle containing 4 l of distilled water to the sun's rays indoors or outdoors until the residual chlorine (as determined with the DPD test following) disappears because of the ultraviolet irradiation.

4.2. Dilute sulfuric acid solution:

- (a) Using a 50-ml graduated cylinder, measure 30 ml chlorine-free distilled water, and pour into a 100-ml beaker.
- (b) With a 10-ml graduated cylinder, measure 10 ml concentrated sulfuric acid.
- (c) While stirring with one hand, slowly and cautiously add the 10 ml sulfuric acid to the 30 ml chlorine-free distilled water. Considerable heat is generated by the mixing of the acid and the water, so pour slowly and mix well to avoid dangerous spattering. Cool to room temperature before use.

4.3. Ferrous ammonium sulfate (FAS) titrant:

- (a) Measure 1,200 ml distilled water into a 2-l pyrex flask and boil for 5 min. Then cover the top of the flask with an inverted 400-ml beaker and allow to cool to room temperature. Place in a cold water bath to speed cooling.
- (b) Pour half of the freshly boiled and cooled distilled water into a 1,500-ml beaker. Add 1 ml dilute sulfuric acid solution (4.2) and mix.
- (c) On an analytical balance weigh 1.106 g ferrous ammonium sulfate, $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$, of reagent-grade quality. Carefully transfer to the 1,500-ml beaker and dissolve in the freshly boiled and cooled distilled water prepared in Step (b).
- (d) Transfer the solution to a 1-l volumetric flask, rinsing the beaker with three 100-ml portions of distilled water. Dilute to the mark, stopper the flask, and mix thoroughly. A 1-ml quantity of this titrant is equivalent to 1.00 mg/l of chlorine in the titration procedure.

(e) Store the titrant in a brown, glass-stoppered bottle away from bright light. Discard after one month.

4.4. N, N-diethyl-p-phenylenediamine (DPD) reagent:

- (a) Place 600 ml chlorine-free distilled water in a 1,500-ml beaker. Add 8 ml dilute sulfuric acid solution (4.2) and mix.
- (b) Weigh 0.2 g disodium ethylenediamine tetraacetate dihydrate [also called (ethylenedinitrilo)tetraacetic acid sodium salt and EDTA], and dissolve by mixing in solution (a).

(c) Weigh either one of the following two chemicals: 1 g N, N-diethyl-p-phenylenediamine oxalate or 1.5 g p-amino-N:N-diethylaniline sulfate, and dissolve by mixing in solution (b).

(d) Transfer the combined solution (c) to a 1-l graduated cylinder and dilute to the 1,000-ml mark with chlorine-free distilled water. Mix thoroughly by pouring back into the beaker and stirring.

(e) Store the reagent solution in a brown, glass-stoppered bottle away from bright light. Discard when the solution becomes discolored.

Handle the oxalate reagent, which is toxic, with extreme caution, and particularly avoid taking into the mouth. Dispense the solution with an automatic, safety, or bulb-operated pipet.

4.5. Phosphate buffer solution:

(a) Weigh the following dry chemicals separately: (1) 24 g disodium hydrogen phosphate (also called sodium dibasic phosphate), Na_2HPO_4 ; and (2) 46 g potassium dihydrogen phosphate (also called potassium monobasic phosphate), KH_2PO_4 .

(b) Transfer the weighed chemicals to a 1,500-ml beaker and dissolve in 600 ml distilled water. If necessary, heat the solution gently and stir to bring all the solid chemicals into solution. If heat is used to dissolve the chemicals, cool the solution to room temperature.

(c) Weigh 0.8 g disodium ethylenediamine tetraacetate dihydrate [also called (ethylenedinitrilo)tetraacetic acid sodium salt and EDTA]. Dissolve in 100 ml distilled water. Then add to solution (b), and mix.

(d) Transfer the mixed solution (c) to a 1-l graduated cylinder and dilute to the 1,000-ml mark with distilled water. Mix thoroughly by pouring back into the beaker and stirring.

(e) Weigh 20 mg mercuric chloride, HgCl_2 , and add to solution (d) for the purpose of preventing mold growth and interference in the free available chlorine test caused by any trace of iodide in the reagents.

4.6. Potassium iodide, KI, crystals.

4.7. Sodium arsenite solution for estimation of manganese interference: Weigh 5.0 g sodium arsenite (also called sodium metaarsenite), NaAsO_2 . Dissolve in 1 l distilled water.

Handle this poison with extreme caution and particularly avoid taking into the mouth. Dispense the solution with an automatic, safety, or bulb-operated pipet.

5. Procedure

5.1. Fill the buret with ferrous ammonium sulfate (FAS) titrant. Record the liquid level in the buret by reading at the bottom of the meniscus. Guard against a leaky stopcock, which can result in the loss of titrant up on standing.

5.2. Selection of sample volume:

Measure the appropriate sample and distilled water volumes for the indicated residual chlorine ranges:

| Residual Chlorine Range mg/l | Original Sample Volume ml | Distilled Water Volume ml |
|---------------------------------|------------------------------|------------------------------|
| 0.0-4.0 | 100 | 0 |
| 4.1-8.0 | 50 | 50 |
| 8.1-16 | 25 | 75 |

(a) If the residual chlorine falls within the range of 0.0-4.0 mg/l, take a 100-ml sample but add no distilled water. Place 5 ml phosphate buffer solution and 5 ml DPD reagent in a 250-ml flask, and mix. Add the 100-ml sample, and mix.

(b) When the total available chlorine exceeds 4.0 mg/l, reduce the sample size and dilute the sample with distilled water to a total volume of 100 ml *in the following manner*: First mix the 5 ml phosphate buffer solution and 5 ml DPD reagent with the specified volume of distilled water. Then add the smaller size sample to bring the total volume to 110 ml.

5.3. If the sample turns pink or red, gradually add FAS titrant from the buret, shaking the flask constantly until the pink color just disappears.

5.4. Read the new buret level at the bottom of the meniscus, and calculate the volume of titrant used by subtracting the initial buret reading (Step 5.1) from the present reading.

5.5. Calculate the free available chlorine by multiplying the result found in Step 5.4 by the appropriate factor:

| Original Sample Volume—ml | Multiply ml Titrant by |
|---------------------------|------------------------|
| 100 | 1 |
| 50 | 2 |
| 25 | 4 |

5.6 Add several crystals (total weight 0.5-1.0 g) of potassium iodide crystals to the flask and mix to dissolve. Let the solution stand for 2 min so that the chloramine can convert the iodide to iodine, as evidenced by the return of the pink or red color.

5.7. Resume titrating with small volumes of FAS titrant until the pink or red color again disappears.

5.8. Read the new buret level at the bottom of the meniscus, and record the total volume of titrant used in both the free chlorine titration (Step 5.4, if carried out) and the total available chlorine titration (Step 5.7). Multiply this total by the appropriate factor given in Step 5.5.

5.9. Subtract the Step 5.5 value from the Step 5.8 value to obtain the combined available chlorine.

5.10. Estimation of manganese interference:

(a) Place 5 ml phosphate buffer solution, one small crystal of potassium iodide, and 0.5 ml sodium arsenite solution into a 250-ml flask, and mix.

(b) Add 100 ml sample and mix.

(c) Add 5 ml DPD reagent and mix.

(d) If the solution turns pink or red, manganese interference is present. Titrate with FAS titrant until the pink color is discharged.

(e) Read the new buret level at the bottom of the meniscus, and calculate the volume of titrant used by subtracting the initial buret reading from the present reading. Multiply the result by the appropriate factor given in Step 5.5 to obtain the manganese interference.

5.11. Subtract the manganese interference from the results in Steps 5.5 and 5.8 to obtain the true free available chlorine and total available chlorine, respectively.

5.12. If chlorine dioxide is used in plant treatment, see Chlorine Dioxide, Method A, Sec. 5 for the applicable determinations and calculations.

Chlorine (Residual) B – Colorimetric Method

1. Purpose of Test

See Chlorine Residual—General.

2. Warning

See Chlorine Residual—General.

3. Apparatus

Except for the titration buret and flasks, all of the apparatus described in Chlorine (Residual), Method A, Sec. 3 as well as the following items are required.

3.1. Nessler tubes, 100 ml, tall form or low form; and support.

3.2. A 10-ml buret or appropriate pipets for measuring dilute potassium permanganate solution.

3.4. Clean rubber stoppers (No. 3 or larger size) for nessler tubes.

3.5. 250-ml beakers.

3.6. Comparator with permanent color standards in the appropriate chlorine range from 0.0 to 1.0 mg/l, 0.0 to 2.0 mg/l or greater chlorine range. Permanent color standards are available commercially* in the form of comparator kits. The compactness and convenience of these kits permit rapid and adequate estimations. However, prudence dictates that a newly arrived comparator be checked for correctness of the values assigned to the individual permanent colors. Such verification can be performed by transferring a portion of each known potassium permanganate solution in the nessler tube prepared in Sec. 5.1(b) to the water-containing cell of the comparator, developing the pink or red color with the manufacturer's reagents, and comparing the resultant color against the appropriate permanent color value. Should the developed color vary appreciably from the permanent standard, the validation process should be repeated several times to rule out gross experimental error. Only then should the necessary correction be applied to the deviant standard.

4. Reagents

Except for the ferrous ammonium sulfate (FAS) titrant, all of the reagents described in Chlorine (Residual), Method A, Sec. 4 as well as the following solution are required.

4.1. Potassium permanganate solution for preparation of color standards:

(a) On an analytical balance weigh 0.891 g dry potassium permanganate, KMnO_4 . Carefully transfer to a 250-ml beaker, and dissolve in 100 ml distilled water.

(b) Transfer the solution to a 1-l volumetric flask, rinsing the beaker with three 100-ml portions of distilled water; dilute to the 1-l mark with distilled water. Stopper and mix thoroughly this stock potassium permanganate solution.

(c) With a volumetric pipet, measure carefully 10 ml stock potassium permanganate solution (b) into a 100-ml volumetric flask.

(d) Dilute to the 100-ml mark with distilled water. Stopper and mix thoroughly

*Hach Chemical Co. of Ames, Iowa 50010, and LaMotte Chemical Products Co. of Chestertown, Maryland 21620.

this dilute potassium permanganate solution. Store the solution in a brown, glass-stoppered bottle away from bright light.

(e) Dilute 1.00 ml of solution (d) with distilled water to 100 ml, in compliance with Sec. 5.1 (a-d), to produce a color equal to 1.00 mg/l chlorine in the DPD reaction.

5. Procedure

5.1. Preparation of color standards:

(a) Transfer the dilute potassium permanganate solution [4.1(d)] to a 10-ml buret and measure the following volumes into separate 100-ml nessler tubes:

| Buret Reading <i>ml</i> | Volume of KMnO_4 <i>ml</i> | Chlorine Equivalent <i>mg/l</i> |
|----------------------------|--|------------------------------------|
| 0.10 | 0.10 | 0.10 |
| 0.30 | 0.20 | 0.20 |
| 0.70 | 0.40 | 0.40 |
| 1.30 | 0.60 | 0.60 |
| 2.10 | 0.80 | 0.80 |
| 3.10 | 1.0 | 1.0 |
| 4.60 | 1.5 | 1.5 |
| 6.60 | 2.0 | 2.0 |
| 9.60 | 3.0 | 3.0 |

(b) Add distilled water to the 100-ml mark of each nessler tube, cap the tube with a clean rubber stopper, and mix the contents thoroughly by inverting the tube four to six times.

(c) Prepare a color blank by filling another nessler tube to the 100-ml mark with distilled water.

(d) Develop the DPD color in each of the standards (b) and the blank (c) in the following manner:

(1) Into a 250-ml beaker measure 5 ml phosphate buffer solution and 5 ml DPD reagent.

(2) Pour the potassium permanganate standard prepared in the 100-ml nessler tube into the beaker with thorough mixing.

(3) Return the developed pink or red colored solution to the nessler tube.

5.2. Free available residual chlorine:

(a) Place 5 ml phosphate buffer solution and 5 ml DPD reagent in a 100-ml nessler tube.

(b) Measure 100 ml of sample with a graduated cylinder, and immediately pour the sample into the nessler tube.

(c) Cap the tube with a clean rubber stopper and mix the contents thoroughly by quickly inverting the tube four to six times.

(d) Promptly compare the developed pink or red color against the color standards prepared in Sec. 5.1 (d3).

(e) Record the result as milligrams per liter of free available residual chlorine.

5.3. Total available residual chlorine:

(a) Add several crystals (total weight 0.5-1.0 g) of potassium iodide to the nessler tube.

(b) Dissolve the crystals by inverting the stoppered tube four to six times.

(c) Let the solution stand for 2 min so that the chloramine can convert the iodide to iodine, as evidenced by an increase in the color intensity.

(d) Again compare the pink or red color against the color standards prepared in Sec. 5.1(d3).

(e) Record the result as mg/l total available residual chlorine.

(f) Subtract the result obtained in Step 5.2(e) from the result obtained in Step 5.3(e) and record the difference as mg/l combined available residual chlorine.

5.4. Estimation of manganese interference:

(a) Place 5 ml phosphate buffer solution, one small crystal of potassium iodide, and 0.5 ml sodium arsenite solution into a 100-ml nessler tube, and mix by swirling.

(b) Measure a 100-ml sample in a graduated cylinder, and immediately pour the sample into the nessler tube.

(c) Cap the tube with a clean rubber stopper and mix the contents thoroughly by quickly inverting the tube four to six times.

(d) Add 5 ml DPD reagent and again invert the stoppered tube four to six times to mix the contents.

(e) If the solution turns pink or red, compare the color, which is due to manganese interference, against the color standards prepared in Sec. 5.1(d3).

(f) Subtract the manganese interference from the results in Steps 5.2(e) and 5.3(e) to obtain the true free available chlorine and total available chlorine, respectively.

5.5. Reduction in sample and reagent volumes for work with comparator kits: Although a 100-ml sample is specified in the preceding tests, smaller samples can be taken with a proportionate scaling down in reagent quantities. For example, a 10-ml sample will require only one tenth the reagent volumes normally applied in the course of the procedure.

5.6. If chlorine dioxide is used in plant treatment, see Chlorine Dioxide, Method B., Sec. 5 for the applicable determinations and calculations.

Chlorine (Residual) C – Field Method Using Commercial Comparator Kit

1. Purpose of Test

This method (called the drop dilution method) is suitable for the approximate estimation of total residual chlorine concentrations above 10 mg/l, such as are applied in the disinfection of water mains and tanks.

2. Warning

The limitations described in Sec. 2 of the preceding laboratory methods for residual chlorine apply. Read that section carefully before proceeding with the field method.

The results of this method are only approximate.

3. Apparatus

3.1. A graduated cylinder for measuring distilled water.

3.2. Two automatic, safety, or bulb-operated pipets for dispensing phosphate buffer solution and DPD reagent.

3.3. A dropping pipet for measuring the water sample. Select a pipet that delivers 1.0 ml of sample per 20 drops. Set this pipet aside for such measurements exclusively.

3.4. A commercial comparator kit containing a suitable range of standards. Study carefully Sec. 3.6 of the preceding Method B and Sec. 6 of the General Introduction to this manual regarding the advantages and limitations of comparator kits.

4. Reagents

DPD reagent and phosphate buffer solution. Prepare as described in Sec. 4.4, 4.5 of the laboratory method for residual chlorine (Method A).

5. Procedure

5.1. Ascertain the volume of the comparator cell, and using automatic, safety, or bulb-operated pipets, add the proper volume of phosphate buffer solution and DPD reagent (0.5 ml for each 10 ml of distilled water to be added).

5.2. Using a graduated cylinder, add a measured volume of distilled water.

5.3. With a dropping pipet, add the water sample a drop at a time, with mixing, until a pink or red color is formed that matches one of the color standards.

5.4. Record the total number of drops used and the final chlorine value obtained.

5.5. Calculate the mg/l residual chlorine as follows:

(a) Multiply by 20 the number of milliliters of distilled water used in Step 5.2.

(b) Multiply the result in Step 5.5(a) by the final chlorine value (mg/l) recorded in Step 5.4.

(c) Divide the result found in Step 5.5(b) by the total number of drops of water sample recorded in Step 5.4.

Chlorine (Residual) D – Orthotolidine Test

1. Purpose of Test

This plant control method is designed for clear and colorless potable waters which are chlorinated to a predominantly free available chlorine residual or a predominantly combined available chlorine residual. The word predominantly means that three-fourths or more of the residual is either in the free available form or the combined available form. Since most drinking waters are chlorinated to yield a final residual of less than 1 mg/l, the preparation of permanent color standard for the 0-1.0 mg/l range alone is described. The latest edition of *Standard Methods* should be consulted for directions on preparing permanent color standards in the chlorine range of 1-10 mg/l, should they be necessary at a particular plant.

2. Warning

For best results samples containing a high proportion of combined available chlorine should be rapidly chilled to as near 1°C as possible before the orthotolidine reagent is added for the determination of the free available residual; otherwise the free available chlorine reading may be erroneously high.

The determination of the combined available residual, on the other hand, should be performed on samples which have been brought to room temperature (20-25°C) in order to insure full color development.

Fortunately, the strong oxidizing agents which might seriously affect the orthotolidine-arsenite method are normally absent in drinking waters.

Since the residual chlorine in a sample may decrease rapidly on standing or in sunlight, the determination should be performed immediately after collection and out of the sunlight. The results grow more questionable as the time between collection and analysis increases.

The nessler tubes in which the chlorine color is developed should be thoroughly cleaned at least twice a week, and oftener if they become dirty for any reason.

3. Apparatus

3.1. Nessler tubes, 100 ml, matched, tall form; and support.

3.2. A 50 ml buret or appropriate pipets for measuring dilute chromate-dichromate solution.

3.3. Two 50 ml automatic or safety pipets.

3.4. Clean rubber stoppers (No. 3 size) for nessler tubes.

3.5. Filter paper.

4. Reagents

4.1. Solutions for permanent color standards:

(a) Strong phosphate buffer solution:

(1) On an analytical balance weigh the following two dry chemicals separately: 22.86 g disodium hydrogen phosphate (also called sodium dibasic phosphate), Na_2HPO_4 ; and 46.16 g potassium dihydrogen phosphate (also called potassium monobasic phosphate), KH_2PO_4 .

(2) Carefully transfer the two weighed chemicals to a 1 l beaker and dissolve in 600 ml distilled water. If necessary, heat the solution gently and stir to bring all the solid chemicals into solution. If heat is used to dissolve the chemicals, cool the solution to room temperature.

(3) Transfer the solution to a 1 l volumetric flask, rinsing the beaker with three 100 ml portions of distilled water. Dilute further to the 1 l mark with distilled water. Stopper and mix thoroughly.

(4) Set this solution aside to age for several days. Remove the precipitate which forms in the solution during that time by pouring the solution through a filter paper.

(b) Dilute phosphate buffer solution. With a 100 ml volumetric pipet, measure two portions of the filtered strong phosphate buffer solution, 4.1(a), into a 1 l volumetric flask. Dilute to the 1 l mark with distilled water. Stopper and mix thoroughly.

(c) Strong chromate-dichromate solution:

(1) On an analytical balance, weigh the following two dry chemicals separately: 1.55 g potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) and 4.65 g potassium chromate (K_2CrO_4).

(2) Carefully transfer the two weighed chemicals to a 500 ml beaker and dissolve in 300 ml dilute phosphate buffer solution, 4.1(b).

(3) Transfer the solution to a 1 l volumetric flask, rinsing the beaker with three 100 ml portions of dilute phosphate buffer solution. Dilute further to the 1 l mark with dilute phosphate buffer solution. Stopper and mix thoroughly.

(d) Dilute chromate-dichromate solution. With a volumetric pipet, measure carefully 100 ml of the strong chromate-dichromate solution, 4.1(c), into a 1 l volumetric flask. Dilute to the 1 l mark with dilute phosphate buffer solution, 4.1(b). Stopper and mix thoroughly.

4.2. Orthotolidine reagent:

(a) Weigh 1.35 g orthotolidine dihydrochloride (also called o-tolidine dihydrochloride). Avoid inhalation, ingestion, and contact with the skin by handling this potentially harmful powder so as to prevent the light particles from becoming airborne.

(b) Carefully transfer the weighed powder to a 1 l beaker and dissolve in 500 ml distilled water.

(c) With a 500 ml graduated cylinder, measure 350 ml distilled water and 150 ml concentrated hydrochloric acid. Pour both the measured distilled water and the hydrochloric acid into a 1,500 ml beaker.

(d) While stirring with one hand, slowly pour Solution 4.2(b) into Solution 4.2(c).

(e) Store the reagent in a tightly stoppered brown bottle away from bright light, preferably in a cool cupboard. Dispense this corrective reagent with an automatic, safety, or bulb-operated pipet.

4.3. Sodium arsenite solution. Weigh 5.0 g sodium arsenite (also called sodium metaarsenite), NaAsO_2 . Dissolve in 1 l distilled water.

Handle both the orthotolidine and sodium arsenite reagents with extreme caution, and particularly avoid taking them into the mouth. Dispense both solutions with automatic, safety, or bulb-operated pipets.

5. Procedure

5.1. Preparation of color standards:

(a) Prepare the following series of color standards by measuring the indicated volumes of the dilute chromate-dichromate solution, 4.1(d), into separate 100 ml nessler tubes:

| Chromate-Dichromate Solution ml | Chlorine Equivalent mg/l | Chromate-Dichromate Solution ml | Chlorine Equivalent mg/l |
|------------------------------------|-----------------------------|------------------------------------|-----------------------------|
| 0 | 0 | 35 | 0.35 |
| 1 | 0.01 | 40 | 0.40 |
| 2 | 0.02 | 45 | 0.45 |
| 5 | 0.05 | 50 | 0.50 |
| 7 | 0.07 | 60 | 0.60 |
| 10 | 0.10 | 70 | 0.70 |
| 15 | 0.15 | 80 | 0.80 |
| 20 | 0.20 | 90 | 0.90 |
| 25 | 0.25 | 100 | 1.0 |
| 30 | 0.30 | | |

Select and prepare any appropriate smaller number of standards from this series if the residual chlorine variation at the plant falls within a narrower range.

(b) Add dilute phosphate buffer solution, 4.1(b), to the 100 ml mark, and mix by inverting 10 times.

(c) Protect these standards by capping the tubes with clean rubber stoppers if the standards are to be used over a period of several months.

5.2. Determination of free available residual chlorine:

(a) Rapidly chill the water sample to as low a temperature as possible by cooling in ice water.

(b) With an automatic or safety pipet, transfer 5 ml orthotolidine reagent to a 100 ml nessler tube.

(c) Add 95 ml of the water sample by filling the nessler tube to the 100 ml mark.

(d) Cap the tube with a clean rubber stopper and mix the contents thoroughly by quickly inverting the tube four to six times. Take no more than 10 seconds for this entire step.

(e) With an automatic or safety pipet, immediately add 5 ml sodium arsenite solution to the tube.

(f) Recap the tube with the rubber stopper and again mix the contents by quickly inverting the tube four to six times.

(g) Promptly compare the developed yellow color against the permanent color standards.

(h) Record the result as mg/l free available residual chlorine.

Note: If chlorine dioxide is used in plant treatment, see Chlorine Dioxide, Sec. 2, for the applicable calculations.

5.3. Determination of total available residual chlorine:

(a) Rapidly bring the water sample to a temperature of 20-25°C.

(b) With an automatic or safety pipet, transfer 5 ml orthotolidine reagent to a 100 ml nessler tube.

(c) Add 95 ml of the water sample by filling the nessler tube to the 100 ml mark.

(d) Cap the tube with a separate clean rubber stopper and mix the contents thoroughly by inverting the tube four to six times.

(e) Compare the developed yellow color against the permanent color standards after the yellow color reaches a maximum, normally within 5 minutes.

(f) Record the result as mg/l total available residual chlorine.

5.4. Combined available residual chlorine. Subtract the result obtained in Step 5.2(h) from the result obtained in Step 5.3(f) and record the difference as mg/l combined available residual chlorine.

Chlorine (Residual) E – Field Method Using Nessler Tube

. Purpose of Test

This method (called the drop dilution method) is suitable for the approximate estimation of total residual chlorine concentrations above 10 mg/l, such as are applied in the disinfection of water mains or tanks.

. Warning

The limitations described in Sec. 2 of the laboratory method (Method A) for residual chlorine determination apply. Read that section carefully before proceeding with the field method. The field method does not distinguish between free and

combined chlorine residuals (see Sec. 1 of lab method for term explanations.)

The results of this method are only approximate.

3. Apparatus

3.1. Nessler tubes, 100 ml, matched, tall form or low form; and support.

3.2. A 50 ml buret or appropriate pipets for measuring dilute chromate-dichromate solution.

3.3. A 5 ml automatic or safety pipet.

3.4. A dropping pipet for measuring the water sample. Select a pipet that delivers 1.0 ml sample in 20 drops. Set this pipet aside for such measurements only.

3.5. Clean rubber stoppers (No. 3 size) for nessler tubes.

3.6. Filter paper.

4. Reagents

4.1. Solutions for permanent color standards. Prepare as in Sec. 4.1 of the laboratory method for residual chlorine. (Method A).

4.2. Orthotolidine reagent. Prepare as described in Sec. 4.2 of the laboratory method for residual chlorine.

5. Procedure

5.1. Preparation of color standards. Proceed as described in Sec. 5.1 of the laboratory method for residual chlorine.

5.2. Determination of total available chlorine:

(a) With an automatic or safety pipet, transfer 5 ml orthotolidine reagent to a 100 ml nessler tube.

(b) Add 95 ml distilled water by filling the nessler tube to the 100 ml mark. Mix the contents thoroughly.

(c) With a dropping pipet, add the water sample a drop at a time, mixing the contents of the nessler tube thoroughly after each addition. When mixing, close the mouth of the tube with a clean rubber stopper or place it against a clean wrist; do not use thumb or finger to close the tube mouth.

(d) Continue adding the water sample a drop at a time until a yellow color is formed in the nessler tube.

(e) Compare the color immediately with the permanent color standards. If the chlorine equivalent is less than 0.1 mg/l, continue adding water sample dropwise, with mixing, until the yellow color produced corresponds to at least 0.1 mg/l chlorine in the matching color standard.

(f) Record the total number of drops used and the final chlorine value obtained.

(g) Calculate the mg/l residual chlorine by first multiplying the final chlorine value by 1,900 and then dividing the result by the total number of drops used.

Chlorine Demand

1. Purpose of Test

Chlorine is added to a water supply to insure its bacteriologic acceptability or to improve the chemical, physical, and taste and odor characteristics.

This control method is suitable for the determination of the approximate amount of chlorine needed to produce a chlorine residual in source waters of potable quality containing comparatively little pollution. The bacteriologic safety of a water is in most cases assured when a slight excess of chlorine is present.

The procedures described in the latest edition of *Standard Methods* should be followed where pollution is known to be great.

2. Warning

The test conditions are subject to considerable variation. Temperature, contact time, and chlorine dosage markedly influence the chlorine demand, and must be mentioned in reporting all chlorine demand test results. Every precaution should be taken to keep all of the chlorine solutions and samples out of the range of direct sunlight and away from chlorine-consuming fumes like ammonia and sulfur dioxide.

If the test has a bacteriologic objective, all glassware should be thoroughly cleaned and sterilized.

If the chlorine dosing solution is unstandardized, the results can only be approximate.

Water samples within a chlorine demand in excess of 10 mg/l are best analyzed by the methods described in the latest edition of *Standard Methods*. The same book should be consulted when more reliable data are desired or for information on the proper method of standardizing the chlorine dosing solution.

3. Apparatus

In addition to the apparatus described under Chlorine (Residual), Method A, and/or B, Sec. 3, the following are also needed.

3.1. Five or more clean quart-size bottles or flasks.

3.2. A dropping pipet or medicine dropper for dispensing the chlorine dosing solution. Select a pipet or dropper which delivers 1.0 ml water in 20 drops. Set this pipet or dropper aside for exclusive use in the chlorine demand determination.

3.3. A 500 ml graduated cylinder for measuring the sample.

4. Reagents

In addition to all of the reagents described under Chlorine (Residual), Method A or Method B, Sec. 4, the following are also needed.

4.1. Stock chlorine solution. Purchase a bottle of household bleaching solution ("Clorox," "Roman Cleanser," or a similar product). Store in a dark, cool place such as a refrigerator to maintain the chemical strength indefinitely. These products contain approximately 5 per cent available chlorine, which represents 50,000 mg/l.

4.2. Strong chlorine dosing solution:

(a) Pour 20 ml stock chlorine solution into a 25 ml graduated cylinder.

(b) Pour 80 ml distilled water into a 100 ml graduated cylinder. Use separate graduated cylinders to prevent the possible contamination of the distilled-water bottle with chlorine fumes.

(c) Mix the two solutions. Store in a tightly stoppered brown bottle in a refrigerator to maintain the chemical strength for a month. Each drop of this solution represents a chlorine dosage of 1 mg/l when added to a 500 ml water sample.

4.3. Dilute chlorine dosing solution:

(a) Pour 10 ml strong chlorine dosing solution (4.2) into a 10 ml or 25 ml graduated cylinder.

(b) Pour 90 ml distilled water into a 100 ml graduated cylinder.

(c) Mix the two solutions. Store in a tightly stoppered brown bottle in a refrigerator to maintain the chemical strength for a week. Each drop of this solution represents a chlorine dosage of 0.1 mg/l when added to a 500 ml water sample.

(d) If possible, check the strength of Solution (c) by the method described in the Appendix (page 43).

5. Procedure

5.1. With a graduated cylinder, measure a 500 ml sample into each of 5 to 10 bottles or flasks.

5.2. Bring these bottled samples to the desired temperature (either room temperature or the temperature of the water entering or leaving the treatment plant).

5.3. Add the following number of drops of dilute chlorine dosing solution (4.3) to the separate bottles or flasks:

| Drops of Dilute Chlorine Dosing Solution | Chlorine mg/l |
|--|---------------|
| 2 | 0.2 |
| 4 | 0.4 |
| 6 | 0.6 |
| 8 | 0.8 |
| 10 | 1.0 |
| 12 | 1.2 |
| 14 | 1.4 |
| 16 | 1.6 |
| 18 | 1.8 |
| 20 | 2.0 |

Select and prepare any appropriate smaller number of samples from this series. Set up the dosing schedule so that the first sample will show no residual chlorine at the end of the desired contact time, which may be 1 hour, 2 hours, or even 24 hours.

5.4. Mix the contents of each bottle or flask and set aside at the temperature used in Step 5.2. Keep the samples in the dark.

5.5. Choose an appropriate contact time. At the end of the selected contact time, mix the contents of the first bottle or flask and determine the total available residual chlorine as described under Chlorine Residual, Methods A and/or B, Sec. 5. If desired, also determine free available chlorine as described under Chlorine (Residual), Methods A and/or B, Sec. 5.

5.6. Record the chlorine dosage added, the total or free available residual chlorine found, the contact time, and the temperature at which the bottled sample was kept during the contact period.

5.7. Repeat Steps 5.5 and 5.6 with each bottled sample.

5.8. Calculate the chlorine demand by subtracting the residual chlorine found from the dosage added, in each case.

5.9. If no residual chlorine (total or free, depending on which is of interest) is found in the sample dosed with 2 mg/l chlorine, use the strong chlorine dosing solution (4.2) to prepare the following series of samples:

| Drops of Strong Chlorine Dosing Solution | Chlorine mg/l |
|--|---------------|
| 2 | 2 |
| 4 | 4 |
| 6 | 6 |
| 8 | 8 |
| 10 | 10 |

5.10. Complete the determination as described in Steps 5.4 - 5.8.

Appendix

Estimation of Strength of Chlorine Dosing Solution

1. Apparatus

- 1.1. A 50 ml buret and support.
- 1.2. A 100 ml graduated cylinder.
- 1.3. A 1 ml measuring pipet for dispensing the starch indicator solution.
- 1.4. A 250 ml erlenmeyer flask.

2. Reagents

- 2.1. Starch indicator solution. Follow the directions in Oxygen (Dissolved), Sec. 4.4.
- 2.2. Boiled distilled water. Follow the directions in Carbon Dioxide, Sec. 4.1. Prepare the boiled distilled water immediately before needed in the preparation of sodium thiosulfate titrant.
- 2.3. Sodium thiosulfate titrant, 0.025N Na₂S₂O₃. Follow the directions in Oxygen (Dissolved), Sec. 4.6.
- 2.4. Sulfuric acid, concentrated, H₂SO₄.
- 2.5. Potassium iodide (KI) crystals or powder.

3. Procedure

3.1. Fill a 50 ml buret with sodium thiosulfate titrant and record the liquid level by reading the bottom of the meniscus.

3.2. Place 1 g potassium iodide crystals or powder in a 250 ml erlenmeyer flask.

3.3. With a graduated cylinder, measure 100 ml distilled water, add to the flask, and swirl to dissolve the crystals.

3.4. Cautiously add 2 ml sulfuric acid while swirling the flask.

3.5. Measure as accurately as possible 25 ml of the well-mixed dilute chlorine dosing solution, Sec. 4.3(c) above, and add to the flask, swirling rapidly during the addition. A reddish-brown color will appear at this stage.

3.6. Gradually add small portions of the sodium thiosulfate titrant from the buret while constantly swirling the liquid in the flask, until the sample changes to a pale yellow or straw color.

3.7. With a measuring pipet, add to the flask 1 or 2 ml starch indicator solution. A blue color will appear.

3.8. Continue adding sodium thiosulfate titrant drop by drop until the blue color just disappears.

3.9. Record the new buret level by reading at the bottom of the meniscus.

3.10. Calculate the volume of titrant used by subtracting the buret reading in Step 3.1 from the buret reading in Step 3.9.

3.11. Calculate the mg chlorine present in each 1.0 ml of the dilute chlorine dosing solution by multiplying the result obtained in Step 3.10 by 0.03545. If the net sodium thiosulfate titration is exactly 28.2 ml, the dilute chlorine dosing solution will be the proper strength of 1.0 mg per 1.0 ml. However, the dilute chlorine dosing solution is of acceptable strength when the net sodium thiosulfate titration ranges between 25.5 and 31.0 ml, in which case the chlorine content falls between 0.9 and 1.1 mg per 1.0 ml. Such a strength will give a chlorine dosage of 0.09 to 0.11 mg/l when 1 drop of the dilute chlorine dosing solution is added to a 500 ml water sample.

Chlorine Dioxide – General

1. Purpose of Test

Chlorine dioxide is added to water primarily for the destruction of such taste-producing bodies as phenols. Some iron and manganese problems are improved by this agent's ability to oxidize these substances to an insoluble state. Recently, greater attention has been directed to chlorine dioxide's disinfection power and nonreactivity with ammonia. The compound is generated at the site of use by reacting strong chlorine and sodium chlorite solutions.

Two test modifications are available for plant control of clear and colorless potable waters that are treated with chlorine dioxide. The determination can be performed according to individual preference: titrimetrically as in Method A or colorimetrically as in Method B.

2. Warning

All of Sec. 2 under Chlorine (Residual) should be read carefully. The same considerations apply to the chlorine dioxide determination.

Chlorine Dioxide A – Titrimetric Method

1. Purpose of Test

See Chlorine Dioxide—General.

2. Warning

See Chlorine Dioxide—General.

3. Apparatus

In addition to all of the apparatus described in Chlorine (Residual), Method A, Sec. 3, the following items are required.

3.1. Two dropping pipets or medicine droppers of 1-ml capacity for dispensing malonic acid and sulfuric acid solutions.

3.2. A 5-ml measuring pipet for dispensing sodium bicarbonate.

4. Reagents

The following reagents are required in addition to those described in Chlorine (Residual), Method A, Sec. 4.

4.1. Malonic acid solution: Weigh 1.0 g malonic acid, $\text{CH}_2(\text{COOH})_2$. Dissolve in 100 ml distilled water.

4.2. Sulfuric acid solution: With a 100-ml graduated cylinder, separately measure 88 ml distilled water and 12 ml dilute sulfuric acid solution prepared as in Sec. 4.2, Chlorine (Residual), Method A. While stirring with one hand, slowly and cautiously add the dilute sulfuric acid solution to the distilled water in a 250-ml beaker.

4.3. Sodium bicarbonate solution: Weigh 4.0 g sodium bicarbonate (also called sodium hydrogen carbonate and sodium acid carbonate), NaHCO_3 . Dissolve in 100 ml distilled water.

5. Procedure

5.1. Chlorine dioxide fraction:

(a) Fill the buret with ferrous ammonium sulfate (FAS) titrant. Record the liquid level in the buret by reading at the bottom of the meniscus. Guard against a leaky stopcock, which can result in the loss of titrant up on standing.

(b) Measure 100 ml sample into a 250-ml flask.

(c) Add 1 ml malonic acid solution and mix. Let the solution stand for 2 min so that the malonic acid can deactivate the chlorine.

(d) Add 5 ml phosphate buffer solution and 5 ml DPD reagent, and mix.

(e) If the sample turns pink or red, gradually add FAS titrant from the buret, shaking the flask constantly until the pink color just disappears.

(f) Read the new buret level at the bottom of the meniscus, and calculate the volume of titrant used by subtracting the initial buret reading in Step (a) from the present reading to yield one fifth the amount of chlorine dioxide in the sample.*

(g) Multiply the result obtained in step (f) by five to obtain the approximate total available chlorine content (in mg/l) of the chlorine dioxide.

5.2. Fraction including free available chlorine, one fifth of the chlorine dioxide, and combined available chlorine:

*This particular titration can only discern this amount.

(a) Refill the buret with FAS titrant. Record the liquid level in the buret by reading at the bottom of the meniscus.

(b) Measure 100 ml sample into a second 250-ml flask.

(c) Add 5 ml phosphate buffer solution and 5 ml DPD reagent, and mix.

(d) If the sample turns pink or red, gradually add FAS titrant from the buret, shaking the flask constantly until the pink color just disappears.

(e) Read the new buret level at the bottom of the meniscus, and record the total volume of titrant used in this titration, which embodies all of the free available chlorine plus one fifth of the chlorine dioxide. Subtract the net titration for chlorine dioxide found in Step 5.1(f) from the present titration to obtain the free available chlorine.

(f) Proceed immediately to Step (g) for the determination of the combined available chlorine component.

(g) Add several crystals (total weight about 0.5-1.0 g) of potassium iodide, mix to dissolve, and allow to stand for 2 min so that the chloramine can convert the iodide to iodine, as evidenced by the return of the pink or red color.

(h) Resume titrating with small volumes of FAS titrant until the pink or red color again disappears.

(i) Read the new buret level at the bottom of the meniscus, and subtract the step (e) reading from the present reading to obtain the combined available chlorine.

5.3. Total available chlorine:

(a) Refill the buret with FAS titrant. Record the liquid level in the buret by reading at the bottom of the meniscus.

(b) Measure 100 ml sample into a third 250-ml flask.

(c) Add 1 ml sulfuric acid solution and mix.

(d) Add several crystals (total weight about 0.5-1.0 g) of potassium iodide, and let the solution stand for about 1 min to enable the chlorine, chlorine dioxide, chloramine, and chlorite to convert the iodide to iodine.

(e) Add 5 ml phosphate buffer solution, 5 ml DPD reagent, and 5 ml sodium bicarbonate solution, and mix.

(f) If the sample turns pink or red, gradually add FAS titrant from the buret, shaking the flask constantly until the pink color just disappears.

(g) Read the new buret level at the bottom of the meniscus, and record the total volume of titrant needed to react with the total available chlorine.

Chlorine Dioxide B – Colorimetric Method

1. Purpose of Test

See Chlorine Dioxide--General.

2. Warning

See Chlorine Dioxide--General.

3. Apparatus

All of the apparatus described in Chlorine (Residual), Method B, Sec. 3, and the pipets listed in Chlorine Dioxide, Method A, Sec. 3 are required.

4. Reagents

Except for the ferrous ammonium sulfate (FAS) titrant, all of the reagents described in Chlorine (Residual), Methods A and B, Sec. 4, together with the reagents described in Chlorine Dioxide, Method A, Sec. 4 are required.

5. Procedure**5.1. Chlorine dioxide fraction:**

- (a) Fill a 100-ml nessler tube to the mark with sample.
- (b) Add 1 ml malonic acid solution.
- (c) Cap the tube with a clean rubber stopper and mix the contents.
- (d) Let the solution stand 2 min so that the malonic acid can deactivate the chlorine.
- (e) Add 5 ml phosphate buffer solution and 5 ml DPD reagent, and again invert the stoppered tube four to six times to mix the contents.
- (f) If the solution turns pink or red, compare the color, which is due to one fifth of the chlorine dioxide, against the color standards prepared in Sec. 5.1(d3), Chlorine (Residual), Method A.

(g) Multiply the reading obtained in Step (f) by five to obtain the approximate total available chlorine content (in mg/l) of the chlorine dioxide.

5.2. Fraction including free available chlorine, one fifth of the chlorine dioxide, and combined available chlorine:

- (a) Place 5 ml phosphate buffer solution and 5 ml DPD reagent in another 100-ml nessler tube.
- (b) Measure 100 ml of sample with a graduated cylinder, and immediately pour the sample into the nessler tube.
- (c) Cap the tube with a clean rubber stopper and mix the contents thoroughly by quickly inverting the tube four to six times.
- (d) Promptly compare the developed pink or red color against the color standards.

(e) Record the result as milligrams per liter of free available residual chlorine and one fifth of the residual chlorine dioxide. Subtract the chlorine dioxide reading found in Step 5.1(f) from the present reading to obtain the free available chlorine.

(f) Continue by adding several crystals (total weight 0.5-1.0 g) of potassium iodide to the nessler tube.

- (g) Dissolve the crystals by inverting the stoppered tube four to six times.
- (h) Let the solution stand for 2 min so that the chloramine can convert the iodide to iodine, evidenced by an increase in the color intensity.
- (i) Again compare the pink or red color against the color standards.
- (j) Record the present milligram-per-liter residual chlorine reading. Subtract the result obtained in step (e) from the present reading, and record the difference as milligrams-per-liter combined available residual chlorine.

5.3. Total available residual chlorine:

- (a) Fill another 100-ml nessler tube to the mark with sample.
- (b) Add 1 ml sulfuric acid solution and several crystals (total weight 0.5-1.0 g) of potassium iodide and seal with a clean rubber stopper.
- (c) Dissolve the crystals by inverting the stoppered tube four to six times.
- (d) Let the solution stand for 1 min, which enables all of the residual chlorine, chlorine dioxide, and chlorite to convert the iodide to iodine.
- (e) Add 5 ml phosphate buffer solution, 5 ml DPD reagent, and 5 ml sodium bicarbonate solution, and again invert the stoppered tube four to six times to mix the contents.
- (f) Compare the pink or red color due to the total available residual chlorine against the color standards.

5.4. Reduction in sample and reagent volumes: Although a 100-ml sample is specified in the preceding tests, smaller samples can be taken with a proportionate scaling down in reagent quantities. For example, a 10-ml sample will require only one tenth the reagent volumes normally applied in the course of the procedure.

Color

1. Purpose of Test

The most common colors which occur in a raw water are yellow and brown. The colors are usually due to organic matter of vegetable or soil origin. In such cases pH exerts an important effect on the nature of the color. Colloidal and soluble iron and manganese, however, can also confer a yellow-to-brown color on a water, while chromate wastes can impart a yellow color.

Two types of color are recognized in water. True color results from the presence of dissolved or colloidal organic substances. Suspended materials can add an apparent color. On a plant level, true color is often removed from a colored soft water by coagulating with alum in the pH acid range. Free residual chlorination and superchlorination may assist in the reduction of color.

This control method is designed to measure the true color in water and is useful for plants which must treat a colored water supply.

2. Warning

This method is suitable only for the measurement of color in clear waters. Since turbidity increases the apparent color of a water, this method should be used to determine the color of a water containing no more than 5 units of turbidity and preferably less than 1 unit of turbidity. When greater amounts of turbidity are present, the procedure recommended in the latest edition of *Standard Methods* should be followed. Sometimes turbidity can be reduced by a sufficient period of settling. Filtering is not recommended because some of the true color may be removed by the filter paper.

3. Apparatus

- 3.1. Ten or more 50 ml nessler tubes, matched, tall form; and support.
- 3.2. A 25 ml buret or appropriate pipets for measuring stock color solution.
- 3.3. Ten or more clean rubber stoppers (No. 2 size).

4. Reagent

Stock color solution:

(a) On an analytical balance, weigh separately 1.246 g potassium chloroplatinate (K_2PtCl_6) and 1.0 g cobaltous chloride ($CoCl_2 \cdot 6H_2O$). Use glass counterpoised balance pans or watch glasses for weighing these chemicals because metallic pans may be attacked by them.

(b) Carefully transfer the two weighed chemicals to a 250 ml beaker and dissolve in 50 ml distilled water.

(c) Add 100 ml concentrated hydrochloric acid and mix thoroughly.

(d) Transfer the solution to a 1 l volumetric flask, rinsing the beaker with three 100 ml portions of distilled water.

(e) Dilute further to the 1 l mark with distilled water. Stopper and mix thoroughly.

5. Procedure

5.1. Preparation of color standards:

(a) Prepare the following series of color standards by measuring the indicated volumes of stock color solution into separate 50 ml nessler tubes:

| Color Solution <i>ml</i> | Color Units |
|-----------------------------|-------------|
| 0 | 0 |
| 0.5 | 5 |
| 1 | 10 |
| 2 | 20 |
| 3 | 30 |
| 4 | 40 |
| 5 | 50 |
| 6 | 60 |
| 7 | 70 |

(b) Add distilled water to the 50 ml mark, and mix.

(c) Protect these standards by capping with clean rubber stoppers if the standards are to be used over a period of several months.

5.2. Determination of sample containing less than 70 units. Place 50 ml of the clear sample in a separate 50 ml nessler tube. Compare the sample with the standards and estimate the color units in the sample. If the color of the sample is greater than that of the standards (more than 70 units) proceed as described in Sec. 5.3.

5.3. Determination of sample containing more than 70 units:

(a) Place a 25 ml portion of the clear sample in a separate 50 ml nessler tube. Dilute to the mark with distilled water. Compare the diluted sample with the standards and estimate the color units in the diluted sample. If the sample color is still outside the range of the standards, repeat the procedure, using smaller and smaller portions of the original sample, until a match is obtained.

(b) Calculate the total color by multiplying the estimated color of the final diluted sample by the appropriate factor:

| Volume of Original Sample <i>ml</i> | Multiply Color Units by: |
|--|-----------------------------|
| 25 | 2 |
| 20 | 2.5 |
| 10 | 5 |
| 5 | 10 |

Copper

1. Purpose of Test

This test is designed to determine soluble copper in water. Copper may be present after the copper sulfate treatment of water for the control of plankton and other aquatic growths.

It may also be picked up by water passing through copper and brass pipes and fixtures. Small amounts of copper in a water have been known to impart a green stain to porcelain basins.

2. Warning

Few of the common constituents of natural water affect the test. The sample must be free of turbidity and color. The development of turbidity in the sample or standards following the addition of mixed cuprethol reagent indicates either that the reagent is too old and must be replaced or that interference is present in excessive concentration.

The latest edition of *Standard Methods* should be consulted for directions on how to cope with samples containing turbidity and color.

3. Apparatus

- 3.1. Eight or more 100 ml nessler tubes, matched, tall form; and support.
- 3.2. A 25 ml buret or appropriate pipets for measuring the standard copper solution.
- 3.3. Clean rubber stoppers (No. 3 size) for nessler tubes.
- 3.4. A dropping pipet or medicine dropper for adding the hydrochloric acid solution.
- 3.5. Pipets for measuring sodium pyrophosphate and sodium acetate solutions.
- 3.6. A safety or automatic pipet for adding the mixed cuprethol reagent.

4. Reagents

- 4.1. Stock copper solution:
 - (a) On an analytical balance weigh 0.393 g dry cupric sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$).
 - (b) Carefully transfer the weighed chemical to a 250 ml beaker and dissolve in 100 ml distilled water.
 - (c) Cautiously add 1 drop of concentrated sulfuric acid, and mix.
 - (d) Transfer the acidified solution to a 1 l volumetric flask, rinsing the beaker with three 100 ml portions of distilled water. Dilute further to the 1 l mark with distilled water. Stopper and mix thoroughly.
- 4.2. Standard copper solution. With a volumetric pipet, measure carefully 10 ml stock copper solution (4.1) into a 100 ml volumetric flask. Dilute to the 100 ml mark with distilled water. Stopper and mix thoroughly. Prepare the standard copper solution on the day of use because of questionable stability.

4.3. Hydrochloric acid solution:

(a) Using a graduated cylinder, measure 100 ml distilled water into a 250 ml bottle.

(b) In the same graduated cylinder, measure 100 ml concentrated hydrochloric acid (HCl) and transfer it to the bottle.

(c) Stopper and shake the bottle to mix the contents.

4.4. Sodium pyrophosphate solution. Weigh 30 g sodium pyrophosphate ($\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$) and place in a 1,500 ml beaker. Dissolve in 1,000 ml distilled water which has been measured with a graduated cylinder.

4.5. Sodium acetate solution:

(a) Weigh 400 g sodium acetate trihydrate ($\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$) and place in a 1,500 ml beaker. Use a graduated cylinder to measure 600 ml distilled water and pour into the beaker.

(b) Heat and stir the solution to dissolve the crystals.

4.6. Cuprethol solution (I):

(a) Use a 250 ml graduated cylinder to measure 200 ml methyl alcohol or methanol and place in a 250 ml glass-stoppered bottle.

(b) Weigh 4.0 g 2,2' iminodiethanol (also called diethanolamine) and dissolve in the methyl alcohol. Tightly stopper this reagent to maintain a maximum period of stability.

Keep away from flame.

4.7. Cuprethol solution (II):

(a) Use a 250 ml graduated cylinder to measure 200 ml methyl alcohol or methanol and place in a 250 ml glass-stoppered bottle.

(b) With a safety or automatic pipet, measure 3 ml carbon disulfide and dissolve in the methyl alcohol. Tightly stopper this reagent to maintain a maximum period of stability.

Keep away from flame.

4.8. Mixed cuprethol reagent:

(a) With a 10 ml graduated cylinder, measure 10 ml cuprethol solution (I) and place in a 20 x 150 mm test tube.

(b) With the same graduated cylinder measure 10 ml cuprethol solution (II) and add to the test tube.

(c) Mix the contents of the test tube.

Keep away from flame.

Prepare this reagent in amounts no greater than needed for a week's use. Keep the storage bottle tightly stoppered to maintain stability.

5. Procedure

5.1. Preparation of copper standards:

(a) Prepare the following series of copper standards by measuring the indicated volumes of standard copper solution into separate 100 ml nessler tubes as indicated in the table on the following page:

Simplified Procedure for Water Examination

| Copper Solution <i>ml</i> | Copper <i>mg/l</i> | Copper Solution <i>ml</i> | Copper <i>mg/l</i> |
|------------------------------|-----------------------|------------------------------|-----------------------|
| 0 | 0 | 3.0 | 0.3 |
| 0.5 | 0.05 | 4.0 | 0.4 |
| 1.0 | 0.1 | 5.0 | 0.5 |
| 2.0 | 0.2 | | |

(b) Add distilled water to the 100 ml mark, and mix.

5.2. Measure the appropriate sample volume for the indicated copper range:

| Sample Volume <i>ml</i> | Copper Range <i>mg/l</i> |
|----------------------------|-----------------------------|
| 100 | 0.05-0.5 |
| 50 | 0.55-1.0 |
| 25 | 1.1-2.0 |

Place the clear and colorless sample in a 100 ml nessler tube. If necessary, dilute to the 100 ml mark with distilled water.

5.3. To all tubes (including each copper standard and sample) add 0.5 ml hydrochloric acid solution with a dropping pipet, and mix by inverting the tube four times.

5.4. With a measuring pipet, add 2 ml sodium pyrophosphate solution, and mix by inverting each tube four times.

5.5. With a measuring pipet, add 10 ml sodium acetate solution, and mix by inverting each tube four times. If possible, check the treated sample at this point to make sure that the pH is between 5 and 6. If necessary, add more sodium acetate solution to bring the pH within the desired 5 to 6 range.

5.6. Allow the tubes to stand for 5 minutes.

5.7. With a dropping pipet, safety pipet, or automatic pipet add 1 ml mixed cuprethol reagent (4.8). Mix each tube by inverting four to six times.

5.8. Let the tubes stand at least 10 minutes but no more than 30 minutes.

5.9. Compare the sample with the standards and determine from the yellow color the amount of copper present.

5.10 Calculate the mg/l copper by multiplying the result found in Step 5.9 by the appropriate factor:

| Sample Volume <i>ml</i> | Multiply mg/l Copper by: |
|----------------------------|--------------------------------|
| 100 | 1 |
| 50 | 2 |
| 25 | 4 |

Fluoride

1. Purpose of Test

Fluoride is artificially added to many water supplies in order to reduce the incidence of dental carries. This method is designed for both naturally and artificially fluoridated water supplies.

2. Warning

This method is suitable for the measurement of fluoride in clear and colorless waters. None of the following substances should be present in excess of the indicated amounts:

| Substance | Amount <i>mg/l</i> |
|--|-----------------------|
| Chloride | 2,000 |
| Total alkalinity (as calcium carbonate) | 400 |
| Sulfate | 300 |
| Phosphate | 5 |
| Iron | 2 |
| Sodium hexametaphosphate (also called Calgon) | 1.0 |
| Aluminum | 0.25 |

If the water fails to meet any of these conditions, the procedure in the latest edition of *Standard Methods* should be followed.

3. Apparatus

- 3.1. Eight or more 100 ml nessler tubes, matched, tall form; and support.
- 3.2. A 25 ml buret or appropriate pipets for measuring the standard fluoride solution.
- 3.3. A 5 ml volumetric pipet for measuring the acid-zirconyl reagent.
- 3.4. A dropping pipet or medicine dropper of 0.5 to 1 ml capacity for dispensing the sodium arsenite solution.
- 3.5. Appropriate volumetric pipets for measuring the sample.

4. Reagents

4.1. Acid-zirconyl reagent:

(a) Weigh 0.30 g zirconium oxychloride (also called zirconyl chloride), $ZrOCl_2 \cdot 8H_2O$, and dissolve in 50 ml distilled water in a 1 l volumetric flask. Use glass counterpoised balance pans or watch glasses for weighing this chemical because metallic pans may be attacked and etched.

(b) Weigh 0.07 g sodium alizarin monosulfonate (also called alizarin red S) and dissolve in 50 ml distilled water in a 250 ml beaker.

(c) Add the alizarin solution to the zirconium solution in the volumetric flask, while swirling. Allow the resulting solution to stand for a few minutes.

(d) Use a 500 ml graduated cylinder to measure 390 ml distilled water. Transfer the distilled water to a 1 l beaker. With a 250 ml graduated cylinder, measure 101 ml concentrated hydrochloric acid (HCl). While stirring the distilled water with one hand, pour the hydrochloric acid into it.

(e) With a 500 ml graduated cylinder, measure 400 ml distilled water. Then transfer the distilled water to a 1 l beaker. With a 50 ml graduated cylinder, measure 33 ml concentrated sulfuric acid (H₂SO₄) into the 400 ml distilled water. Considerable heat is generated by the mixture, so pour slowly and mix well to avoid dangerous spattering. Allow the solution to cool to room temperature.

(f) Slowly pour Solution (d) into Solution (e) while stirring with one hand.

(g) To the clear zirconium-alizarin solution (c) in the 1 l volumetric flask, add the mixed acids (f) to the 1 l mark. Stopper and mix thoroughly. Allow the mixed solution to stand 1 hour before use. When protected from sunlight, the reagent is stable for at least 6 months.

4.2. Stock fluoride solution:

(a) On an analytical balance, weigh carefully 0.2210 g dry sodium fluoride (NaF). Carefully transfer the weighed chemical to a 250 ml beaker and dissolve in 100 ml distilled water.

(b) Transfer the solution to a 1 l volumetric flask, rinsing the beaker with three 100 ml portions of distilled water.

(c) Dilute further to the 1 l mark with distilled water. Stopper and mix thoroughly.

4.3. Standard fluoride solution. With a volumetric pipet, measure carefully 10 ml of the stock fluoride solution into a 100 ml volumetric flask. Dilute to the 100 ml mark with distilled water. Stopper and mix thoroughly.

4.4. Sodium arsenite solution. Weigh 5.0 g sodium arsenite (also called sodium metaarsenite), NaAsO₂. Dissolve in 1 l distilled water.

Handle this poison with extreme caution and avoid taking into the mouth. Dispense the solution with a dropping pipet or a medicine dropper.

5. Procedure

5.1. Preparation of fluoride standards:

(a) Prepare the following series of fluoride standards by measuring the indicated volumes of the standard fluoride solution (4.3) into separate 100 ml nessler tubes:

| Standard Fluoride Solution ml | Fluoride mg/l |
|-------------------------------|---------------|
| 0 | 0 |
| 2 | 0.2 |
| 4 | 0.4 |
| 6 | 0.6 |

| Standard Fluoride Solution ml | Fluoride mg/l |
|-------------------------------|---------------|
| 8 | 0.8 |
| 10 | 1.0 |
| 12 | 1.2 |

(b) Add distilled water to the 100 ml mark, and mix by inverting each tube four to six times.

5.2. Measure the appropriate sample volume for the indicated fluoride range:

| Sample Volume <i>ml</i> | Fluoride Range <i>mg/l</i> |
|----------------------------|-------------------------------|
| 100 | 0.1-1.2 |
| 50 | 1.3-2.4 |
| 25 | 2.5-4.8 |

Place the clear and colorless sample in a 100 ml nessler tube. If necessary, dilute to the 100 ml mark with distilled water.

5.3. Remove any residual chlorine from the sample by adding 1 drop (0.05 ml) of sodium arsenite solution for each 1 mg/l of residual chlorine present in the 100 ml sample. Mix the sample tube by inverting four to six times.

5.4. Allow the standards and sample to come to the same temperature, because the color development depends critically on the temperature. Keep the temperature difference between the tube that is warmest and that which is coolest at no greater than 2°C.

5.5. With a volumetric pipet, add 5 ml acid-zirconyl reagent to each of the standards and the sample. Complete the addition of reagent to the entire series of nessler tubes within 5 minutes. Mix the contents of each tube thoroughly by inverting four to six times. Allow the tubes to stand at least 60 minutes.

5.6. Compare the sample with the standards and determine from the yellow-to-pink color the amount of fluoride present.

5.7. Calculate the mg/l fluoride by multiplying the result found in Step 5.6 by the appropriate factor:

| Sample Volume <i>ml</i> | Multiply mg/l Fluoride by: |
|----------------------------|----------------------------------|
| 100 | 1 |
| 50 | 2 |
| 25 | 4 |

Hardness

1. Purpose of Test

The presence of calcium and magnesium causes hardness in a water. The hardness can be of two types: carbonate and noncarbonate. Hardness can be removed by treatment with lime, soda ash, cation exchanger, or a combination of all three.

This method is designed for routine hardness determinations of potable water.

2. Warning

The water should be free of color or turbidity which might obscure or affect the indicator response. Fortunately, substances which cause errors in this titration are seldom present in potable water supplies. Barium, strontium, cadmium, lead, zinc, and manganese titrate like hardness, while limited amounts of copper, iron, cobalt, nickel, and aluminum can affect the hardness results. The latest edition of *Standard Methods* should be consulted for the concentrations of all these substances which can be tolerated in a sample.*

The titration should be performed on a sample which is at room temperature. The reaction is slow in a cold sample, the indicator decomposes in heat.

The titration should be completed within 5 minutes of the time the buffer is added to avoid trouble arising from the precipitation of calcium carbonate.

The buffer and solid indicator mixture are subject to deterioration and should be kept tightly stoppered when not in use.

A poor or green off-color endpoint means a need for fresh indicator.

3. Apparatus

- 3.1. A 25 ml buret and support.
- 3.2. A 50 ml graduated cylinder or appropriate volumetric pipets for measuring.
- 3.3. Two or more 250 ml flasks or porcelain casseroles.
- 3.4. Two or more stirring rods for casseroles.
- 3.5. A dropping pipet or medicine dropper, 0.5-1 ml, for dispensing the buffer.
- 3.6. Two small measuring spoons, 0.2-0.3 ml capacity, for dispensing the sodium cyanide crystals and the dry indicator mixture.

4. Reagents

The following prepared solutions and solid mixtures are commercially available:

- 4.1. Sodium cyanide crystals, NaCN.

Handle this poison carefully with a small spoon or spatula. Avoid ingestion and inhalation of deadly cyanide fumes.

- 4.2. Buffer solution:

(a) On an analytical balance, carefully weigh separately: 1.179 g dry disodium ethylene-diaminetetraacetate dihydrate (abbreviated EDTA or Na_2EDTA) of reagent grade quality; and 0.780 g magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$). Carefully transfer the two weighed chemicals to a 100 ml beaker and dissolve in 50 ml distilled water.

(b) Weigh 16.9 g ammonium chloride (NH_4Cl) and place in a 400 ml beaker.

*Two United States patents (No. 2,583,890 and 2,583,891) have been issued to G. Schwarzenbach disclosing titration and complexometric methods for quantitative determination of water hardness. Nothing contained in this Manual is to be construed as granting any right, by implication or otherwise, for manufacture, sale, or use in connection with any method, apparatus, or product covered by patent, nor as insuring anyone against liability for infringement of patent.

With a 250 ml graduated cylinder, measure 143 ml concentrated ammonium hydroxide and add to the 400 ml beaker. Dissolve the ammonium chloride in the concentrated ammonium hydroxide.

(c) Pour Solution (a) into Solution (b) while stirring.

(d) Pour Solution (c) back into the 250 ml graduated cylinder and dilute with distilled water to the 250 ml mark. Mix the resulting solution thoroughly by pouring back and forth between the 400 ml beaker and the 250 ml graduated cylinder. Store in a tightly stoppered bottle.

4.3. Solid indicator mixture. Weigh separately 0.5 g Eriochrome Black T dye and 100 g sodium chloride (NaCl). Place both chemicals in a mortar and grind together with a pestle until the dark dye is uniformly distributed throughout the white salt. Store in a tightly stoppered bottle.

4.4. EDTA titrant:

(a) On an analytical balance, carefully weigh 3.723 g dry disodium ethylenediaminetetraacetate dihydrate (abbreviated EDTA or Na_2EDTA) of reagent grade quality. Carefully transfer the chemical to a 250 ml beaker and dissolve in 150 ml distilled water.

(b) Carefully transfer the solution to a 1 l volumetric flask, rinsing the beaker with three 100 ml portions of distilled water. Dilute further to the 1 l mark with distilled water. Stopper and mix thoroughly.

EDTA titrant loses strength slowly; the loss is generally negligible during the first 6 months but becomes noticeable after a year.

5. Procedure

5.1. Fill the buret with EDTA titrant (4.4). Record the liquid level in the buret by reading at the bottom of the meniscus. Guard against a leaky stopcock which results in the loss of titrant on standing.

5.2. Measure the appropriate sample volume for the indicated hardness ranges:

| Sample Volume <i>ml</i> | Hardness Range <i>mg/l as CaCO₃</i> |
|----------------------------|---|
| 50 | 0-300 |
| 25 | 301-600 |
| 10 | 601-1,500 |

If a sample of only 25 ml is needed, add 25 ml distilled water to bring the total volume to 50 ml; with a 10 ml sample, add 40 ml of distilled water. Measure the additional distilled water with a graduated cylinder. Place the sample (and extra distilled water if needed) in a 250 ml flask (or porcelain casserole).

5.3. Prepare a color comparison blank by placing 50 ml distilled water (measured with a graduated cylinder) in a similar 250 ml flask (or porcelain casserole).

5.4. With a small spoon, measure out 0.25 g sodium cyanide crystals and add to the color comparison blank and the sample. Dissolve the crystals by mixing.

5.5. Add 1-2 ml of buffer solution to the color comparison blank and the sample and mix.

5.6. With the small spoon, add 1 measure (0.2 g) solid indicator mixture to the

color comparison blank and the sample; and then mix to achieve dissolution.

5.7. To the color comparison blank, carefully add from the buret 1 drop of EDTA titrant at a time until the purplish color changes to a bright blue. Sometimes no drops will be needed; at other times as many as 3 drops will be required. Record the new buret level by reading at the bottom of the meniscus.

5.8. If the sample turns a red or purple color in Step 5.6, gradually add EDTA titrant from the buret. Shake the flask constantly (or stir the contents of the porcelain casserole constantly). Continue adding the titrant until the red color turns to a purplish tinge. Stop the titrant addition at this point for 10 seconds but continue the shaking (or stirring).

5.9. Resume adding the EDTA titrant drop by drop until the purple color turns to the same bright blue color as in the color comparison blank. Shake the flask (or stir the contents of the porcelain casserole) as before throughout the new addition. The color change from purple to bright blue will occur within a span of 1 to 4 drops. If you have difficulty recognizing the change when adding 1 drop at a time, add the titrant 2 drops at a time near the endpoint. This will intensify the color change, and the slight loss in accuracy is not significant.

5.10. Record the new buret level by reading at the bottom of the meniscus.

5.11. Calculate the gross volume of titrant used by subtracting the initial buret reading (Step 5.7) from the last reading (Step 5.10).

5.12. Calculate the blank correction by subtracting the buret reading in Step 5.1 from the buret reading in Step 5.7.

5.13. Calculate the net volume of titrant used for the sample alone by subtracting the result found in Step 5.12 from the result found in Step 5.11.

5.14. Calculate the hardness in terms of mg/l as calcium carbonate by multiplying the result found in Step 5.13 by the appropriate factor:

| Sample Volume <i>ml</i> | Multiply ml EDTA Titrant by: |
|----------------------------|---------------------------------------|
| 50 | 20 |
| 25 | 40 |
| 10 | 100 |

Iron

1. Purpose of Test

Iron concentrations in excess of 1 mg/l may occur naturally in well water or river water which receives industrial wastes. Removal treatment is undertaken on those water supplies where iron causes color, staining, bacterial, and taste and odor problems in the distribution system. Complexing with polyphosphates is one way of coping with iron in some well waters. Iron coagulants may be added in water treatment for the removal of turbidity. In such cases, significant amounts of iron in

the finished water indicate that an incorrect coagulant dosage is being applied. A knowledge of the total iron content of a water is adequate for most purposes.

This method is designed to measure the total iron that is normally present in water or that may be introduced as a result of treatment with iron coagulants or by corrosion of iron pipe.

2. Warning

The sample must be free of non-iron turbidity or color. The fact that iron may be present in a soluble, colloidal, complexed, or particulate form as well as in a ferrous or ferric state makes this determination most difficult. Good judgment must be exercised in collecting the iron sample, particularly when looking for evidence of corrosion. A glass container with a glass or plastic top is preferable to one with a metal cover. Thorough shaking of the bottle is necessary in order to suspend and distribute all of the iron uniformly throughout the sample before a withdrawal is made for the total-iron analysis. Even so, some iron deposit may remain on the sides of the container when the determination is delayed for several days. Such adsorbed iron must be dissolved with hydrochloric acid for an accurate estimate. For consistent results in a particular laboratory, the determination should always be performed in exactly the same way from the first to the last step. Otherwise the results may vary with the variations in manipulative technique.

In order to prevent contamination a flask, tube, beaker, or any other glassware which has held chemicals with a high iron content (such as ferrous sulfate or ferric sulfate or chloride), should be cleansed by boiling for at least 1/2 hour with a solution containing equal volumes of concentrated hydrochloric acid and distilled water.

Although few of the common constituents of natural water affect the test, the following substances can cause errors: zinc concentrations greater than 10 times the iron value; copper and cobalt greater than 5 mg/l; more than 2 mg/l nickel; and bismuth, silver, cadmium, mercury, molybdate, and large amounts of strong oxidizing agents. The latest edition of *Standard Methods* should be consulted for information on the determination of ferrous and dissolved iron because special handling is required in such cases.

3. Apparatus

3.1. Ten or more 100 ml nessler tubes, matched, tall or low form; and support.

3.2. A 25 ml buret or appropriate pipets for measuring the standard iron solution.

3.3. A 100 ml graduated cylinder for measuring the sample. A 50 ml or 25 ml graduated cylinder may be used for smaller samples with particulate matter which may clog a pipet.

3.4. One or more 250 ml erlenmeyer flasks.

3.5. A safety or automatic pipet for measuring concentrated hydrochloric acid.

3.6. Pipets for measuring hydroxylamine hydrochloride, acetate buffer, and phenanthroline solutions.

3.7. A gas burner or electric hot plate.

3.8. One or more wire gauze squares, 20 mesh.

3.9. Four or more glass beads.

- 3.10. One or more glass stirring rods.
- 3.11. A wash water bottle for rinsing flask, beads, and stirring rod.

4. Reagents

4.1. Stock iron solution:

(a) With a 50 ml graduated cylinder, measure 50 ml distilled water and pour into a 250 ml beaker.

(b) With the same 50 ml graduated cylinder, measure 20 ml concentrated sulfuric acid.

(c) While stirring with one hand, slowly and cautiously add the sulfuric acid to the distilled water. Considerable heat is generated by the mixing of the sulfuric acid and the distilled water, so pour slowly and mix well to avoid dangerous spattering. Allow the solution to cool to room temperature.

(d) On an analytical balance, weigh 0.7022 g dry ferrous ammonium sulfate, $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$, and add to Solution (c). Dissolve the crystals by stirring the solution.

(e) Weigh 1 g potassium permanganate (KMnO_4), place in a 250 ml beaker, and dissolve in 100 ml distilled water.

(f) Add Solution (e) dropwise to Solution (d) with constant stirring until a faint pink color persists.

(g) Transfer Solution (f) to a 1 l volumetric flask, rinsing the beaker with three 100 ml portions of distilled water, and dilute further to the 1 l mark with distilled water. Stopper and mix thoroughly.

4.2. Standard iron solution. With a volumetric pipet, measure carefully 10 ml stock iron solution into a 100 ml volumetric flask. Dilute to the 100 ml mark with distilled water. Stopper and mix thoroughly. Prepare the standard iron solution on the day of use because the solution is unstable.

4.3. Concentrated hydrochloric acid, HCl .

4.4. Hydroxylamine hydrochloride solution:

(a) Weigh 10 g hydroxylamine hydrochloride ($\text{NH}_2\text{OH} \cdot \text{HCl}$) and place in a 250 ml beaker.

(b) With a graduated cylinder, measure 100 ml distilled water and pour into the 250 ml beaker. Stir to dissolve the crystals.

4.5. Acetate buffer solution:

(a) Weigh 250 g ammonium acetate ($\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$), place in a 1 l beaker, and dissolve in 150 ml distilled water.

(b) Add 700 ml glacial (or concentrated) acetic acid, and mix.

(c) Transfer the mixed ammonium acetate/acetic acid solution to a 1 l graduated cylinder and dilute to the 1,000 ml mark with distilled water. Mix thoroughly by pouring back into the beaker and stirring.

4.6. Phenanthroline solution:

(a) On an analytical balance, weigh 0.1 g 1,10 phenanthroline monohydrate ($\text{C}_{12}\text{H}_8\text{N}_2 \cdot \text{H}_2\text{O}$) and transfer to a 250 ml beaker.

(b) With a graduated cylinder measure 100 ml distilled water and add to the beaker.

(c) Add 2 drops of concentrated hydrochloric acid to the beaker.

(d) Stir the solution to dissolve. (If the crystals fail to dissolve quickly, place

the beaker over a hot-water bath to hasten dissolution.) Discard the solution when it darkens on prolonged standing.

5. Procedure

5.1. Preparation of iron standards:

(a) Prepare the following series of iron standards by measuring the indicated volumes of standard iron solution (4.2) into separate 100 ml nessler tubes:

| Standard Iron Solution ml | Iron mg/l |
|---------------------------|-----------|
| 0 | 0 |
| 0.5 | 0.05 |
| 1.0 | 0.10 |
| 2.0 | 0.20 |
| 3.0 | 0.30 |
| 4.0 | 0.40 |
| 6.0 | 0.60 |
| 8.0 | 0.80 |
| 10.0 | 1.0 |

(b) Add distilled water to the 100 ml mark, and mix.

5.2. Mix the sample thoroughly and measure the appropriate sample volume for the indicated range:

| Sample Volume ml | Iron Range mg/l |
|------------------|-----------------|
| 100 | 0.05-1.0 |
| 50 | 1.2-2.0 |
| 25 | 2.1-4.0 |

Place the sample in a 250 ml flask.

5.3. Add 2 ml concentrated hydrochloric acid, and mix.

5.4. Add 1 ml hydroxylamine hydrochloride solution, and mix.

5.5. Heat the solution to boiling and then boil for 5 minutes. Prevent spattering and bumping of the boiling liquid by placing a wire gauze over the electric or gas heat source and then resting the bottom of the flask on the wire gauze. For further protection, add 4 or 5 glass beads to the flask, or place a glass stirring rod in the flask.

5.6. Cool the sample to room temperature.

5.7. Transfer the sample to a 100 ml nessler tube. Rinse the flask, glass beads, and stirring rod with 5 to 10 ml distilled water, and add the wash to nessler tube.

5.8. Add sufficient distilled water to bring the volume to the 100 ml mark.

5.9. To the sample and the series of standards prepared in Step 5.1, add 10 ml acetate buffer solution with a measuring pipet, and mix thoroughly by inverting each tube four to six times.

5.10. Add 5 ml phenanthroline solution with a measuring pipet, and mix thoroughly by inverting each tube four to six times.

5.11. Allow the tubes to stand at least 15 minutes for the color to develop.

5.12. Compare the sample with the standards and determine from the pink color the amount of total iron present.

5.13. Calculate the mg/l total iron by multiplying the result found in Step 5.12 by the appropriate factor:

| Sample Volume <i>ml</i> | Multiply mg/l Iron by: |
|----------------------------|------------------------------|
| 100 | 1 |
| 50 | 2 |
| 25 | 4 |

Jar Tests

1. Purpose of Test

One of the objectives of a water treatment plant is to produce a finished water which is clear and colorless, and, therefore, of an appearance and taste acceptable to the consumer.

Surface waters generally contain suspended matter called turbidity (dirt for the most part) which varies in size and amount. When an untreated turbid water is applied alone to a rapid sand filter, only a portion of the dirt will be removed. Turbidity removal is improved by adding a coagulant like filter alum (aluminum sulfate) to the water, mixing rapidly for a brief interval, stirring slowly for a longer period (a process variously termed flocculation or coagulation), and then settling for an even longer time. During these steps the coagulant forms a "floc" which attracts and enmeshes the small particles of color, dirt, algae, and other particulate matter. The resulting combination of floc and particulate matter, being heavier than water, largely settles out in the settling chambers. The unsettled portion, now grown to greater particle size than the original turbidity, is more readily removed from the water by means of a sand filter. Thus, coagulation converts a "fog" of turbidity with poor settling and filtering qualities into a "snowstorm" of much larger particles which settle and filter satisfactorily.

Alkalinity and pH both influence the coagulation process. Color is often more readily removed at an acid pH, following which the water is usually made alkaline. Turbidity, on the other hand, is usually coagulated under alkaline conditions. It

should be emphasized, though, that all coagulants do not react similarly at the same pH.

Acid is produced when aluminum sulfate dissolves in water, and an alkaline compound must be present to form an alum floc. If the alkaline compound does not exist naturally in the water, it must be added artificially. Lime and soda ash are frequently added to soft waters which contain less than 25 mg/l total alkalinity. Neutralization of each 1 mg/l alum is achieved by 0.25 mg/l pure lime or 0.46 mg/l pure soda ash (sodium carbonate).

The jar tests are designed to show the nature and extent of the chemical treatment which will prove effective in the plant. Many of the chemicals added to a water supply can be evaluated on a laboratory scale by means of jar tests. Among the most important of these chemicals are coagulants, coagulant aids, alkaline compounds, softening chemicals, and activated carbon for taste and odor removal. When coagulants are added during the jar test, the terms flocculation test, coagulation test, or floc test may be used to describe the operation.

The flocculation test attempts to approximate the coagulant addition, flash mix, and flocculation and settling conditions prevailing in a given plant. By this means the operator can arrive at the correct dosage for plant use when varying amounts of turbidity, color, or other factors dictate a change in the coagulant dose. The test also permits the appraisal of the relative merits of the aluminum and iron coagulants, alone or in conjunction with such coagulant aids as activated silica, polyelectrolytes, clays, stone dust, activated carbon, settled sludge, lime, and soda ash. The test results have considerable value in the design of new plants where no prior information is available on the coagulant most suited to the water and the type of floc to be expected. By the same token, the test can demonstrate the wisdom and necessity of modernizing an old plant through the installation of improved flash mixers or mechanical flocculators and remodeling the settling basins.

2. Warning

Even the smallest detail may have an important influence on the result of a jar test. Therefore, all samples in a series of tests should be handled as nearly alike as possible.

The purpose of the test will determine such experimental conditions as the speed of the stirring and the length of the flash mix, flocculation, and settling intervals. The various conditions described in the following procedure are a good starting point for a laboratory trying the test for the first time. Some or all of the times and speeds may have to be changed to reflect the deficiencies of an old plant or the improved operation of a new or remodeled plant.

Since temperature plays an important role in coagulation, the raw-water samples should be collected and measured only after all other preparations have been made, in order to reduce the effect that room temperature might have on the sample.

3. Apparatus

3.1. A stirring machine with three to six paddles, capable of operation at variable speeds (from 0 to 100 revolutions per minute). Multiple stirring units like that illustrated in Fig. 7 are commercially available and generally superior to similar homemade outfits which may entail considerable construction time by a mechanic.

3.2. A floc illuminator. This item, located at the base of the laboratory stirrer shown in Fig. 7, enables the observation of small floc particles.

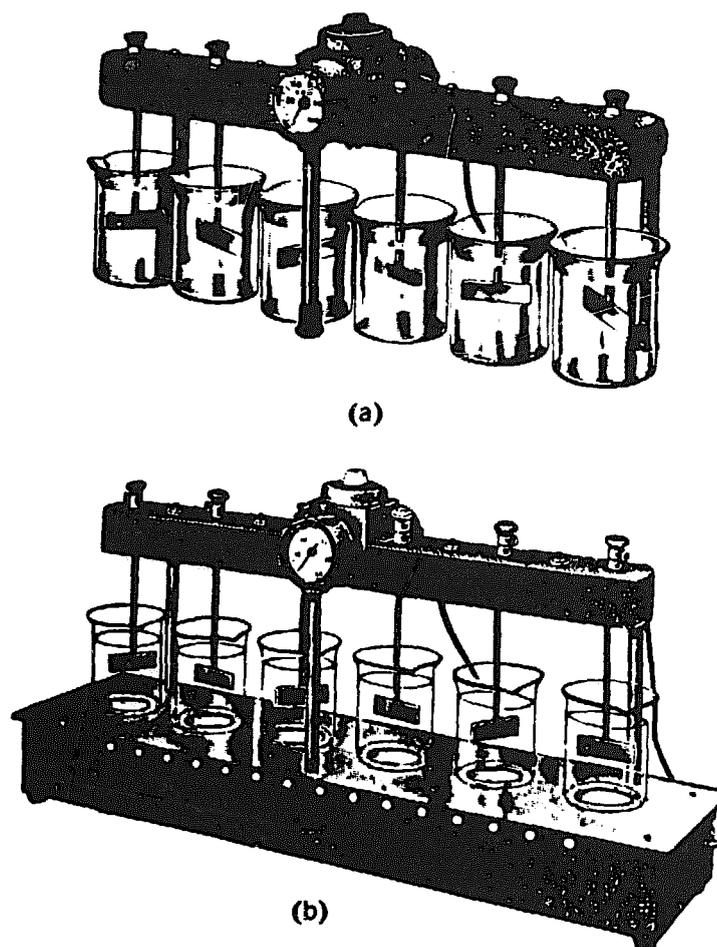


Fig. 7. Stirrer for Jar Test

Key: (a) stirring machine; (b) floc illuminator with stirrer in place.

3.3. Beakers, 1,500 ml, low form, pyrex. Beakers of 500 ml or other capacity can be used with stirring machines of smaller size.

3.4. A plastic pail, household type, with a capacity in excess of 2 gallons, for collecting the sample.

3.5. A 1,000 ml graduated cylinder. A 500 ml graduate can be used for measuring samples into smaller-size beakers.

3.6. Measuring pipets, 1, 5, and 10 ml, all graduated in 0.1 ml steps, for dosing samples rapidly with coagulant, suspensions, and other necessary solutions. Rinse these pipets thoroughly with tap water or distilled water to prevent caking with coagulant, suspensions, or other solutions being used.

3.7. A 100 ml pipet for withdrawing the coagulated and softened sample.

3.8. Apparatus for determining color, turbidity, pH, and phenolphthalein and total alkalinity.

3.9. Special apparatus for softening test:

- (a) Filter funnel.
- (b) Filter paper of medium retentiveness. Whatman No. 40 or Schleicher & Schull No. 589 are satisfactory.

3.10. Special apparatus for test of taste and odor removal with activated carbon:

- (a) Filter tube.
- (b) Glass wool. Wash thoroughly with odor-free water.

4. Dosing Solutions and Suspensions

A number of chemicals and other materials are used in the coagulation of turbid or colored water. They fall into three classes: coagulants, coagulant aids, and carriers of alkalinity.

The common coagulants are: aluminum sulfate (also called filter alum), $\text{Al}_2(\text{SO}_4)_3 \cdot 14\text{H}_2\text{O}$; ferrous sulfate (also called copperas), $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$; ferric sulfate (also called ferrisul), $\text{Fe}_2(\text{SO}_4)_3$; sodium aluminate, NaAlO_2 .

Coagulant aids include: activated silica; artificial organic materials known as polyelectrolytes; clay, kaolin, or stone dust.

Alkalinity is needed for the coagulant to produce the desired hydrous oxide floc. A small amount of strong alkali is added to a soft water to supply the necessary alkalinity. The chemicals used for this purpose are: Calcium oxide (also called lime, quicklime, unslaked lime, or burnt lime), CaO ; calcium hydroxide (also called hydrated lime or slaked lime), $\text{Ca}(\text{OH})_2$; sodium carbonate (also called soda ash), Na_2CO_3 .

Dosing solutions or suspensions should be prepared from the stock materials actually used in plant treatment. Distilled water used for the preparation of lime suspensions should be boiled for 15 minutes to expel the carbon dioxide and then cooled to room temperature before the lime is added.

4.1. Coagulant dosing solution or suspension:

- (a) Weigh 10.0 g of material. Dissolve or suspend in 1 l distilled water. Record the date of preparation on the bottle label.
- (b) Shake the suspension immediately before use.
- (c) Each 0.1 ml of this solution or suspension represents a dosage of 1 mg/l when added to a 1 l water sample, while each 1.0 ml of dosing solution represents a dosage of 10 mg/l in 1 l of sample. (See Sec. 6 for the test procedure to follow when using this suspension.)

Note: If 17.1 g of material is used in Step 4.1(a), each 1.0 ml of the resulting solution represents a dosage of 1 grain per US gallon when added to 1 l of sample. If 14.3 g is used in Step 4.1(a), each 1.0 ml represents 1 grain per Imperial gallon when added to 1 l of sample.

4.2. Activated silica dosing solution. Activated silica is prepared by acidifying a stock silica solution with any of the following substances: ammonium sulfate, aluminum sulfate, sulfuric acid, sulfur dioxide, chlorine, or carbon dioxide. The materials available at the particular treatment plant largely dictate the acidifying agent selected. The activated silica is prepared in two stages. The alkaline sodium silicate solution is prepared first and then reacted with the acidifying substance to form the activated silica solution. Ammonium sulfate has been selected as the acidifying substance for purposes of illustration in this procedure:

(a) Stock sodium silicate solution. Weigh 348.4 g sodium silicate solution ("N" brand sodium silicate solution, a product of Philadelphia Quartz Co., Philadelphia, Pa.). Dissolve in 500 ml distilled water. Dilute to 1 l with distilled water. This solution is stable indefinitely.

(b) Stock ammonium sulfate solution. Weigh 66.0 ammonium sulfate, $(\text{NH}_4)_2\text{SO}_4$. Dissolve in 980 ml distilled water. This solution is stable indefinitely.

(c) Preparation of activated silica solution:

(1) Place 20 ml distilled water in a 100 ml graduated cylinder. Add 10 ml stock sodium silicate solution and invert four times to mix.

(2) Add 10 ml stock ammonium sulfate solution and invert 10 times to mix. Let this mixture stand for 5 minutes. Dilute to 100 ml with distilled water, and invert 10 times to mix. Prepare the activated silica solution daily.

(3) Each 0.1 ml of this activated silica solution represents a dosage of 1 mg/l when added to a 1 l water sample, while each 1.0 ml of activated silica solution represents a dosage of 10 mg/l in 1 l of sample. See Sec. 6.14(c) for the test procedure to follow when using this solution.

4.3. Activated carbon dosing suspension:

(a) Weigh 1.0 g activated carbon. Carefully transfer the weighed powder to a glass-stoppered bottle.

(b) Add 1,000 ml of odor-free water. Stopper the bottle and shake well to suspend all of the carbon completely. Shake the suspension immediately before use.

(c) Each 1 ml of this suspension represents a dosage of 1 mg/l when added to a 1 l water sample. (See Sec. 8 for the test procedure to follow when using this suspension).

4.4. Prepare the dosing solutions or suspensions with the following frequency (use airtight stoppers on the bottles to prevent the oxidation or carbonation of those solutions which must be prepared on a daily or weekly schedule):

(a) Daily: ferrous sulfate, activated silica, ferric sulfate, lime, sodium aluminate.

(b) Weekly: soda ash, polyelectrolytes.

(c) Monthly: clay, kaolin, stone dust.

(d) Semiannually: aluminum sulfate, activated carbon.

5. Reagents

See the appropriate sections of this Manual for the reagents needed in determining color, turbidity, pH, phenolphthalein and total alkalinity, hardness, calcium, and free carbon dioxide, when such determinations are necessary.

6. Test Procedure for Coagulation Treatment

The basic procedure is described in Sec. 6.1-6.13. Variations involving the addition of alkaline agents, clay suspensions, or activated silica are covered in Sec. 6.14. When the water contains natural color, add the alum dose before the other agents or coagulant aids.

6.1. Rinse six 1,500 ml beakers with tap water and let the beakers drain for a few minutes in an upside-down position. Beakers that have had several days of use should be scrubbed inside and out with a brush and a household dishwashing detergent, finishing with a thorough rinse of tap water.

6.2. Clean the stirring machine paddles with a damp cloth.

6.3. Collect a sample of raw water and complete Steps 6.4 through 6.8 within 20 minutes. Throw away the sample and collect a fresh sample if the work must be interrupted during this critical stage. Otherwise, the settling of high turbidity and an increase in the sample temperature from the heat of the laboratory may cause erroneous results.

6.4. Stand the beakers right side up and pour more than 1 l of raw water into each one.

6.5. Taking one beaker at a time, pour some of the raw water back and forth between the beaker and a 1 l graduated cylinder. Finally, fill the graduated cylinder to the 1 l mark, and discard the excess raw water in the beaker. Return the measured 1 l sample to the beaker.

6.6. Place all the beakers containing the measured 1 l samples on the stirring machine.

6.7. With a measuring pipet, add increasing doses of coagulant solution (4.1) as rapidly as possible. Select a series of doses so that the first beaker will represent undertreatment and the last beaker will represent overtreatment. When a proper series is set up, the succession of beakers will show poor, fair, good, and excellent coagulation at the end of the run. It will be necessary to repeat the jar test once or twice to ascertain the proper series of doses for the desired results. (The suggestion of a specific series of coagulant doses in this Manual is inadvisable because coagulation depends on the pH and the amount of turbidity, color, alkalinity, and dissolved minerals in the water, as well as on the nature of the coagulant being added.)

6.8. Lower the stirring paddles into the beakers, start the stirring machine, and operate it for 1 minute at a speed of 60 to 80 revolutions per minute.

6.9. Reduce the stirring speed over the next 30 seconds to 30 revolutions per minute, and continue stirring at that speed for exactly 15 minutes. (See Sec. 9.1 for a discussion of variations in stirring time.)

6.10. Observe each beaker for the appearance of "pinpoint" floc and record the time and order of such appearance.

6.11. Stop the stirring machine and allow the samples to settle for 5, 15, 30, or 60 minutes. Observe the floc and record the order of settling. Describe the results as poor, fair, good, or excellent. A hazy sample indicates poor coagulation. Properly coagulated water contains floc particles that are well formed, and the liquid between the particles is clear. The lowest coagulant dosage which brings down the turbidity during the jar test should first be tried in plant operation. (See Sec. 9.2 for a discussion of variations in settling time.)

6.12. Using a 100 ml pipet, withdraw a portion of the top 1.5 inches of sample from each beaker.

6.13. Determine the color, turbidity, pH, and phenolphthalein and total alkalinity of the coagulated sample according to the directions given in the appropriate sections of this Manual.

6.14. Variations involving addition of other agents:

(a) Addition of alkaline agents. When the water is deficient in natural alkalinity, add a well-shaken lime dosing solution or soda ash solution, or both, to the water. (Prepare as directed in Sec. 4.1; see Table 3 for the amounts of soda ash and lime needed to neutralize the various coagulants.) Determine by experiment whe-

ther the lime or soda ash should be added before or after the coagulant to achieve the best results. Then follow the procedure in Sec. 6.1-6.13.

(b) Addition of clay suspensions. Where clay is used to improve the coagulation of low-turbidity water, add a well-shaken clay, kaolin, or stone dust dosing suspension (prepared as directed in Sec. 4.1) just before the coagulant. Then follow the procedure in Sec. 6.1-6.13.

(c) Addition of activated silica. Where activated silica is employed as a coagulant aid, use the activated silica solution (4.2) in amounts to give a dosage range of 1 to 7 mg/l. On the average, activated silica dosages of 2 to 5 mg/l yield satisfactory results. Determine by experiment whether the activated silica solution should be added before or after the coagulant for best results. Then follow the procedure in Sec. 6.1-6.13.

7. Test Procedure for Lime-Soda Ash Softening Treatment

7.1. Determine the hardness, phenolphthalein and total alkalinity, calcium, and free carbon dioxide as described under the appropriate sections of this Manual.

7.2. Calculate the chemical dosages of lime, soda ash, or both needed to soften the water (see Table 4 for the chemical requirements of each constituent). Based on these calculations, prepare appropriate dosing suspensions as directed in Sec. 4.1.

7.3. With a graduated cylinder, measure 1 l samples of raw water into one or more 1,500 ml beakers.

7.4. Lower the stirring paddle into the sample, start the stirring machine, and set at 30 revolutions per minute.

7.5. With a measuring pipet, add the well-shaken lime dosing suspension and (if used) the soda ash dosing solution.

7.6. Continue to operate the stirring machine for 30 minutes at 30 revolutions per minute.

7.7. Stop the stirring machine and allow the sample to settle until the liquid becomes fairly clear (usually 10 to 15 minutes).

7.8. Using a 100 ml pipet, withdraw two or three portions of the top 3 inches of sample from the beaker.

7.9. Warm the combined withdrawn portions to a temperature of 25° C.

7.10. Filter through filter paper of medium retentiveness.

7.11. Determine the hardness, phenolphthalein and total alkalinity, and pH as described in this Manual.

8. Test Procedure for Activated Carbon Treatment

8.1. Clean and scrub the beakers, filter tubes, collecting pail, and stirring machine paddles with a nonodorous detergent. Rinse thoroughly with odor-free water.

8.2. With a graduated cylinder, measure 1 l samples of the odor-bearing water collected at a plant site just ahead of the point of carbon application.

8.3. Pour the measured odor-bearing water samples into five separate beakers. Use one of the samples as a blank.

8.4. With a measuring pipet, add increasing doses of well-shaken activated carbon dosing suspension (4.3) to four of the samples. Select a series of doses so that

TABLE 3

Alkaline Chemicals Required to Neutralize Coagulants

| To Neutralize 1 mg/l of the Following | Requires the Following Amount of: | | | | |
|--|-----------------------------------|--|---|-----------------------------|--|
| | Pure Lime mg/l | Com- mercial Quicklime (90% Pure) mg/l | Com- mercial Hydrated Lime (93% Pure) mg/l | Pure Soda Ash mg/l | Com- mercial Soda Ash (98% Pure) mg/l |
| Aluminum sulfate (filter alum) | 0.25 | 0.28 | 0.35 | 0.48 | 0.49 |
| Ferrous sulfate (copperas) | 0.20 | 0.22 | 0.28 | 0.38 | 0.39 |
| Ferric sulfate (90%) | 0.38 | 0.42 | 0.54 | 0.72 | 0.73 |
| Ferric sulfate- ferric chloride (chlorinated copperas) | 0.30 | 0.33 | 0.42 | 0.57 | 0.58 |

TABLE 4

Softening Chemicals Required to Reduce Hardness

| To Reduce 1 mg/l of the Following | Requires the Following Amount of: | | | | |
|-----------------------------------|-----------------------------------|--|---|-----------------------------|--|
| | Pure Lime mg/l | Com- mercial Quicklime (90% Pure) mg/l | Com- mercial Hydrated Lime (93% Pure) mg/l | Pure Soda Ash mg/l | Com- mercial Soda Ash (98% Pure) mg/l |
| Noncarbonate hardness* | | | | 1.06 | 1.08 |
| Bicarbonate hardness ⁺ | 0.56 | 0.62 | 0.79 | | |
| Free carbon dioxide | 1.27 | 1.41 | 1.79 | | |
| Magnesium ‡ | 2.31 | 2.56 | 3.26 | | |

+ Same as bicarbonate alkalinity (see Alkalinity, Table 1). Concentrations are measured in terms of "mg/l as calcium carbonate."

* Noncarbonate hardness exists if the total hardness exceeds the total alkalinity, and is calculated by subtracting the total alkalinity from the EDTA total hardness (when both are expressed in terms of "mg/l as calcium carbonate").

‡ To calculate the magnesium content in mg/l, subtract the calcium hardness (in "mg/l as calcium carbonate") from the total hardness (in "mg/l as calcium carbonate") and multiply the result by 0.243.

the first beaker will represent undertreatment and the last beaker will represent overtreatment. It will be necessary to repeat the jar test once or twice to ascertain the proper doses for the desired results.

8.5. Lower the stirring paddles into the samples, start the stirring machine, and mix the samples for 30 minutes at 30 revolutions per minute.

8.6. With a measuring pipet, add to all five samples (including the blank) the same amount of coagulant and other chemicals being used in plant operation.

8.7. Increase the mixing rate for 1 minute to a speed of 60 to 80 revolutions per minute.

8.8. Reduce the stirring speed over the next 30 seconds to 30 revolutions per minute, and continue stirring at 30 revolutions per minute for 15 minutes.

8.9. Stop the stirring machine and allow the samples to settle for 5 to 15 minutes.

8.10. Filter each of the samples through a filter tube containing a generous plug of glass wool that has previously been washed with odor-free water.

8.11. Determine the taste and odor quality of each filtrate as described under Taste and Odor, Sec. 5.

8.12. On the basis of this laboratory test, begin the plant scale treatment at the minimum dosage which will produce a satisfactory water. Through plant trial, reduce the activated carbon dosage until the smallest dose required for palatability has been reached.

9. Practical Applications of Jar Tests

9.1. Variation in stirring time. The procedure specifies a stirring time of exactly 15 minutes at 30 rpm. This arbitrary figure is of real value only when two water plants at some distance from each other desire to compare research on a particular coagulant or coagulant aid. The 15 minute stirring period will be too short in water plants with a flocculation period of 40 to 60 minutes, and too long in an old plant where only 5 minute flocculation is provided. The operator should increase or decrease the 15 minute stirring time to match the plant flocculation time.

Plant operating conditions may also dictate a change in the laboratory stirring time. For example, the flocculation period in a plant operating at 5 mgd in winter may be 40 minutes, while a summer pumpage rate of 10 mgd will reduce the flocculation period to 20 minutes. The stirring time should be reduced accordingly in the second case. Similar downward adjustments should be made when a plant has three flocculation-settling basins in parallel, with a normal flocculation period of 30 minutes, but one basin is taken out of service for repairs or cleaning, thereby reducing the effective flocculation period.

9.2. Variation in settling period. A 5 minute settling period can be useful in supporting a decision made at the end of the stirring period. For example, a sample may show promise during stirring but after the stirring is stopped the large floc may not settle in 10 to 20 minutes. On the other hand, a sample with a larger dosage and having exactly the same appearance during the stirring will start to settle well in less than 5 minutes, and will quickly show a "skin" of approximately 1/2 inch of clear water at the top of the beaker. The larger dosage might be preferable under such circumstances. The general idea is to employ the lowest dosage in plant operation that gives a well-formed, rapidly settling floc which shows clear water between the particles.

9.3. Comparison of various coagulants. When the optimum dosage is found for each of the different coagulants tested, a final jar test can be run in which each coagulant is applied at its optimum dosage to a separate beaker. At the end of the test the coagulants can be compared on the basis of speed of floc formation, size of

floc, floc strength, rate of settling, apparent clarity of water between floc particles, turbidity, pH, alkalinity, color of the water after settling, and cost per thousand or million gallons.

9.4. Assessment of need for coagulant aid. Very turbid waters generally coagulate quite well in both winter and summer. Relatively clear, cold waters, however, present coagulation problems because the lack of floc nuclei and the low temperature slow down the coagulation process. A colloidal clay-like turbidity occurring during winter can be particularly troublesome. Activated silica and a number of commercial materials loosely termed "polyelectrolytes" have proved of value under such conditions.

Activated silica is often used as a coagulant aid with filter alum. To check the need and effectiveness of this or any other coagulant combination, the jar test is run in the normal manner, using the alum coagulant alone in the first series. The optimum alum dosage and the turbidity of the water after settling are determined. Then the test is repeated and 5 mg/l of activated silica suspension is applied to each 1,000 ml sample of raw water prior to the addition of the various alum dosages. The test series is repeated a third time and the activated silica is added after the various alum dosages. The results from all these runs should reveal whether the application of activated silica actually improves coagulation through the formation of a faster, larger, more rugged, and quicker-settling floc. The best order of adding the activated silica and any saving in the alum dosage should be noted. A fourth test series is finally run to determine the optimum activated silica dosage. All samples receive the same economical alum dosage, and the activated silica dose alone is varied from 1 to 7 mg/l and is added before or after the alum, as found best from the previous experiments.

9.5. Check of plant efficiency. Jar tests can help to uncover deficiencies in plant design. The first series of experiments is run in the accepted manner to determine the actual coagulant dose needed for plant operation. In the second series, the optimum plant coagulant dose found in the first series is added to all beakers, and single beakers are taken from the stirring machine at 5 or 10 minute intervals over a 40 minute span to find the optimum stirring period. Using the optimum stirring period found in the second series, a third series involving varied coagulant doses is run to see if a more economical coagulant dose can be used with the new stirring time. These experiments can reveal shortcomings in the mixing, flocculating, and settling processes of an old plant, and the need for introducing such improvements as more accurate coagulant feeders, better mechanical mixing and flocculation equipment, and longer times for mixing, flocculating, and settling. Obviously, any conclusions drawn from a series of jar tests would have to be related and confined to the particular treatment plant and water supply. Generalizations about all water plants and water supplies can prove dangerous in this regard.

9.6. Softening reactions. The jar test checks the calculated lime and soda ash dosages needed for plant treatment. Gentle stirring is used throughout the softening jar test because of the slow reaction which takes place between the hardness components and the lime and soda ash.

A complete discussion of the various lime-soda ash softening treatments is beyond the scope of this book. The softening treatment must be tailored to the particular water supply. The usual practice is to soften a water to a hardness of 50 to

100 mg/l as calcium carbonate and to reduce the carbonate hardness as calcium carbonate to 35 to 40 mg/l. Soda ash is the more expensive softening chemical and is usually reserved for the removal of noncarbonate hardness. Table 3 shows how to calculate the approximate amounts of softening chemicals required.

Manganese

1. Purpose of Test

Manganese causes laundry and staining problems similar to iron except that the stains and deposits are darker and often more persistent. The problems are accentuated because manganese seldom occurs alone but usually coexists with iron, especially in well waters. Removal steps are instituted whenever the manganese concentration reaches a troublesome level, which may be as little as 0.1 mg/l.

2. Warning

Few of the common constituents of natural water affect the test. The sample must be free of turbidity or color which cannot be overcome by the preliminary boiling described in Step 5.4. The latest edition of *Standard Methods* should be consulted for instructions on how to cope with samples containing considerable organic matter or chloride in excess of 1,000 mg/l.

3. Apparatus

- 3.1. Ten or more 250 ml erlenmeyer flasks.
- 3.2. A 25 ml buret or appropriate pipets for measuring the standard manganese solution.
- 3.3. A 100 ml graduated cylinder for measuring distilled water and sample.
- 3.4. A pipet for measuring the mixed reagent solution.
- 3.5. A gas burner or electric hot plate.
- 3.6. One or more wire gauze squares, 20 mesh.
- 3.7. Glass beads.
- 3.8. One or more glass stirring rods.
- 3.9. Ten or more 100 ml nessler tubes, matched, tall form; and support.
- 3.10. Wash water bottle for rinsing flask, beads, and stirring rod.

4. Reagents

- 4.1. Stock manganese solution:
 - (a) On an analytical balance, weigh 0.308 g dry manganous sulfate monohydrate ($\text{MnSO}_4 \cdot \text{H}_2\text{O}$). Carefully transfer the weighed chemical to a 250 ml beaker and dissolve in 100 ml distilled water.
 - (b) Cautiously add 1 ml concentrated sulfuric acid (H_2SO_4) with constant mixing.
 - (c) Transfer the acid solution to a 1 l volumetric flask, rinsing the beaker with three 100 ml portions of distilled water. Dilute further to the 1 l mark with distilled water. Stopper and mix thoroughly.

4.2. Standard manganese solution. With a volumetric pipet, measure carefully 10 ml stock manganese solution into a 100 ml volumetric flask. Dilute to the 100 ml mark with distilled water. Stopper and mix thoroughly. Prepare the standard manganese solution on the day of use because of questionable stability.

4.3. Mixed reagent solution:

(a) With a 500 ml graduated cylinder, measure 200 ml distilled water and pour into a 1,500 ml beaker.

(b) With the same graduated cylinder, measure 400 ml concentrated nitric acid.

(c) While stirring with one hand, slowly and cautiously add the nitric acid to the distilled water.

(d) Weigh 75 g mercuric sulfate (HgSO_4) and dissolve in Solution (c).

(e) With the 500 ml graduated cylinder, measure 200 ml 85 per cent phosphoric acid (H_3PO_4), and mix into Solution (d).

(f) On an analytical balance, weigh 0.035 g silver nitrate (AgNO_3), add to Solution (e), and mix to dissolve.

(g) Allow Solution (f) to cool to room temperature. Then transfer to a 1 l graduated cylinder, and dilute to the 1,000 ml mark with distilled water. Mix thoroughly by pouring back into the beaker and stirring.

4.4. Ammonium persulfate crystals or powder, $(\text{NH}_4)_2\text{S}_2\text{O}_8$.

5. Procedure

5.1. Preparation of manganese standards:

(a) Prepare the following series of manganese standards by measuring the indicated volumes of standard manganese solution (4.2) into separate 250 ml flasks:

| Standard Manganese Solution <i>ml</i> | Manganese <i>mg/l</i> |
|---|--------------------------|
| 0 | 0 |
| 0.5 | 0.05 |
| 1.0 | 0.1 |
| 2.0 | 0.2 |
| 3.0 | 0.3 |
| 4.0 | 0.4 |
| 6.0 | 0.6 |
| 8.0 | 0.8 |
| 10.0 | 1.0 |

(b) With a graduated cylinder, measure 75 ml distilled water and add to each flask.

5.2. Mix the sample thoroughly and measure 100 ml into a 250 ml flask which has been marked with a line at the 90 ml level.

5.3. Add 5 ml mixed reagent solution to the sample.

5.4. Heat the sample to boiling, and boil until the liquid level reaches the 90 ml level. Prevent spattering and bumping of the boiling liquid by placing a wire gauze over the electric or gas heat source and then resting the bottom of the flask on the wire gauze. As an added safeguard, add 4 or 5 glass beads to the flask, or place a glass stirring rod in the flask.

5.5. To each manganese standard prepared in Step 5.1 add 5 ml mixed reagent solution, and mix.

5.6. Weigh 1 g portions of ammonium persulfate crystals, and add to each manganese standard and to the sample.

5.7. Heat the contents of each flask to boiling; then boil gently for 1 minute more. A pink-to-purple color will develop in the manganese standards at this stage.

5.8. Remove each flask from the heat source, and allow to stand for 1 minute longer.

5.9. Cool each flask to room temperature under running tap water. Do not delay, since slow cooling may result in some loss of permanganate color.

5.10. Transfer the contents of each flask to a separate 100 ml nessler tube. Rinse the flask, glass beads, and stirring rod with 5 to 10 ml distilled water, and add the wash to the nessler tube.

5.11. Add sufficient distilled water to bring the volume to the 100 ml mark.

5.12. Mix each nessler tube by inverting four to six times.

5.13. Compare the sample with the standards and determine from the pink-to-purple color the mg/l manganese present.

Oxygen (Dissolved)

1. Purpose of Test

Aeration is an accepted water treatment process. Oxygen is introduced into some ground waters as the first step in removing iron and manganese prior to filtration. Aeration also lowers dissolved gases such as carbon dioxide and hydrogen sulfide to manageable levels in certain well waters. The taste and odor of some water supplies are improved by aeration.

Water in close contact with the air is often saturated with oxygen at the prevailing temperature. On the other hand, water which has been separated from contact with the air (such as deep well water or water from the deep parts of stratified lakes) will contain little or no dissolved oxygen (DO). The DO content can also be increased through the oxygen produced by aquatic plants during photosynthesis activity. A decrease in the DO of a surface water can occur when the temperature of the water rises or when the pollution load of a stream goes up.

Dissolved oxygen is important in water for a number of reasons. The palatability of water is improved by it. Fish and other smaller organisms which live in water need DO for their survival. And its presence can cause corrosion, especially in hot water systems.

2. Warning

This method (called the azide modification of the Winkler method) is applicable for most water supplies intended for potable purposes. When a water contains

more than 0.1 mg/l nitrate nitrogen or more than 1 mg/l ferrous iron, or contains other oxidizing or reducing agents, the latest edition of *Standard Methods* should be consulted.

The sampling point should be selected with the following thoughts in mind: The sample should be representative of the water supply. Care should be taken against the introduction of air into the water during all pumping operations ahead of the sampling site. Chlorine or chlorine dioxide treatment of the water in advance of sampling can contribute a measurable error to the determination, the extent of the error depending on the amount of the chlorine residual. If possible, therefore, the sampling site should be located ahead of the chlorination point.

The latest edition of *Standard Methods* should be consulted for details regarding the special sampling devices needed for collecting samples from streams, ponds, tanks, and other unusual locations.

3. Apparatus

3.1. One or more standard 250- to 300 ml BOD bottles (Fig. 1) with narrow mouths and glass stoppers.

3.2. A 250 ml graduated cylinder.

3.3. One or more wide-mouth 500-ml flasks.

3.4. A 25 ml buret and support.

3.5. Four measuring pipets for transferring 2 ml portions of reagents.

4. Reagents

4.1. Manganous sulfate solution:

(a) Weigh 480 g $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ or 400 g $\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$ or 364 g $\text{MnSO}_4 \cdot \text{H}_2\text{O}$.

(b) Place the salt in a 1,500 ml beaker and dissolve in 600 ml distilled water.

(c) Dilute to 1,000 ml with distilled water in a 1 l graduated cylinder. Mix thoroughly by pouring back into the beaker and stirring.

4.2. Alkali-iodide-azide solution:

(a) Weigh 500 g sodium hydroxide (NaOH) pellets, place in a 1,500 ml beaker, and dissolve in 600 ml distilled water. Perform this operation carefully because considerable acrid fumes and heat are generated.

(b) Weigh 150 g potassium iodide (KI), place in a 250 ml beaker, and dissolve in 150 ml distilled water.

(c) Add Solution (b) to Solution (a) with constant and thorough mixing.

(d) Transfer the mixed solution (c) to a 1 l graduated cylinder and dilute to the 1,000 ml mark with distilled water. Mix thoroughly by pouring back into the beaker and stirring.

(e) Weigh 10 g sodium azide (NaN_3), transfer to a 150 ml beaker, and dissolve in 40 ml distilled water.

(f) Add Solution (e) to Solution (d) with constant and thorough mixing. Store in a bottle which has a rubber or plastic stopper.

4.3. Sulfuric acid, concentrated, H_2SO_4 .

4.4. Starch indicator solution:

(a) Weigh 1 g potato, arrowroot, or soluble starch, and place in a 150 ml beaker.

(b) Add 10 ml distilled water and stir until the starch is wet and suspended as a milky liquid.

(c) Pour this suspension into a 250 ml beaker containing 200 ml boiling distilled water. Stir and boil the solution for 3 minutes.

(d) Cool the solution and allow to settle overnight.

(e) Pour off the clear liquid. Preserve the solution by adding 4 drops of toluene. Discard the solution when molds and debris begin to form at the bottom of the bottle.

4.5. Boiled distilled water. Follow the directions in Carbon Dioxide, Sec. 4.1. Prepare the boiled distilled water immediately before needed in the preparation of sodium thiosulfate titrant (4.6).

4.6. Sodium thiosulfate titrant, 0.0375*N* :

(a) On an analytical balance, carefully weigh 9.307 g sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$) of reagent grade quality. Carefully transfer to a 600-ml beaker and dissolve in 300-ml boiled distilled water (4.5). If necessary, heat gently to dissolve all the solid.

(b) Carefully transfer the cooled solution to a 1 l volumetric flask, rinsing the beaker with three 100 ml portions of boiled distilled water (4.5).

(c) Preserve the titrant by adding 0.4 g sodium hydroxide pellets. Mix the contents of the volumetric flask to dissolve the pellets.

(d) Dilute to the 1 l mark with boiled distilled water (4.5). Stopper and mix thoroughly.

5. Procedure

5.1. Collect the sample as described in Carbon Dioxide, Sec. 5.1 and 5.2. Use a narrow-mouth, glass-stoppered BOD bottle of 250 - 300 ml capacity.

5.2. Measure the temperature of the water being sampled as described in Temperature. Record the temperature.

5.3. Remove the glass stopper from the sample bottle. Using a measuring pipet, add 2 ml manganous sulfate solution. Place the tip of the pipet below the surface of the water so as to allow the heavy solution to flow in without contact with the air.

5.4. In the same way, add 2 ml alkali-iodide-azide solution.

5.5. Carefully replace the glass stopper so that no air becomes entrapped below it.

5.6. Mix by inverting the bottle several times over a period of 3 minutes.

5.7. Allow the resulting precipitate to settle at least to one half the bottle height.

5.8. Invert the bottle several times more, then set aside until the precipitate has settled at least halfway in the bottle.

5.9. Remove the stopper again, and, with a measuring pipet, add 2 ml concentrated sulfuric acid.

5.10. Replace the stopper carefully to prevent air from entering the bottle. Rinse the outside of the stoppered bottle with tap water. Then mix by inverting several times until the precipitate completely dissolves and the brown or yellow color is distributed uniformly.

5.11. Transfer the entire contents of the 300-ml BOD bottle to a wide-mouth 500-ml Erlenmeyer flask.

5.12. Fill the buret with sodium thiosulfate titrant, and record the liquid level

by reading the bottom of the meniscus. Guard against a leaky stopcock which results in the loss of titrant on standing.

5.13. Gradually add small portions of sodium thiosulfate titrant from the buret while constantly swirling the liquid in the flask, until the sample changes to a pale yellow or straw color.

5.14. With a measuring pipet, add to the flask 1 or 2 ml starch indicator solution, which will cause the solution to turn blue.

5.15. Continue adding sodium thiosulfate titrant drop by drop until the blue color disappears. Ignore any reappearance of the blue color on standing.

5.16. Record the new buret level by reading at the bottom of the meniscus.

5.17. Calculate the volume of titrant used by subtracting the buret reading in Step 5.12 from the buret reading in Step 5.16. The result is the dissolved-oxygen concentration in mg/l.

pH Value

1. Purpose of Test

The pH scale bears some resemblance to a thermometer. A thermometer scale measures the intensity of heat. The pH scale, on the other hand, denotes the intensity of acidity and alkalinity. The pH scale runs from 0 to 14. A water having a pH of 7.0 is at the midpoint of the scale and is considered neutral. Such a water is neither alkaline nor acidic. A water containing an acid will yield a pH reading below 7.0. The stronger the intensity of the acidity, the lower the pH will be. A pH of 0 means that the sample is very acid. The opposite holds true with alkalies. Alkalies raise the pH values above 7.0. A pH of 14 means that the sample is intensely alkaline.

pH plays an important role in such treatment processes as chlorination, coagulation, softening, and corrosion control. The pH test enables the plant operator to detect changes in the raw-and finished-water quality, and accordingly to add the proper chemical doses for the best coagulation and softening reactions. The finished water of some plants is adjusted with lime or soda ash to the slightly alkaline pH of 8 or more for the purpose of minimizing corrosion in the distribution system.

2. Warning

This method is suitable only for the approximate estimation of pH in clear and colorless waters in the pH range of 5.8 to 11.0. The best results are obtained on samples containing no more than 5 units of turbidity, and preferably less than 1 unit of turbidity. The color value of the water should also be less than 5 units.

The indicator can contribute its own pH to a slightly buffered water low in bicarbonate. In such an event, the pH should be checked by using a second indicator of somewhat different range. A 50 ml sample in a nessler tube, instead of the usual 10 ml sample, allows dilution of the indicator solution and possible correction of this difficulty. Adjustment of the pH of the indicator solution itself to the midpoint of its range by adding 0.02*N* acid or alkali is another way of minimizing this trouble.

Chlorine destroys or changes the sulfonephthalein indicators, thereby causing

erroneously low readings. For this reason, the residual chlorine concentration of the sample should not exceed 0.5 mg/l when present as free available chlorine and 1.0 mg/l when present as combined available chlorine.

These important shortcomings of the colorimetric method have led to the growing adoption of the electrometric or instrumental method of determining pH in recent years.

Temperature exerts an important effect on buffer solutions and pH measurements, a situation which neither colorimetric nor electrometric methods can overcome. For example, the same solution may give a reading of pH 7.6 at 20° C, 7.8 at 40° C, and 8.0 at 60° C. The buffer solutions described in Sec. 5.1 are intended to yield the best results in the temperature range of 18-25° C, which is room temperature in most laboratories.

3. Apparatus

3.1. Test tubes, 16 x 150 mm, matched; and support.

3.2. Burets, 25 or 50 ml, or appropriate pipets for measuring the reagents in the preparation of the buffer solutions.

3.3. A 1 ml measuring pipet, graduated in 0.1 ml, for dispensing the indicator solution.

3.4. Clean rubber stoppers to fit the test tubes.

3.5. A 10 ml pipet for measuring the sample.

4. Reagents

4.1. Reagents for preparation of buffer solution:

(a) Potassium dihydrogen phosphate solution:

(1) On an analytical balance, weigh 13.62 g dry potassium dihydrogen phosphate (also called potassium monobasic phosphate), KH_2PO_4 . Carefully transfer the weighed chemical to a 250 ml beaker and dissolve in 175 ml distilled water.

(2) Transfer the solution to a 1 l volumetric flask, rinsing the beaker with three 100 ml portions of distilled water. Dilute further to the 1 l mark with distilled water. Stopper and mix thoroughly.

(b) Borax solution:

(1) On an analytical balance, weigh 19.10 g sodium borate (also called sodium tetraborate), $\text{Na}_2\text{B}_4\text{O}_7$. Carefully transfer the weighed chemical to a 250 ml beaker and dissolve in 175 ml distilled water. Heat gently to dissolve all the solid.

(2) Transfer the cooled solution to a 1 l volumetric flask, rinsing the beaker with three 100 ml portions of distilled water. Dilute further to the 1 l mark with distilled water. Stopper and mix thoroughly.

(c) Sodium carbonate solution:

(1) On an analytical balance, weigh 5.30 g dry sodium carbonate (Na_2CO_3). Carefully transfer the weighed chemical to a 250 ml beaker and dissolve in 150 ml distilled water.

(2) Transfer the solution to a 1 l volumetric flask, rinsing the beaker with three 100 ml portions of distilled water. Dilute further to the 1 l mark with distilled water. Stopper and mix thoroughly.

4.2. Indicator solutions:

(a) Bromthymol blue indicator solution for pH range of 6.0 to 7.6. On an

analytical balance, weigh 0.100 g bromthymol blue sodium salt powder (also called 3'3''-dibromothymolsulfonephthalein sodium salt powder). Dissolve in 250 ml distilled water.

(b) Phenol red indicator solution for pH range of 6.8 to 8.4. On an analytical balance, weigh 0.100 g phenol red sodium salt powder (also called phenosulfonephthalein sodium salt powder). Dissolve in 250 ml distilled water.

(c) Thymol blue indicator solution for pH range of 8.0 to 9.6. On an analytical balance, weigh 0.100 g thymol blue sodium salt powder (also called thymolsulfonephthalein sodium salt powder). Dissolve in 250 ml distilled water.

5. Procedure

5.1. Preparation of buffer solutions:

(a) Prepare the following series of buffer solutions in the pH range of 5.8 to 9.2 by measuring into separate test tubes the indicated volumes of potassium dihydrogen phosphate solution and borax solution:

| Potassium Dihydrogen Phosphate Solution <i>ml</i> | Borax Solution <i>ml</i> | Buffer Solution <i>pH</i> |
|---|--------------------------------|---------------------------------|
| 9.3 | 0.7 | 5.8 |
| 8.9 | 1.1 | 6.0 |
| 8.4 | 1.6 | 6.2 |
| 7.9 | 2.1 | 6.4 |
| 7.3 | 2.7 | 6.6 |
| 6.7 | 3.3 | 6.8 |
| 6.3 | 3.7 | 7.0 |
| 5.9 | 4.1 | 7.2 |
| 5.6 | 4.4 | 7.4 |
| 5.3 | 4.7 | 7.6 |
| 5.1 | 4.9 | 7.8 |
| 4.8 | 5.2 | 8.0 |
| 4.5 | 5.5 | 8.2 |
| 4.0 | 6.0 | 8.4 |
| 3.4 | 6.6 | 8.6 |
| 2.8 | 7.2 | 8.8 |
| 1.8 | 8.2 | 9.0 |
| 0 | 10.0 | 9.2 |

(b) Prepare the following series of buffer solutions in the pH range of 9.2 to 11.0 by measuring into separate test tubes the indicated volumes of sodium carbonate solution and borax solution using the table on page 78.

(c) Select and prepare any appropriately smaller number of buffer solutions from the series shown if the pH variation at the plant falls within a narrower range.

(d) With a 1 ml measuring pipet, add 0.40 ml of the appropriate indicator solu-

tion to each buffer solution. Shake gently to accomplish complete mixing. Note that:

(1) The series of buffer solutions containing bromthymol blue indicator solution, 4.2(a) changes gradually from a yellow color at pH 6.0 and below to a blue color at pH 7.6 and above.

(2) The series of buffer solutions containing phenol red indicator solution, 4.2(b), changes gradually from a yellow color at pH 6.8 and below to a red color at pH 8.4 and above.

(3) The series of buffer solutions containing thymol blue indicator solution, 4.2(c), changes gradually from a yellow color at pH 8.0 and below to a blue color at pH 9.6 and above.

(e) Protect these buffer solutions by tightly capping with clean rubber stoppers if the solutions are to be used for a month or more.

5.2. Determination of sample:

(a) With a 1 ml measuring pipet, transfer 0.40 ml of the appropriate indicator solution to a perfectly clean test tube.

(b) With a pipet, add 10 ml of sample. Mix the sample and indicator gently with the pipet.

(c) Compare the sample with the buffer solutions prepared in Step 5.1(d). Place a white sheet of paper behind the tubes and look through the sides of the tubes. For best results move the colored sample from hole to hole in the test tube stand until the color of the sample matches one of the colored buffer solutions or falls between two consecutive buffer solutions showing the closest color approximation.

| Sodium Carbonate Solution <i>ml</i> | Borax Solution <i>ml</i> | Buffer Solution <i>pH</i> |
|--|-----------------------------|------------------------------|
| 3.6 | 6.4 | 9.4 |
| 5.6 | 4.4 | 9.6 |
| 6.7 | 3.3 | 9.8 |
| 7.5 | 2.5 | 10.0 |
| 8.2 | 1.8 | 10.2 |
| 8.7 | 1.3 | 10.4 |
| 9.2 | 0.8 | 10.6 |
| 9.5 | 0.5 | 10.8 |
| 9.7 | 0.3 | 11.0 |

Phosphate

1. Purpose of Test

Phosphate is seldom present in significant concentrations in natural waters used for drinking purposes but may be added in one of several forms during the treatment process. Sodium metaphosphate and related polyphosphates some-

times are applied in small doses to waters to prevent or delay iron precipitation or corrosion, or to control the deposition of calcium carbonate scale throughout the distribution system. Boiler feedwaters are sometimes treated with phosphate salts in order to reduce scale formation.

The procedure in Sec. 5 is divided into two parts. Section 5.1, Determination of Orthophosphate, describes the steps to be followed when simple phosphate salts are used for the treatment of boiler feedwaters. Section 5.2, Determination of Total Phosphate, should be followed when sodium metaphosphate and polyphosphates are used during the treatment process.

2. Warning

The procedures are suitable for clear and colorless waters. For brines with a high chloride content and waters containing more than 1 mg/l of ferric iron, nitrite, or oxidizing agents like chromate, consult the latest edition of *Standard Methods*.

3. Apparatus

- 3.1. Eight or more 100 ml nessler tubes, matched, tall form; and support.
 - 3.2. A 25 ml buret or appropriate pipets for measuring the standard phosphate solution.
 - 3.3. A pipet for measuring the acid molybdate reagent.
 - 3.4. A dropping pipet or medicine dropper for adding the stannous chloride reagent.
- In addition, the following are needed for the total-phosphate determination:
- 3.5. A 100 ml graduated cylinder or appropriate volumetric pipets for measuring the sample.
 - 3.6. One or more 250 ml erlenmeyer flasks.
 - 3.7. Dropping pipets or medicine droppers, capacity 0.5-1 ml, for dispensing phenolphthalein indicator solution, acid solution, and sodium hydroxide solution.
 - 3.8. A gas burner or electric hot plate.
 - 3.9. One or more wire gauze squares, 20 mesh.
 - 3.10. Four or more glass beads.
 - 3.11. One or more glass stirring rods.
 - 3.12. A wash water bottle for rinsing flask, beads, and stirring rod.

4. Reagents

- 4.1. Stock phosphate solution:
 - (a) On an analytical balance, weigh 0.7165 g dry potassium dihydrogen phosphate (also called potassium monobasic phosphate), KH_2PO_4 . Carefully transfer to a 250 ml beaker, and dissolve in 100 ml distilled water.
 - (b) Transfer the solution to a 1 l volumetric flask, rinsing the beaker with three 100 ml portions of distilled water, and dilute further to the 1 l mark with distilled water. Stopper and mix thoroughly.
- 4.2. Standard phosphate solution. With a volumetric pipet, measure carefully 10 ml stock phosphate solution into a 1 l volumetric flask. Dilute to the 1 l mark with distilled water. Stopper and mix thoroughly.
- 4.3. Acid-molybdate solution:

(a) With a 500 ml graduated cylinder, measure 400 ml distilled water and pour into a 1,500 ml beaker.

(b) With a 500 ml graduated cylinder, measure 280 ml concentrated sulfuric acid, H_2SO_4 .

(c) While stirring constantly with one hand, slowly and cautiously add the sulfuric acid to the distilled water. Considerable heat is generated by the mixing of the sulfuric acid and the distilled water, so pour slowly and mix well to avoid dangerous spattering.

(d) Allow Solution (c) to cool to room temperature.

(e) Weigh 25 g ammonium molybdate, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, place in a 250 ml beaker, and dissolve in 175 ml distilled water.

(f) Add the molybdate solution to the cooled acid solution, dilute to 1 l with distilled water, and mix thoroughly.

4.4. Stannous chloride solution:

(a) Weigh 2.5 g stannous chloride ($SnCl_2 \cdot 2H_2O$) and place in 250 ml pyrex bottle.

(b) With a graduated cylinder, measure 100 ml reagent grade glycerol or glycerine and add to the bottle. Place the bottle in a hot-water bath and stir the chemicals with a glass rod until the stannous chloride completely dissolves.

In addition, the following are needed for the total-phosphate determination:

4.5. Phenolphthalein indicator solution. Weigh 0.5 g phenolphthalein disodium salt powder and dissolve in 100 ml distilled water.

4.6. Acid solution:

(a) With a 1 l graduated cylinder, measure 600 ml distilled water and pour into a 1,500 ml beaker.

(b) With a 500 ml graduated cylinder, measure 300 ml concentrated sulfuric acid, H_2SO_4 .

(c) While stirring constantly with one hand, slowly and cautiously add the sulfuric acid to the distilled water. Considerable heat is generated by the mixing of the sulfuric acid and the distilled water, so pour slowly and mix well to avoid dangerous spattering.

(d) Allow the solution to cool to room temperature.

(e) Add 4.0 ml concentrated nitric acid, HNO_3 , to the cooled solution and mix well.

(f) Transfer Solution (e) to a 1 l graduated cylinder and dilute to the 1,000 ml mark with distilled water. Mix thoroughly by pouring back into the beaker and stirring.

4.7. Sodium hydroxide solution, 1N NaOH. Weigh 40 g sodium hydroxide pellets. Dissolve in 1,000 ml distilled water.

5. Procedure

5.1. Determination of orthophosphate:

(a) Prepare the following series of phosphate standards by measuring the indicated volumes of standard phosphate solution (4.2) into separate 100 ml nessler tubes using the upper table, page 81.

(b) Add distilled water to the 100 ml mark, and mix.

(c) Place 100 ml of the clear, colorless sample in another 100 ml nessler tube.

| Standard Phosphate Solution <i>ml</i> | Phosphate <i>mg/l</i> |
|--|--------------------------|
| 0 | 0 |
| 1 | 0.05 |
| 2 | 0.10 |
| 3 | 0.15 |
| 4 | 0.20 |
| 5 | 0.25 |
| 6 | 0.30 |

(d) Allow the standards and sample to come to the same temperature (room temperature).

(e) With a measuring pipet, add 4 ml acid-molybdate solution (4.3) to each of the standards and the sample, and mix thoroughly by inverting each tube four to six times.

(f) With a dropping pipet or medicine dropper, add 10 drops of stannous chloride solution and mix by inverting each tube four to six times.

(g) Allow the tubes to stand 10 minutes for the color to develop.

(h) Compare the sample with the standards and determine from the blue color the amount of phosphate present.

5.2. Determination of total phosphate:

(a) Measure the appropriate sample volume for the indicated sodium metaphosphate range:

| Sample Volume <i>ml</i> | Sodium Metaphosphate Range <i>mg/l</i> |
|----------------------------|---|
| 100 | 0.1-2.0 |
| 50 | 2.1-4.0 |
| 25 | 4.1-6.0 |

If a sample of only 50 ml is needed, add 50 ml distilled water to bring the total volume to 100 ml; with a 25 ml sample, add 75 ml distilled water. Measure the additional distilled water with a graduated cylinder. Place the sample (and extra distilled water if needed) in a 250 ml flask which has been previously marked with lines at the 50 ml and 25 ml levels.

(b) Add 1 drop of phenolphthalein indicator solution.

(c) If a red or pink color develops, add acid solution (4.6) one drop at a time until the red color disappears. (Stir the sample constantly while adding the acid.)

(d) Then add 1 ml extra of the acid solution (4.6).

(e) Boil the acid-treated sample gently for 90 minutes. Add distilled water from time to time to keep the volume between the 25 and 50 ml lines on the flask. Prevent spattering and bumping of the boiling liquid by placing a wire gauze over the electric or gas heat source and then resting the bottom of the flask on the wire gauze. For further protection add 4 or 5 glass beads to the flask, or place a glass stirring rod in the flask.

(f) Cool the sample to room temperature.

(g) Stirring the sample constantly, add sodium hydroxide solution until a faint pink color reappears.

(h) Transfer the sample to a 100 ml nessler tube. Rinse the flask, glass beads, and stirring rod with 10 ml distilled water, and add the wash to the nessler tube.

(i) Add sufficient distilled water to bring the volume to the 100 ml mark.

(j) Complete the determination as described in Sec. 5.1.

(k) Calculate the mg/l total phosphate by multiplying the result found in Step 5.2(j) by the appropriate factor:

| Sample Volume <i>ml</i> | Multiply mg/l Phosphate by: |
|----------------------------|-----------------------------------|
| 100 | 1 |
| 50 | 2 |
| 25 | 4 |

Residue (Filtrable)

1. Purpose of Test

A highly mineralized water is less acceptable than a water of little or moderate mineral content for drinking, household, and special industrial purposes. A good indication of the quantity of minerals in a water can be found by weighing the material left after evaporation. If the sample is filtered before evaporation, the residue is called "filtrable residue"; if not, "total residue."

2. Warning

This simple method is intended to give an approximate indication of the dissolved solids in a potable water. In many instances this result will prove sufficiently accurate. However, for samples which are highly alkaline or highly mineralized, use the procedure described in the latest edition of *Standard Methods*.

3. Apparatus

3.1. A filter funnel.

3.2. Filter paper, acid washed, ashless, hard finished, of fine retentiveness. Whatman No. 42, 44, or 50 or Schleicher & Schull No. 589 is satisfactory.

3.3. An evaporating dish, about 90 to 110 mm in diameter, with a capacity

of 120-200 ml. The dish may be made of platinum, nickel, porcelain, silica, Vycor, or pyrex.

3.4. An infrared heat lamp, 250 watts; with support.

3.5. A desiccator containing a desiccant which changes color when the moisture content becomes too high.

3.6. An analytical balance capable of accurate weighing to 1 mg.

4. Procedure

4.1. Filter a turbid sample through a retentive filter paper. Discard the first 25 ml of the filtrate and save the rest.

4.2. Clean the evaporating dish thoroughly and dry under the infrared heat lamp for 15 to 30 minutes.

4.3. Place the evaporating dish in the desiccator.

4.4. As soon as the temperature of the evaporating dish reaches that of the room and the balance, weigh the dish as accurately as possible.

4.5. With a graduated cylinder, measure 100 ml of the filtered sample and transfer to the weighed evaporating dish.

4.6. Place the evaporating dish under the heat lamp and evaporate the sample to dryness. A distance of 1½ to 2 inches between the bottom of the heat lamp and the lip of the evaporating dish usually gives a satisfactory rate of evaporation. If necessary, adjust the distance upward or downward to avoid boiling or spattering of the sample.

4.7. If necessary, add a second and a third 100 ml portion of filtered sample to the evaporating dish, so that the weighed residue will be at least 10 to 25 mg but no more than 250 mg.

4.8. After all the water has evaporated, keep the evaporating dish under the heat lamp for 1 hour more.

4.9. With a clean cloth, lightly wipe off any dust, debris, or moisture that has accumulated on the outside of the evaporating dish. Place the evaporating dish in the desiccator.

4.10. As soon as the temperature of the evaporating dish comes down to that of the room and the balance, weigh the dish as accurately as possible.

4.11. Subtract the weight obtained in Step 4.4 from the weight obtained in Step 4.10.

4.12. Calculate the mg/l filtrable residue by multiplying the result found in Step 4.11 by the appropriate factor:

| Sample Volume <i>ml</i> | Multiply mg Residue by: |
|----------------------------|-------------------------------|
| 100 | 10 |
| 200 | 5 |
| 300 | 3.3 |

Silica

1. Purpose of Test

Silica occurs naturally in most well and river waters and is also applied artificially (in the form of "activated silica") as a coagulant aid in many treatment plants. Activated silica is prepared on the premises by partially or completely neutralizing a sodium silicate solution with sulfuric acid, alum, ammonium sulfate, chlorine, or carbon dioxide. Proper use of activated silica causes no increase in the silica content of the treated water.

This test is designed for the determination of that silica fraction which is already dissolved as well as the fraction which is readily solubilized by sodium bicarbonate treatment. These two silica fractions can be composed of the silica originally present in the water and the activated silica added in the course of treatment.

2. Warning

The results obtained with this method are approximate and should be reported to the nearest mg/l. The latest edition of *Standard Methods* should be consulted if greater sensitivity and accuracy are desired in the results, as well as for ways of overcoming turbidity and color in a sample.

3. Apparatus

- 3.1. Eight or more 50 ml nessler tubes, matched, tall form; and support.
- 3.2. A 25 ml buret or appropriate pipets for measuring the potassium chromate solution.
- 3.3. A 25 ml graduated cylinder for measuring the borax solution.
- 3.4. Clean rubber stoppers (No. 2 size) for nessler tubes.
- 3.5. A 50 ml graduated cylinder for measuring the sample.
- 3.6. A 100 ml evaporating dish. Although a platinum dish is preferable, dishes made of nickel or porcelain may be substituted.
- 3.7. An infrared heat lamp, 250 watts, and support.
- 3.8. Measuring pipets for adding sulfuric acid, ammonium molybdate, and oxalic acid solutions.
- 3.9. A dropping pipet for adding hydrochloric acid solution.

4. Reagents

- 4.1. Potassium chromate solution:
 - (a) On an analytical balance, weigh 0.63 g dry potassium chromate (K_2CrO_4). Transfer the weighed chemical to a 250 ml beaker and dissolve in 100 ml distilled water.
 - (b) Transfer the solution to a 1 l volumetric flask, rinsing the beaker with three 100 ml portions of distilled water, and dilute further to the 1 l mark with distilled water.
- 4.2. Sodium borate solution. Weigh 10 g sodium borate (also called sodium tetraborate), $Na_2B_4O_7 \cdot 10H_2O$, and place in a 1,500 ml beaker. Dissolve in 1,000 ml distilled water which has been measured with a graduated cylinder.

4.3. Sodium bicarbonate powder, silica-free, NaHCO_3 .

4.4. Sulfuric acid solution, $1N \text{H}_2\text{SO}_4$:

(a) Using a graduated cylinder, measure 980 ml distilled water, and pour into a 1,500 ml beaker.

(b) Using a 50 ml graduated cylinder, measure 28 ml concentrated sulfuric acid.

(c) While stirring with one hand, slowly and cautiously add the measured sulfuric acid to the distilled water. Considerable heat is generated by the mixing of the sulfuric acid and the distilled water, so pour slowly and mix well to avoid dangerous spattering.

4.5. Hydrochloric acid solution:

(a) Using a graduated cylinder, measure 100 ml distilled water into a 250 ml bottle.

(b) In the same graduated cylinder, measure 100 ml concentrated hydrochloric acid (HCl) and transfer it to the bottle.

(c) Stopper and shake the bottle to mix the contents.

4.6. Ammonium molybdate solution:

(a) Weigh 10 g ammonium molybdate, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$, and place in a 250 ml beaker. Dissolve in 100 ml distilled water which has been measured with a graduated cylinder.

(b) Heat and stir the solution to dissolve the crystals. Store the solution in a polyethylene bottle to keep it silica-free. Discard the solution when a precipitate begins to form.

4.7. Oxalic acid solution. Weigh 10 g oxalic acid ($\text{H}_2\text{C}_2\text{O}_4\cdot 2\text{H}_2\text{O}$) and place in a 250 ml beaker. Dissolve in 100 ml distilled water which has been measured with a graduated cylinder. Store the solution in a polyethylene bottle.

5. Procedure

5.1. Preparation of silica standards:

(a) Prepare the following series of color standards by measuring the indicated volumes of potassium chromate solution into separate 50 ml nessler tubes:

| Potassium Chromate Solution <i>ml</i> | Silica Equivalent <i>mg/l</i> |
|--|----------------------------------|
| 0.0 | 0 |
| 1.0 | 2 |
| 2.0 | 4 |
| 4.0 | 8 |
| 5.0 | 10 |
| 7.5 | 15 |
| 10.0 | 20 |

(b) Using a 25 ml graduated cylinder, measure 25 ml sodium borate solution into each tube.

(c) Add distilled water to each tube to bring the volume to the 50 ml mark, and mix by inverting the tube four to six times.

(d) Protect these standards by capping with clean rubber stoppers if the standards are to be used over a period of several months.

5.2. Using a graduated cylinder, measure 50 ml of the clear and colorless sample into a 100 ml evaporating dish.

5.3. Weigh 0.2 g sodium bicarbonate powder, and add to the sample.

5.4. Place the evaporating dish under the infrared heat lamp and warm the sample for 60 minutes. A distance of 1½ to 2 inches between the bottom of the heat lamp and the lip of the evaporating dish usually gives a satisfactory rate of heating. If necessary, adjust the distance upward or downward to avoid boiling or spattering of the sample.

5.5. Turn off the heat lamp and allow the sample to cool to room temperature.

5.6. With a measuring pipet, cautiously and slowly add 2.4 ml sulfuric acid solution. Stir the solution throughout the acid addition.

5.7. Transfer the contents of the evaporating dish to a 50 ml nessler tube. Rinse the evaporating dish with a small volume (5 to 10 ml) of distilled water and add the wash to the nessler tube. Carefully control the volume of distilled water used, in order to keep the volume of the sample plus the wash from exceeding 50 ml.

5.8. Add sufficient distilled water to bring the volume to the 50 ml mark.

5.9. Using a dropping pipet, add 1.0 ml hydrochloric acid solution.

5.10. Quickly add 2.0 ml of ammonium molybdate solution with a measuring pipet. Mix immediately by inverting the tube four to six times.

5.11. Let the tube stand for 5 to 10 minutes.

5.12. With a measuring pipet, add 1.5 ml oxalic acid solution, and mix by inverting the tube four to six times.

5.13. Allow the tube to stand at least 2 minutes but no more than 15 minutes.

5.14. Compare the sample with the standards and determine from the yellow color the amount of silica present.

5.15. Check the silica content of all the reagents as follows:

(a) Weigh 0.2 g sodium bicarbonate powder and add to 50 ml distilled water in the same (or a similar) 100 ml evaporating dish used in Step 5.2.

(b) Continue with all the steps of the procedure from 5.4 through 5.14.

(c) Subtract the silica value thus found in the reagents from the result obtained in Step 5.14 to get the true silica content of the sample.

5.16. Report the results to the nearest mg/l.

Taste and Odor

1. Purpose of Test

Objectionable tastes and odors in water can be caused by plankton, actinomycetes, bacteria, decaying vegetation, inadequately treated domestic wastes, and industrial wastes. Corrective measures designed to reduce or eliminate unpleasant tastes and odors include aeration as well as the application of free residual chlorine, chlorine-ammonia, chlorine dioxide, activated carbon, and copper sulfate. An im-

(c) Add distilled water to each tube to bring the volume to the 50 ml mark, and mix by inverting the tube four to six times.

(d) Protect these standards by capping with clean rubber stoppers if the standards are to be used over a period of several months.

5.2. Using a graduated cylinder, measure 50 ml of the clear and colorless sample into a 100 ml evaporating dish.

5.3. Weigh 0.2 g sodium bicarbonate powder, and add to the sample.

5.4. Place the evaporating dish under the infrared heat lamp and warm the sample for 60 minutes. A distance of 1½ to 2 inches between the bottom of the heat lamp and the lip of the evaporating dish usually gives a satisfactory rate of heating. If necessary, adjust the distance upward or downward to avoid boiling or spattering of the sample.

5.5. Turn off the heat lamp and allow the sample to cool to room temperature.

5.6. With a measuring pipet, cautiously and slowly add 2.4 ml sulfuric acid solution. Stir the solution throughout the acid addition.

5.7. Transfer the contents of the evaporating dish to a 50 ml nessler tube. Rinse the evaporating dish with a small volume (5 to 10 ml) of distilled water and add the wash to the nessler tube. Carefully control the volume of distilled water used, in order to keep the volume of the sample plus the wash from exceeding 50 ml.

5.8. Add sufficient distilled water to bring the volume to the 50 ml mark.

5.9. Using a dropping pipet, add 1.0 ml hydrochloric acid solution.

5.10. Quickly add 2.0 ml of ammonium molybdate solution with a measuring pipet. Mix immediately by inverting the tube four to six times.

5.11. Let the tube stand for 5 to 10 minutes.

5.12. With a measuring pipet, add 1.5 ml oxalic acid solution, and mix by inverting the tube four to six times.

5.13. Allow the tube to stand at least 2 minutes but no more than 15 minutes.

5.14. Compare the sample with the standards and determine from the yellow color the amount of silica present.

5.15. Check the silica content of all the reagents as follows:

(a) Weigh 0.2 g sodium bicarbonate powder and add to 50 ml distilled water in the same (or a similar) 100 ml evaporating dish used in Step 5.2.

(b) Continue with all the steps of the procedure from 5.4 through 5.14.

(c) Subtract the silica value thus found in the reagents from the result obtained in Step 5.14 to get the true silica content of the sample.

5.16. Report the results to the nearest mg/l.

Taste and Odor

1. Purpose of Test

Objectionable tastes and odors in water can be caused by plankton, actinomycetes, bacteria, decaying vegetation, inadequately treated domestic wastes, and industrial wastes. Corrective measures designed to reduce or eliminate unpleasant tastes and odors include aeration as well as the application of free residual chlorine, chlorine-ammonia, chlorine dioxide, activated carbon, and copper sulfate. An im-

portant objective of a water treatment plant is to produce a finished water which is free of unpleasant tastes and odors.

This simple qualitative method is designed for routine determinations in small water plant laboratories. The test is suitable for potable water samples and water supplies undergoing treatment.

2. Warning

The taste test should be applied only to waters known to be safe for drinking. Where possible, the majority opinion of several testers should be sought and recorded.

When a more quantitative appraisal of the character and intensity of the taste and odor is desired, the procedures in the latest edition of *Standard Methods* should be followed.

3. Apparatus

3.1. Two or more 500 ml wide-mouth glass-stoppered bottles or flasks. Remove all trace of taste and odor from the interior and exterior of the bottles or flasks by scrubbing with nonodorous cleansers or hydrochloric acid. Finally, rinse with several portions of odor-free water. Set these bottles or flasks aside for exclusive use in the taste and odor determination. Store, if possible, by immersing the bottles or flasks in odor-free water.

3.2. Thermometer, 0-110° C, of the mercury or metal-stem dial type.

3.3. Odor-free water generator. See Fig. 8.

4. Odor-Free Water

Prepare the odor-free water generator as shown in Fig. 8. Before charging the generator with activated carbon, thoroughly clean the gravel, tubing, and the 1 gal jug with a nonodorous cleanser or hydrochloric acid and rinse several times with distilled water. After the generator is connected to the tapwater line, allow the tap water to pass through for a short time to wash out the fine carbon particles, before starting the collection of the odor-free water. Operate the generator at a rate of 1 l per minute. Use the orthotolidine method - see Chlorine (Residual), Method A - to check the residual chlorine in the collected water if the tap water has been chlorinated. Replace the activated carbon when residual chlorine appears in the collected water.

5. Procedure

5.1. Taste and odor quality at collection temperature:

(a) Fill one bottle or flask half full of odor-free water and replace the glass stopper. Use this water as the comparison blank.

(b) Fill the second bottle or flask half full of sample and replace the glass stopper.

(c) Vigorously shake the contents of the bottle containing the odor-free water, remove the stopper, and immediately sniff the odor.

(d) Without delay, vigorously shake the contents of the bottle containing the sample, remove the stopper, and also sniff the odor. Record the odor by the code shown in Table 5.

Simplified Procedure for Water Examination

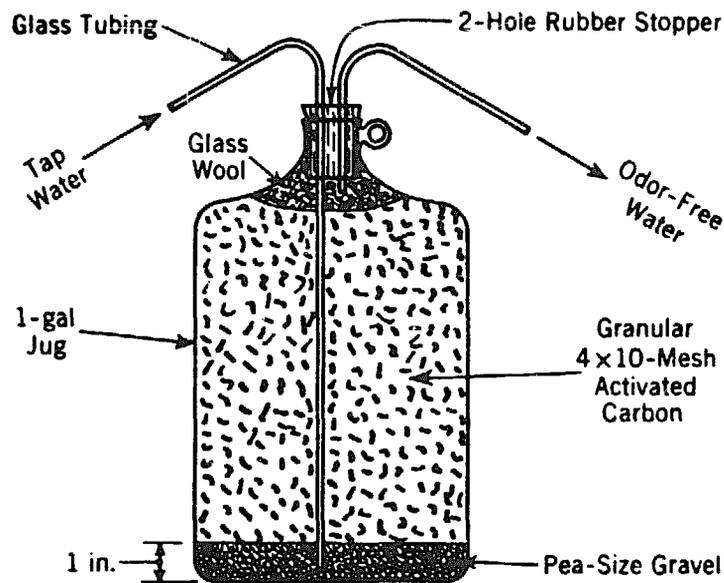


Fig. 8. Odor-Free Water Generator

(e) Pour 10 ml of the sample into a beaker and place the water in the mouth. Hold the water in the mouth for several seconds and then spit it out. Note the taste while the sample is in the mouth, as well as the aftertaste.

Do not taste any sample which may be unsafe for drinking.

TABLE 5

Qualitative Descriptions of Odors

| Code | Nature of Odor | Description (Such as Odors of:) |
|------|--------------------|--|
| A | Aromatic (spicy) | camphor, cloves, lavender, lemon |
| Ac | cucumber | <i>Synura</i> |
| B | Balsamic (flowery) | geranium, violet, vanilla |
| Bg | geranium | <i>Asterionella</i> |
| Bn | nasturtium | <i>Aphanizomenon</i> |
| Bs | sweetish | <i>Coelosphaerium</i> |
| Bv | violet | <i>Mallomonas</i> |
| C | Chemical | industrial wastes or treatment chemicals |
| Cc | chlorinous | free chlorine |
| Ch | hydrocarbon | oil refinery wastes |
| Cm | medicinal | phenol and iodoform |
| Cs | sulfuretted | hydrogen sulfide or rotten eggs |
| D | Disagreeable | (pronouncedly unpleasant) |
| Df | fishy | <i>Uroglenopsis</i> , <i>Dinobryon</i> |
| Dp | pigpen | <i>Anabaena</i> |
| Ds | septic | stale sewage |
| E | Earthy | damp earth |
| Ep | peaty | peat |
| G | Grassy | crushed grass |
| M | Musty | decomposing straw |
| Mm | moldy | damp cellar |
| V | Vegetable | root vegetables |

5.2. Taste and odor quality at elevated temperatures:

(a) Warm the bottles or flasks containing the odor-free water and the sample - Steps 5.1(a) and 5.1(b) - to 40° C.

(b) Carry out Steps 5.1(c) through 5.1(e). (Record the taste due to chlorination as "chlorinous" and that due to chlorophenol as "medicinal.")

(c) Repeat the above procedure at 60° C.

Temperature

1. Purpose of Test

Accurate temperature readings are important in a number of treatment processes and laboratory determinations. For example, temperature is a factor in certain algal blooms, in the degree of dissolved-oxygen saturation, and in carbon dioxide concentration.

2. Warning

For the best results, the temperature must be taken at the same sampling point. The thermometer should be immersed in the flowing stream, or in a large container filled with the sample and held until the mercury level stops moving. The temperature should then be read before withdrawing the thermometer from the sample.

Since mercury is a poison, mercury thermometers should be permanently installed only in a water pipe leading to a drain rather than in one leading into the distribution system, so that breakage of the thermometer can cause no serious spill of mercury into the potable supply.

3. Apparatus

A mercury-filled Centigrade thermometer with a range of about 0 to 100° C. This suffices for most general purposes. The scale should be subdivided into 0.5° or 1° C for ease in reading. Thermometers are calibrated for either "total immersion" or "partial immersion." Total-immersion thermometers must be completely immersed in the water to yield the correct temperature. Partial-immersion thermometers, on the other hand, must be immersed in the water to the depth of the etched circle which appears around the stem below the scale level. For best results, the accuracy of the thermometer in routine use should be checked against a precision thermometer which has been certified by the National Bureau of Standards.

4. Procedure

4.1. Immerse the thermometer in the sample to the proper depth for a correct reading.

4.2. Record the temperature to the nearest fraction of a degree Centigrade which can be estimated from the thermometer available. If a Fahrenheit thermometer is used, convert the readings to Centigrade by referring to Table 6.

TABLE 6

Conversion of Temperature From Fahrenheit to Centigrade Degrees

| °F | °C | °F | °C | °F | °C | °F | °C |
|----|-------|----|------|----|------|-----|------|
| 30 | -1.1 | 49 | 9.4 | 68 | 20.0 | 87 | 30.6 |
| 31 | -0.56 | 50 | 10.0 | 69 | 20.6 | 88 | 31.1 |
| 32 | 0.0 | 51 | 10.6 | 70 | 21.1 | 89 | 31.7 |
| 33 | 0.56 | 52 | 11.1 | 71 | 21.7 | 90 | 32.2 |
| 34 | 1.1 | 53 | 11.7 | 72 | 22.2 | 91 | 32.8 |
| 35 | 1.7 | 54 | 12.2 | 73 | 22.8 | 92 | 33.3 |
| 36 | 2.2 | 55 | 12.8 | 74 | 23.3 | 93 | 33.9 |
| 37 | 2.8 | 56 | 13.3 | 75 | 23.9 | 94 | 34.4 |
| 38 | 3.3 | 57 | 13.9 | 76 | 24.4 | 95 | 35.0 |
| 39 | 3.9 | 58 | 14.4 | 77 | 25.0 | 96 | 35.6 |
| 40 | 4.4 | 59 | 15.0 | 78 | 25.6 | 97 | 36.1 |
| 41 | 5.0 | 60 | 15.6 | 79 | 26.1 | 98 | 36.7 |
| 42 | 5.6 | 61 | 16.1 | 80 | 26.7 | 99 | 37.2 |
| 43 | 6.1 | 62 | 16.7 | 81 | 27.2 | 100 | 37.8 |
| 44 | 6.7 | 63 | 17.2 | 82 | 27.8 | 101 | 38.3 |
| 45 | 7.2 | 64 | 17.8 | 83 | 28.3 | 102 | 38.9 |
| 46 | 7.8 | 65 | 18.3 | 84 | 28.9 | 103 | 39.4 |
| 47 | 8.3 | 66 | 18.9 | 85 | 29.4 | 104 | 40.0 |
| 48 | 8.9 | 67 | 19.4 | 86 | 30.0 | | |

Turbidity

1. Purpose of Test

Turbidity is the expression used to describe the insoluble particles of clay, silt, mineral matter, organic debris, plankton, and other microscopic organisms which impede the passage of light through water. Since turbidity bears no direct relationship to the weight of the miscellaneous material in suspension, it is reported in turbidity units.

The turbidity unit is an empirical quantity which is based on the Jackson candle turbidimeter. This instrument is used for measuring the turbidity of raw waters in the range above 25 units as well as for standardizing stock suspensions which are then diluted to a range below 25 units. Turbidity standards in more than one turbidity range are often necessary for laboratory estimations. For example, one set of standards may encompass the range above 5 turbidity units, another set the region below 5 turbidity units. The latter set is designed for estimating the turbidity of the coagulated and filtered water.

Turbidity in excess of 5 units is noticeable to the consumer and accordingly represents an unsatisfactory condition. Public water supplies are coagulated and filtered in order to reduce the number and size of the suspended particles to an unobjectionable level. Efficient coagulation and filtration should produce a turbidity below 1 unit. The appearance of turbidity in a filtered water may indicate breaks in the filter sand bed or precipitation of a coagulant floc in the filtered water basins as a result of overtreatment or incomplete coagulation. Colloidal turbidity of extremely fine particle size is difficult to remove except by chemical treatment.

The turbidity test is used for controlling the amount of coagulant and accessory

chemical aids that are needed to produce a water of the desired clarity. The procedure in this Manual is divided into three well-defined parts. The first deals with the use of the Jackson candle turbidimeter, the recognized basic instrument for the determination. The second section describes the use of bottle standards. Although the greatest application of bottle standards is in the range from 2 to 25 units, some laboratories prepare and rely on bottle standards up to 50 and 100 units for routine operational control. The usual practice in such cases is to prepare standards at intervals of 5 units up to 40 units, and thereafter of intervals of 10 units up to 100 units. The final section of the procedure touches briefly on the commercial instruments available for estimating turbidity in the range of 1 to 5 units.

2. Warning

The visual nature of the estimation restricts the lower limit to which turbidity can correctly be determined or interpreted. For example, a reading of 0 or 0.0 turbidity does not mean the complete absence of particles in the water. The cotton plug test or complaints of black turbidity from certain areas of the distribution network may indicate that some particles are escaping with the finished water. Moreover, such materials as activated carbon and corrosion products can yield turbidity readings which differ from those produced by a similar amount of silica or naturally occurring debris in the water. The turbidity estimations should, therefore, be regarded as approximate rather than absolute. The results provide a guide for producing an acceptable water. The turbidity readings also depend on the instrument used for the measurement. Experience shows that modern photoelectric devices often give different readings with natural water than the Jackson candle turbidimeter does.

The turbidity estimate should be made in a reasonable time to avoid changes in the suspended particles themselves. Sometimes an immediate determination is advisable when the particles consist mainly of coagulant floc. The estimation of samples containing unreactive mineral particles, on the other hand, may safely be delayed for a week or longer.

All bottles and glassware used for the turbidity estimations should be kept in a scrupulously clean condition, both inside and out. Scratched or etched glassware should be discarded.

The latest edition of *Standard Methods* should be consulted for additional information on turbidity determination.

3. Apparatus

3.1. Sufficient (up to a dozen or more) 1 l reagent-type bottles of clear glass, plain, narrow mouth, with glass stopper. Reserve these bottles for the collection and estimation of samples, and for the storage of all standard turbidity suspensions.

3.2. A Jackson candle turbidimeter, including stand, calibrated glass tube, and special beeswax-spermaceti candle. (See Fig. 9a.)

3.3. A 1,000 ml graduated cylinder for preparing sample dilutions and dilute turbidity standards.

3.4. Measuring pipets or graduated cylinders of appropriate capacity for preparing bottle standards.

3.5. Light source for estimating turbidity by the bottle standard procedure des-

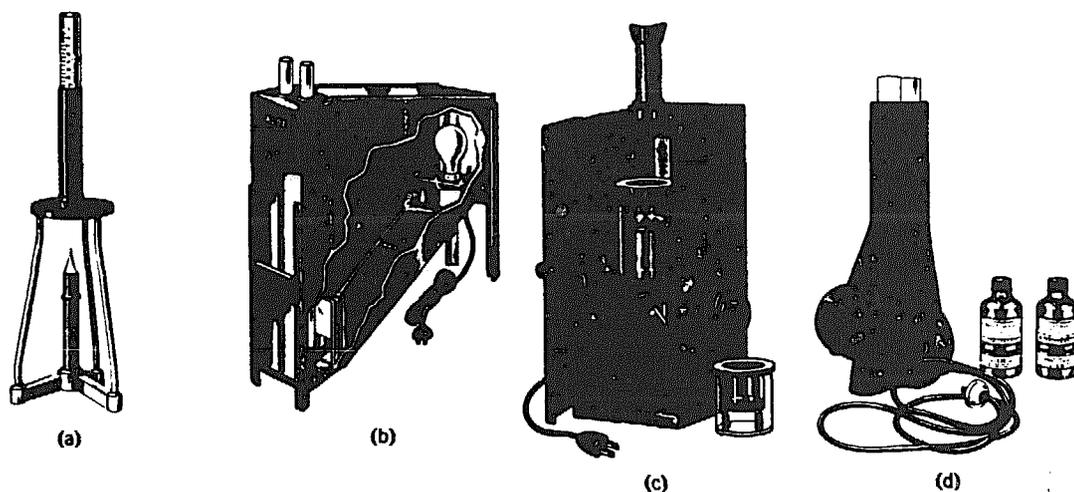


Fig. 9. Turbidimeters

Key: (a) Jackson candle type; (b) Baylis type; (c) Hellige type; (d) St. Louis type.

cribed in Sec. 7. Arrange the light source so that no rays reach the eye directly but at the same time equally illuminate the sample and the bottle standards for the proper estimation of the turbidity. A suitable arrangement is a shielded fluorescent light located below a platform which has an opening down the entire middle of its length to admit the light rays.

3.6. An instrument for the determination of low-level turbidity (below 5 units). Among the instruments widely used for the purpose are the Baylis turbidimeter, the Hellige turbidimeter, and the St. Louis turbidimeter.

4. Stock Suspension for Bottle Standards

4.1. For an improved visual match between the sample and the turbidity standards, prepare the stock suspension from a highly turbid raw water of the same origin as the sample, or from a concentrated suspension made up of the turbidity normally present in the raw water. Determine the turbidity of these stock suspensions by the Jackson candle turbidimeter as described in Sec. 6.

4.2. When a highly turbid water or a concentrated suspension is unavailable or difficult to prepare, purchase a standard 1,000-unit turbidity suspension from a reputable supply house.

4.3. Use either of the above stock suspensions to prepare the weaker turbidity standards needed in the laboratory. Since all turbidity standards deteriorate, prepare the standards weekly or even twice a week if overuse breaks down the particles to smaller sizes. Shake each stock suspension before use.

5. Preliminary Handling of Sample

5.1. Warm a very cold sample to room temperature to avoid the formation of

dew on the outside of the bottle while the turbidity estimation is being made.

5.2. Remove all annoying supersaturated air bubbles from the sample by alternate shaking and standing of the bottle.

6. Procedure Using Jackson Candle Turbidimeter

6.1. Estimation of turbidity between 25 and 1,000 units:

(a) Place the Jackson candle turbidimeter in a shaded area free from drafts so that minimum flickering of the candle flame will occur during the following estimations.

(b) Adjust the candle to its full height in the holder and break off that length of the charred wick which can be easily removed with the fingers.

(c) Shake the sample vigorously and add 1 to 5 ml to the calibrated glass tube of the turbidimeter.

(d) Light the candle. Allow the candle to burn only a few minutes at a time because the flame has a tendency to increase in size after a while.

(e) Shake the sample again. Look down through the calibrated glass tube at the flame of the candle while adding small portions of the sample to the glass tube. Stop the addition when the image of the candle flame can no longer be distinguished. The flame outline gradually fades as the sample is added to the tube, and then is replaced by diffused light. From the scale on the graduated tube, read the turbidity at which the flame just disappears. Record this preliminary reading.

(f) Remove the sample from the tube and rinse the tube with distilled water or clear (0.0 turbidity) tap water.

(g) Shake the sample once more. Add sufficient sample to the tube to approximate 80 per cent of the preliminary reading obtained in Step 6.1(e). Then add small portions until the flame disappears again. Record this reading.

(h) Repeat Steps 6.1(f) and 6.1(g) two more times, and record the reading in each case.

(i) Record the average of the three readings obtained in Steps 6.1(g) and 6.1(h). (See Sec. 9, Reporting of Results.)

6.2. Estimation of turbidity in excess of 1,000 units:

(a) Dilute the sample with clear tap water as follows, so that the resulting turbidity readings will fall in the range of 300 to 700 units, and preferably near 500 units:

(1) Shake the sample vigorously and measure into a 1,000 ml graduated cylinder the appropriate sample volume for the indicated turbidity range:

| Sample Volume <i>ml</i> | Turbidity Range (units) |
|----------------------------|----------------------------|
| 500 | 1,000- 1,200 |
| 250 | 1,300- 2,000 |
| 100 | 3,000- 5,000 |
| 50 | 6,000-10,000 |

(2) Quickly dilute to the 1,000 ml mark with clear (0.0-turbidity) tap water.

(3) Immediately transfer the diluted sample to a 1 l glass-stoppered bottle. Swirl the contents of the graduate during the transfer operation in order to remove as much of the turbidity and sediment as possible.

(b) Shake the diluted sample vigorously and follow the complete procedure in Sec. 6.1.

(c) Calculate the turbidity by multiplying the result found in Step 6.2(b) by the appropriate factor:

| Sample Volume <i>ml</i> | Multiply Turbidity Units by: |
|----------------------------|------------------------------|
| 500 | 2 |
| 250 | 4 |
| 100 | 10 |
| 50 | 20 |

7. Procedure Using Bottle Standards

7.1. Preparation of bottle standards:

(a) Estimate the turbidity of the well-shaken stock suspension (4.1 or 4.2) by the Jackson candle turbidimeter procedure (Sec. 6).

(b) Calculate the volume of stock suspension needed to prepare 1 l of bottle standard as follows: Look in Column A below for the turbidity standard desired. Divide the corresponding figure in Column B by the turbidity of the stock suspension; the result is the number of milliliters of stock suspension required.

| Column A | Column B |
|----------|----------|
| 2 | 2,000 |
| 5 | 5,000 |
| 10 | 10,000 |
| 15 | 15,000 |
| 20 | 20,000 |
| 25 | 25,000 |

Example: To prepare a bottle standard with a turbidity of 2 from a stock suspension with a turbidity of 300, divide 2,000 by 300:

$$2,000/300 = 6.7 \text{ ml of stock suspension.}$$

(c) After shaking the stock suspension vigorously, measure the required volume with a measuring pipet or graduated cylinder.

(d) Immediately transfer the measured stock suspension to a clean 1 l bottle. Select bottles which are the same size, shape, and type as the bottle that will be used for the collection and estimation of the sample.

(e) With a 1,000 ml graduated cylinder, measure the proper volume of distilled water or clear (0.0-turbidity) tap water to bring the total volume to 1,000 ml in each bottle standard.

(f) Weigh 1 g portions of mercuric chloride (HgCl_2) and add to each 1 l bottle standard as a preservative. Shake to dissolve the crystals.

(g) Label each bottle standard with its turbidity value and the date of preparation.

(h) If necessary, pour out a little (5 to 10 ml) of the well-shaken standard from the bottle to allow enough room for thorough mixing of the contents.

7.2. Shake the sample and each bottle standard thoroughly and compare the turbidity by looking horizontally through the bottles at a ruled or printed sheet of paper. Observe the distinctness with which the ruled lines or print is visible through the turbidity of the sample and each standard. Arrange the source of light so that no rays reach the eye directly but at the same time equally illuminate the sample and standards.

7.3. Match the sample with the most nearly comparable turbidity standard. (See Sec. 9, Reporting of Results.)

8. Estimation of Low-Level Turbidity

A number of commercial instruments are suitable for the determination of low-level turbidity (below 5 units) in water. Among these are the Baylis turbidimeter, the Hellige turbidimeter, and the St. Louis turbidimeter. These instruments (Fig. 9) can be purchased from reputable supply houses. The directions supplied by the manufacturer of each instrument should be carefully followed.

9. Reporting of Results

Report the final result of a turbidity determination in accordance with the table below:

| Turbidity as Determined | Report to Nearest: |
|----------------------------|-----------------------|
| 1.0 or less | 0.1 |
| 1-10 | 1 |
| 10-100 | 5 |
| 100-400 | 10 |
| 400-700 | 50 |
| over 700 | 100 |

Also identify the instrument or method used in the determination.

II. Bacteriologic Examination

Introduction

Improperly treated or unprotected water may contain microorganisms that are pathogenic (capable of causing disease). Coliform bacteria, while not themselves disease producers, are often associated with pathogenic organisms and are a good index of the degree of bacteriologic safety of a water. Coliform bacteria occur normally in the intestines of humans and other warm-blooded animals and are discharged in great numbers in human and animal wastes. In polluted water, coliform bacteria are found in densities roughly proportional to the degree of fecal pollution. When members of the coliform group are present, other kinds of microorganisms capable of causing disease also may be present.

Coliform bacteria are more hardy than disease-causing bacteria; therefore, their absence from water is an indication that the water is bacteriologically safe for human consumption. The presence of coliform bacteria, on the other hand, is an indication that disease-causing bacteria also may be present and that the water is unsafe to drink.

The coliform group includes all aerobic and facultatively anaerobic, Gram-negative, nonspore-forming, rod-shaped bacteria which ferment lactose with gas production in prescribed culture media within 48 hours at 35° C. The bacteriologic procedures described in this Manual are designed to show the presence and number of bacteria meeting the definition of the coliform group.

The bacteriologic quality of water supplies used in interstate carriers, and of other water supplies subject to federal control, is governed by the Interstate Quarantine Regulations, which are based on the US Public Health Service "Drinking Water Standards." These regulations stipulate that members of the coliform group are the official indicators of the bacteriologic quality of water. The regulations further state that the procedures used for the detection and enumeration of coliform bacteria must be in accordance with the latest edition of *Standard Methods for the Examination of Water and Wastewater*. Most states, either by law or by regulation, require similar adherence to the provisions of *Standard Methods*.

Routine samples for bacteriologic examination should be collected from representative points on the distribution system. The number of samples examined per

month should be based on the total population served by the water supply. The minimum number of samples per month, based on the population served, is graphically presented in Fig. 10. When the tested water fails to meet bacteriologic standards, daily samples from the same sampling point are required until a satisfactory quality is shown on at least two consecutive days.

The frequency with which samples are collected from the source of supply and from the various stages of the water treatment process will depend on operating conditions.

Two methods are accepted for bacteriologic examination of water; the membrane filter method and the multiple-tube fermentation method.

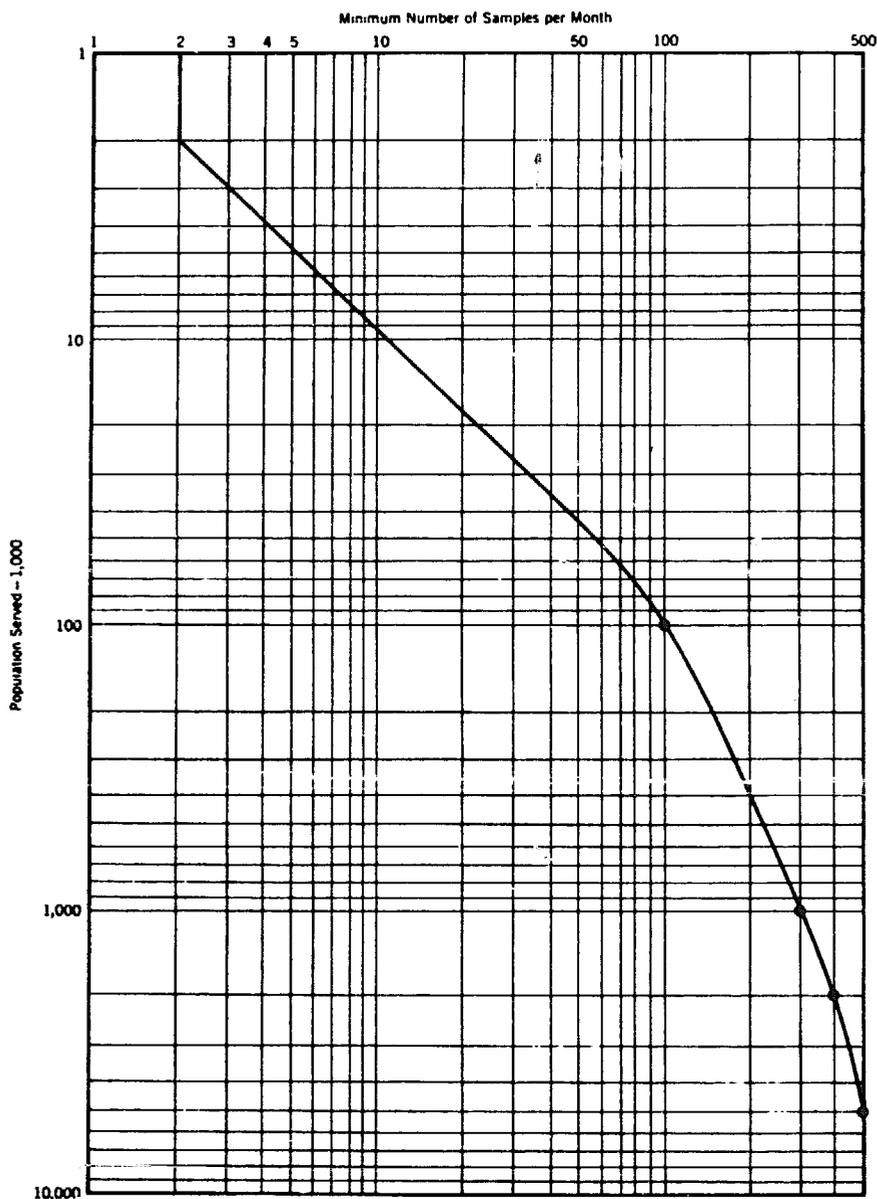


Fig. 10. Minimum Number of Samples Based on Population Served

A. Membrane Filter Method

1. General

A measured volume of water sample is filtered under a vacuum through a membrane filter. The filter is then placed in a sterile container and incubated in contact with a selective, differential culture medium. A colony of coliform bacteria develops at each point on the filter where viable coliform bacteria were collected during filtration. The coliform bacteria colonies are counted, and a simple computation is made to determine the number of coliform colonies per 100 ml of sample.

In tests made to determine the bacteriologic safety of finished water, the standard sample volume should be at least 50 ml – preferably 100 ml – if it is to meet the requirements of the US Public Health Service Drinking Water Standards. When the purpose of the test is quantitative rather than qualitative - for example, when it is desired to know the number of coliform bacteria in a raw water supply or in water undergoing treatment - smaller sample volume may be adequate. For reliable coliform counts, a volume should be selected which will yield 20 to 80 coliform colonies on the membrane filter. Not more than 200 colonies of all types (coliform and noncoliform) should develop on the filter used for counting.

Suggested sample filtration volumes to correspond with expected coliform bacteria counts follow:

| Coliform Colonies per 100 ml | Filtration Volume ml |
|---------------------------------|----------------------------|
| 1 to 80 | 100 |
| 81 to 320 | 25 |
| 321 to 1,300 | 6 |
| 1,001 to 4,000 | 2 |
| 4,001 to 16,000 | 0.5 |

For higher coliform bacteria counts, smaller filtration volumes should be used.

If fluctuations in coliform bacteria density are expected, it may be necessary to filter two or more sample volumes to obtain reliable results. For example, if the coliform bacteria count is expected to fall between 300 and 16,000 per 100 ml, it is better to filter one sample each of 6, 2, and 0.5 ml.

2. Warning

This Manual presents an abridged version of the membrane filter method given in *Standard Methods*. Since *Standard Methods* allows a considerable range of personal choice in equipment, culture media, and testing procedures, the latest edition should be consulted for a complete account of all the permissible options.

3. Apparatus

3.1. Sterilization equipment:

- (a) A sterilizing oven. The oven should be large enough to permit the loose

packing of such items as pipets, petri dishes, test tubes, sample bottles, and other glassware and dry apparatus which must be sterilized by the dry-heat process. Free circulation of heated air around the equipment is essential; therefore, overcrowding must be avoided. If necessary, sterilize in more than one load. The oven should be provided with a temperature regulator and a thermometer, and should operate normally at a temperature of 170° C. Required sterilization time for most glassware is 1 hour at 170° C.

(b) An autoclave. The autoclave should be large enough to permit the free flow of live steam around the normal load to be sterilized by this process. It should be equipped with a pressure gage and a thermometer, with the thermometer bulb properly located in the exhaust line. Operation of the autoclave should be in strict accord with the manufacturer's instructions and in such a way as to replace all the air in the chamber with steam. Sterilizing temperatures should be reached within 30 minutes.

3.2. An incubator, equipped with a temperature control and so designed that the temperature in all usable parts is 35° C \pm 0.5° C. The chamber should be large enough to permit the free flow of air around all cultures when the largest expected work load is being incubated. To check the temperature in a large incubator one or more thermometers should be placed at representative locations in the chamber, and periodic (preferably daily) records of the temperature should be kept. Saturated or near-saturated humidity should be maintained in the incubation chamber. If the incubator is not equipped with a working automatic humidifier, a shallow tray of water should be kept in the chamber at all times.

3.3. A balance, for weighing the powdered culture media and chemicals for solutions. Most weighings are in amounts ranging from 1 to 100. The balance should weigh with an accuracy of \pm 2 g at a 150 g load.

3.4. Water distillation or deionizing apparatus. This should produce a nontoxic water, free from substances which prevent or otherwise interfere with the growth of bacteria.

3.5. Sample bottles, large enough to contain at least 100 ml of sample plus sufficient volume to permit thorough mixing when the sample is shaken.* The bottle caps should fit tightly. If nonglass caps are used, they must be demonstrated to be free of soluble substances which can interfere with bacterial growth. A small amount (20-50 mg) of sodium thiosulfate in the form of solution or powder should be added to each clean sample bottle intended for the collection of a water sample containing residual chlorine. Sample bottles closed by a stopper inserted into the neck require special protection against external contamination while the sample is being collected. A piece of paper should be fastened over the top of the stopper and tied around the neck of the bottle just prior to sterilization (see Fig. 11c). The bottle should then be sterilized in the sterilizing oven.

3.6. Dilution bottles, to hold 99 \pm 2 ml of dilution water with sufficient volume left for good mixing when shaken.* Screw-capped bottles with caps free of soluble toxic substances are excellent for this purpose (Fig. 11b).

*All glassware must be absolutely clean. Consult the introduction to this Manual on the cleaning of glassware (see p. 8.) Borosilicate glassware, sold under the trade names of "Pyrex" or "Kimax," is best for bacteriologic work because of its relative chemical inertness and thermal stability.

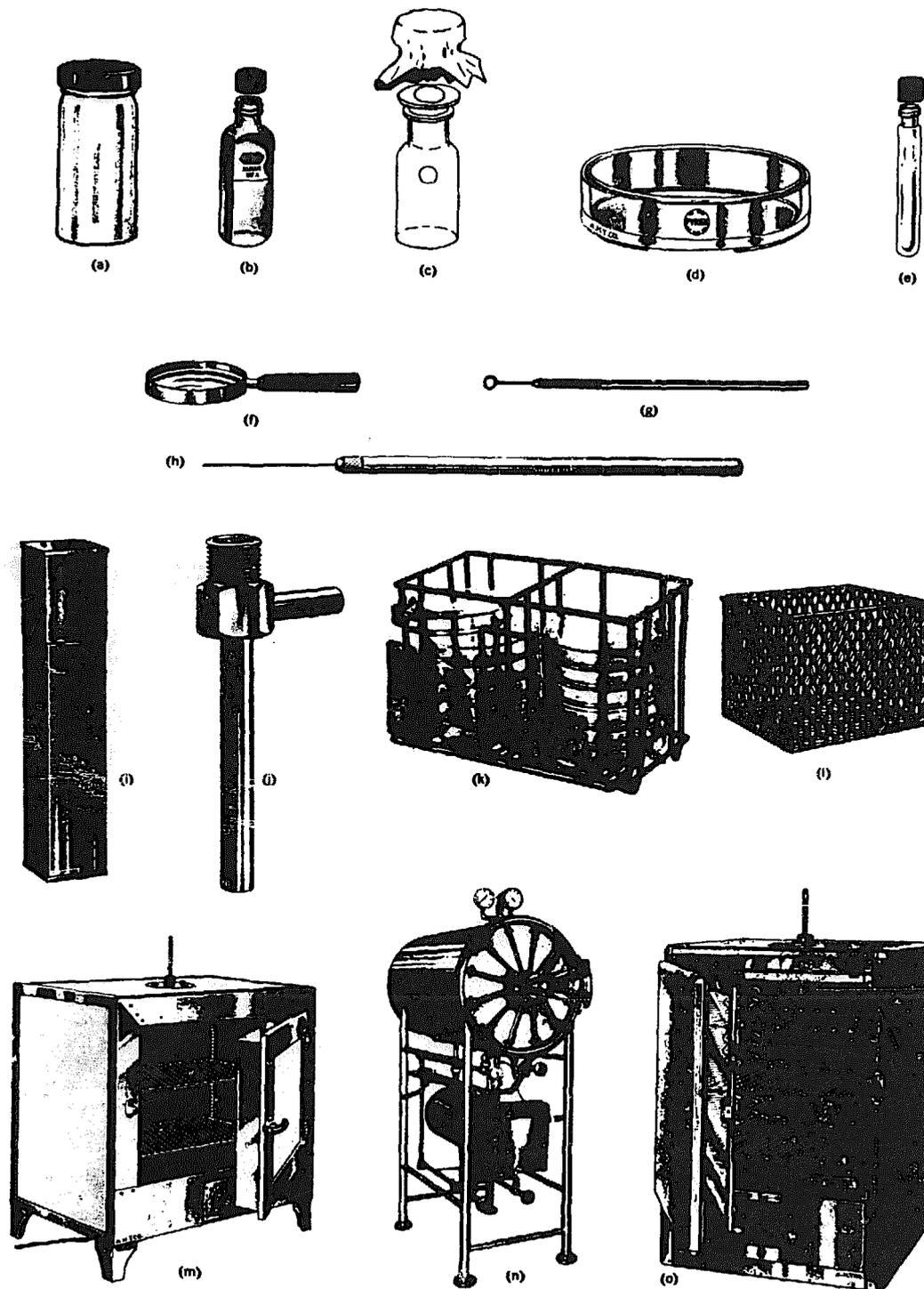


Fig. 11. Bacteriology Apparatus

Key: (a) sample bottle with screw cap; (b) dilution water bottle; (c) glass-stoppered sample bottle with protective covering; (d) petri dish; (e) test tube with screw cap; (f) magnifier; (g) inoculation loop; (h) inoculation needle; (i) pipet box; (j) hydroaspirator (filter pump); (k) petri dish holder; (l) test tube basket; (m) hot-air sterilizer; (n) autoclave; (o) incubator.

3.7. Measuring pipets, 1 ml and 10 ml.* The 1 ml pipet should be graduated in 0.1 ml increments and the 10 ml pipet at least in 0.1 ml increments. Pipets with chipped or cracked tips should be discarded at once. Many workers insert a small plug of cotton just inside the mouthpiece of the pipet. Pipets are conveniently stored in sterilized metal containers specially made for this purpose. A separate container should be used for each size of pipet. Pipets can be wrapped individually in paper and sterilized with heat.

3.8. Media preparation equipment. Glass or stainless metal containers, heating equipment, and stirrers used in the preparation of media should be clean and free of soluble toxic materials.*

3.9. A gas burner, bunsen or similar type.

3.10. Filtration equipment:

(a) A water aspirator, electric vacuum pump, or any convenient means of producing at least one-half atmosphere differential pressure.

(b) A suction flask. A 1 l thick-walled side-arm erlenmeyer flask is suitable. It is connected to the vacuum facility (3.10a) with rubber tubing of sufficient wall thickness to avoid collapse when the vacuum is turned on. Latex tubing with 3/16 in. bore and 3/32 in. wall thickness is excellent. With this type of tubing, the vacuum can remain turned on and can be interrupted by a pinch clamp when needed.

(c) A filter-holding unit. This unit consists of two parts. The lower or basal part, which is mounted in the suction flask with a No.8 rubber stopper, supports the membrane filter in the assembled unit. Its porous surface permits the free passage of filtered water into the suction flask during operation. The upper part, which is clamped or locked to the basal portion during operation, is somewhat funnel shaped to direct the water being filtered onto the proper area of the membrane filter. The filter-holding unit can be constructed of metal or of glass in any of several designs (Fig. 12). If of metal, copper should not be a component of any part exposed to the samples.

The two parts of the filter-holding unit are wrapped separately in paper and sterilized in the autoclave for at least 15 minutes at 121°C. No unit having rubber or plastic parts should be placed in the sterilizing oven.

A ring stand with a split ring provides a convenient support for the upper part of the filter holding unit when it is disassembled (Fig. 12).

3.11. Glass or plastic petri dishes, 60 x 15 mm, to contain cultures. Plastic dishes ordinarily are intended for single use and are discarded without reesterilization. Glass dishes are intended for repeated use.

3.12. Membrane filters, 47 to 50 mm in diameter with a pore diameter suitable for bacterial tests on water (0.4 - 0.7 micron). Acceptable membrane filters can be wrapped in convenient units, in paper packets, sterilized in the autoclave for 10 minutes at 121°C, and dried by rapid exhaustion of the steam after sterilization.

3.13. Nutrient absorbent pads, consisting of filter-paper disks about 1 mm thick, having the same diameter as the membrane filters with which they are used. The same procedure should be followed for their preparation and sterilization as for the membrane filter (3.12).

3.14. Forceps. Sterile forceps are used for the manipulation of the sterile membrane filters and the sterile absorbent pads for the nutrient. The forceps should

*See footnote p.107

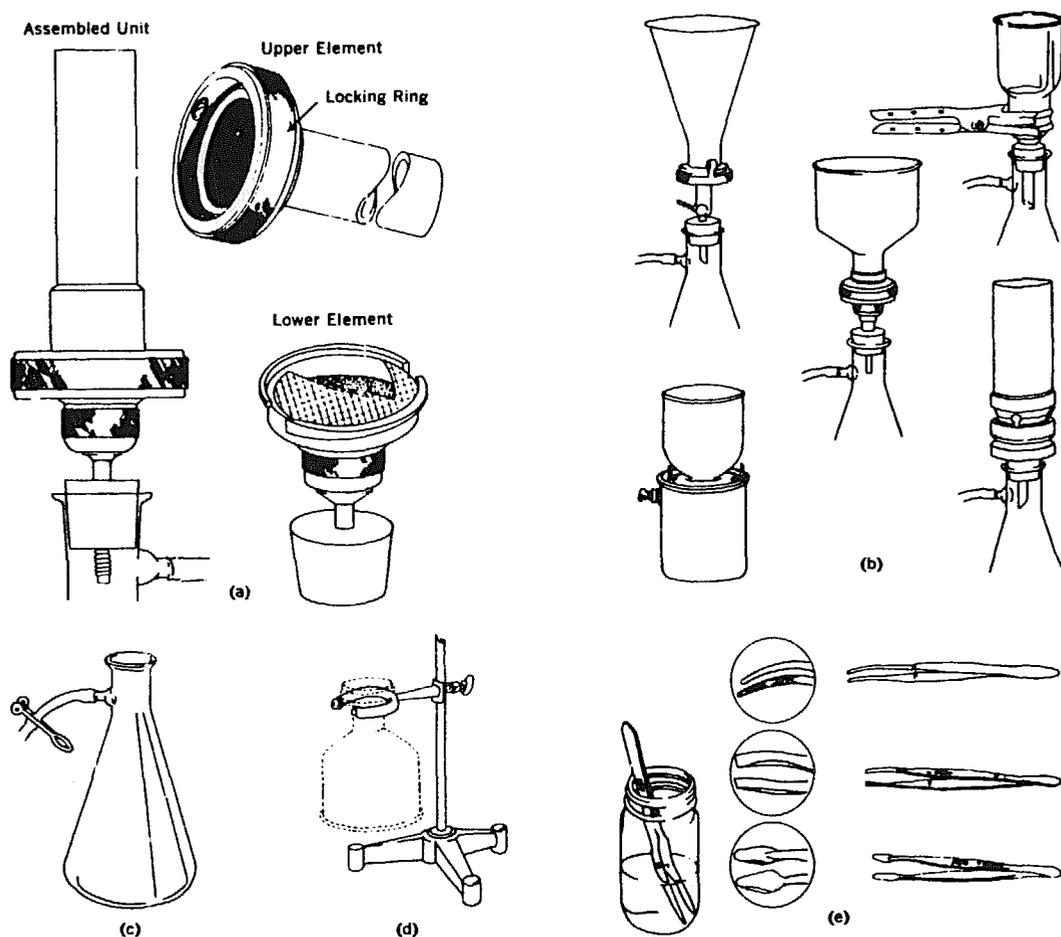


Fig. 12. Membrane Filter Apparatus

Key: (a) elements of filter-holding unit; (b) filter-holding units of various designs; (c) suction flask; (d) ring stand with split ring; (e) suitable types of forceps.

have rounded (not sharp-pointed) tips, conveniently curved at the end (Fig. 12).

3.15. Colony counting apparatus. A simple lens with 4x to 5x magnification can be used to examine and count the number of coliform colonies. A wide-field binocular dissecting microscope gives better results. A fluorescent lamp with two 4-watt bulbs in a reflecting housing is recommended as a light source. It has the advantage of high illumination and low heat, permitting examination of the results close to the source of light.

4. Solutions and Culture Media

4.1. Buffered dilution water. Use sterile buffered dilution water to prepare sample dilutions prior to inoculation into culture medium, and flush the filtration apparatus following sample planting. Prepare the working solution of buffered dilution water from a concentrated stock solution of phosphate buffer, as follows:

(a) Stock solution. Dissolve 34.0 g potassium dihydrogen phosphate (also called potassium monobasic phosphate), KH_2PO_4 , in 500 ml distilled water. Check the pH of the phosphate solution by the method described on page 54. If necessary, add small amounts of 1N sodium hydroxide solution until the pH of the phos-

phate solution reaches 7.2. (Prepare the sodium hydroxide solution by dissolving 4.0 g NaOH in 100 ml distilled water.) Add sufficient distilled water after the pH adjustment to bring the total stock solution volume to 1 l. When not in use, store in a tightly closed container at 4 - 10° C to avoid changes in concentration through evaporation. If evidence of mold or other growth appears on standing, discard and prepare a new stock solution.

(b) Working solution. Add 1.25 ml stock solution to 1 l distilled water, and dispense in dilution water bottles in amounts that will provide 99 ± 2 ml after sterilization in the autoclave. Prior to sterilization, loosely cap the bottles of dilution water. Sterilize the buffered dilution water in the autoclave for 20 minutes at 121° C. After sterilization, tighten the caps and store the dilution water in a clean place until needed.

4.2. M-Endo broth MF medium. Dissolve 2.4 g of the commercially available dehydrated culture medium (Difco 0749-02, Difco Laboratories, Detroit, Mich.; or BBL 01-494, Baltimore Biological Laboratories, Baltimore, Md.) in 50 ml distilled water; then add 1 ml of 95 per cent ethyl alcohol. Sterilize by heating gently just to the boiling point. Do not boil vigorously and do not sterilize the medium in the autoclave. The pH of the finished medium should be between 7.1 and 7.3. The medium can be stored as long as 4 days in the refrigerator at 4-10° C. Use 50 ml of the medium for approximately 25 filtrations.

5. Sampling Procedure

Read carefully the section of sampling in the Introduction to this Manual (page 13) before proceeding. For a more detailed discussion of this important matter consult the latest edition of *Standard Methods*.

5.1. Collect waters having residual chlorine in sample bottles containing sodium thiosulfate in order to dechlorinate the sample. Failure to dechlorinate makes it impossible to interpret results because the chlorine continues to kill bacteria while in the sample bottle. Thus, the laboratory tests cannot measure the quality of the water supply at the time and place of sampling, but can test only the condition of the sample at the time the test is started.

5.2. Keep the sample bottle closed until the moment of collecting the sample. When opening the bottle, avoid touching any part of the bottle which will come in direct contact with the water sample. If a paper strip has been inserted between the stopper and the neck of the bottle, discard it before taking the sample.

5.3. To avoid loss of the sodium thiosulfate in the sample bottle, do not rinse the bottle with the water being collected.

5.4. When collecting the sample, take at least 100 ml but do not fill the bottle more than four-fifths full.

5.5. When collecting a sample from a tap or a pump, allow the water to run freely for 4 - 5 minutes before inserting the bottle into the water stream. If the sample is being taken from a source of supply, such as a river, lake, or impoundment, make the collection as close as possible to the point of intake into the treatment plant. Handle the sample bottle in such a manner as to collect a sample which is truly representative of the source. The sample must not have had any contact with the person, the clothing, or any object used to assist the collector in approaching the sample site.

5.6. Immediately record the temperature of the sample source, and maintain

the sample as nearly as possible at this temperature. Transport the sample to the laboratory as quickly as possible.

5.7. If possible, start the laboratory testing procedures within 1 hour after sample collection. To be valid, the test must be started within 24 hours after sample collection.

5.8. Recording and identification of samples:

(a) Enter all pertinent sample information on the laboratory record form as soon as the sample arrives in the laboratory. (Either a bound book or loose-leaf notebook containing printed forms is suitable; requirements of state or other regulatory authorities having jurisdiction should be complied with.)

(b) Record the following initial information about the sample: the assigned laboratory number, the source of the sample, the date and time of collection, the temperature of the sample source, the name of the sample collector, and the date and time the sample was received in the laboratory.

(c) Record the following test information: the date and time laboratory operations were started, the sample volumes used for the tests, and the name of the laboratory worker making the test. The laboratory form should also contain space for recording all the results which will be obtained during all stages of the laboratory operations, a summary of the results, and quantitative determinations.

6. Laboratory Procedure

6.1. Clean the laboratory bench surface with water, or preferably, with a suitable disinfectant solution. Allow the surface to dry before proceeding.

6.2. Place in a row, or series of rows, all the sterile culture containers to be used in the sample filtrations.

6.3. Label the culture containers to correspond with the sample number shown on the data sheet.

6.4. Open all the culture containers; invert the tops of the containers and place them next to the lower portions. Place one sterile absorbent pad in the lower half of each culture container. Use sterile forceps for all manipulations of the absorbent pads.

6.5. Using a sterile pipet, deliver enough prepared M-Endo Broth MF to saturate each absorbent pad. The amount of culture medium required for each absorbent pad is approximately 2 ml, but cannot be stated precisely. Apply sufficient medium so that, when the culture container is tipped, a good-sized drop of the medium freely drains out of the absorbent pad. Replace the covers of the containers.

6.6. Arrange the following supplies and equipment on the laboratory bench for convenient sample filtration:

(a) Filter-holding unit (sterile at the beginning of the filtration series), with suction flask connected by rubber tubing to the vacuum facility. (Ring stand with split ring to hold the upper part of the filter-holding unit when disassembled is optional.)

(b) Labeled culture containers with absorbent pads saturated with culture medium.

(c) Sterile membrane filters.

(d) An alcohol jar with forceps.

(e) A gas burner.

- (f) A graduated cylinder and pipets for sample measurement.
- (g) Dilution water, sterile, in 99 ml amounts.

6.7. Place a sterile membrane filter, grid side up, on the basal part of the filter-holding unit, centered over the porous part of the membrane support plate. Membrane filters are easily damaged. For manipulation, use sterile forceps and *always* grasp the filter disk at the part outside the portion of the filter through which the sample is to be passed. To keep the forceps sterile, *always* store the working tips immersed in about 1 inch of ethyl alcohol or methyl alcohol. Burn off the alcohol when ready to use the forceps. Do not hold the forceps in the flame longer than is necessary to set fire to the alcohol.

6.8. Assemble the filtration unit, clamping the upper (funnel) portion to the basal part. Use care to avoid damage to the membrane filter in the filtration unit.

6.9. Shake the sample bottle vigorously, approximately 25 times, using an up-and-down motion.

6.10. With the vacuum cut off, deliver the measured test sample into the funnel portion of the filtration unit. If the test sample is less than 10 ml, it should be preceded by approximately (do not measure) 10 ml of sterile dilution water. If the sample is 10 ml or more, no dilution water need be poured into the filtration assembly ahead of the sample.

6.11. Turn on the vacuum to hasten filtration of the sample through the membrane. After the sample has passed through the membrane filter, turn off the vacuum.

6.12. Rinse the funnel with 20 to 30 ml of sterile dilution water. Repeat the rinsing after all of the first rinse has passed through the filter.

6.13. Disassemble the filtration assembly. Use a sterile forceps to remove the membrane filter from the base of the filter holder. Carefully place the filter, grid side up, on the absorbent pad in the appropriate culture container (Fig. 13). In-



Fig. 13. Placement of Membrane on Pad Soaked With Culture Medium

spect the filter for evidence of air bubbles between the absorbent pad and the filter. If necessary, reposition the filter on the absorbent pad. Since air bubbles interfere with the diffusion of culture medium from the absorbent pad through the membrane filter, reduce the entrapment of bubbles by having enough culture medium on the absorbent pad for nutrient, and by rolling the membrane filter into proper position on the absorbent pad.

6.14. After the completion of each filtration, proceed to the next filtration in the series without reesterilization of the filtration unit. The unit requires reesterilization only after completion of all filtrations of a consecutive series. If more than 15 minutes elapse between the filtration of successive samples, reesterilize the unit by immersing it in boiling water for 2 minutes and cooling before further filtration.

6.15. Upon completion of the filtrations, invert the tightly closed culture containers and place them in a 35° C incubator, in an atmosphere with saturated humidity, for 18 - 22 hours. If the entire incubator does not have saturated humidity, place the cultures in a tightly closed container with wet paper towels or other moist material.

6.16. After incubation, remove the cultures and count the coliform bacteria colonies as follows: Use a wide-field dissecting microscope or (less desirably) a simple lens. Position a light with a large surface close to the bacteria colonies. Adjust it so that the light is reflected directly off the colony surfaces into the microscope or lens.

Coliform bacteria colonies are red or pink, with a green-gold or metallic surface sheen. This sheen may cover the entire colony, or may appear only in the center of the colony. Noncoliform bacteria colonies range from colorless to pink or red, but do not have the characteristic surface sheen.

6.17. Record the coliform bacteria colony counts on the data sheets.

7. Interpretation of Results

7.1. In answering the question, "Is a water bacteriologically safe to drink?" the limits established in the US Public Health Service Drinking Water Standards are a good guide. These limits are based on the use of a standard sample with a minimum volume of 50 ml (100 ml is preferred). The Standards state that, when the membrane filter technique is used, the average (that is, the arithmetic mean) coliform bacteria density of all standard samples examined per month must not exceed 1 colony per 100 ml. Further, with regard to individual standard samples, the number of colonies must not exceed 3 per 50 ml (or 4 per 100 ml, 7 per 200 ml, or 13 per 500 ml):

- (a) In two consecutive samples; or
- (b) In more than one sample if less than 20 samples are examined per month; or
- (c) In more than 5 per cent of the samples if 20 or more samples are examined per month.

When coliform bacteria colonies in a single standard sample exceed the above values, daily samples from the same sampling point must be collected promptly and examined until the results obtained from at least two consecutive samples show the water to be of satisfactory quality.

7.2. When a quantitative rather than a qualitative interpretation is desired, the following calculations can be used to determine the number of coliform colonies:

(a) If more than one sample volume was initially filtered, select the one producing between 20 and 80 coliform bacteria colonies but not more than 200 colonies of all types. Divide the number of colonies by the number of milliliters of sample filtered and multiply the result by 100. This will give the number of colonies per 100 ml of sample. Round out the answer to two significant figures.

Example: Assume that 34 coliform bacteria colonies were counted on a membrane filter through which 25 ml of sample had been filtered; then $34 \times 100 \div 25 = 136$. So the number of coliform bacteria colonies per 100 ml (to two significant figures) is 140.

(b) If none of a series of sample volumes produces between 20 and 80 coliform bacteria colonies on a filter, select the filter most nearly meeting the requirements, and make the calculation as above. In this case, make a special notation to show that the count was based on a less-than-ideal filtration. Many workers designate such results as "estimated counts."

(c) If two membrane filters of a series of sample filtrations produce between 20 and 80 coliform bacteria colonies, divide the total number of colonies found on both by the total milliliters filtered and multiply by 100.

Example: Assume that 22 coliform bacteria colonies were counted on a filter representing 5 ml of sample, and 75 coliform bacteria colonies were counted from a 15 ml portion. Then:

$$\frac{22 + 75}{5 + 15} \times 100 = \frac{97}{20} \times 100 = \frac{9700}{20} = 485$$

So the number of coliform bacteria colonies per 100 ml (to two significant figures) is 490.

B. Multiple - Tube Fermentation Method

1. General

The multiple-tube fermentation method determines the presence and number of coliform bacteria through the planting of a series of measured sample portions into tubes containing favorable culture media. The test progresses through three distinct stages: the Presumptive Test, the Confirmed Test, and the Completed Test. It is possible to stop the examination of a water sample at the end of any of these stages - provided the purpose of the test has been fulfilled - or the examination may proceed directly from one stage to the following stage. Figure 14 shows the relationship of the three stages. The Confirmed Test and the Completed Test increase the certainty that positive results obtained in the Presumptive Test are, in fact, due to coliform bacteria and not to the activity of other kinds of bacteria.

The Completed Test is the standard test for the determination of the bacteriologic safety of a water in accordance with the US Public Health Service Drinking Water Standards. Under those standards, termination of the examination after the Confirmed Test is permissible, but only if the Confirmed Test has been proved to give results equivalent to the Completed Test by a series of parallel tests. In routine practice, bacteriologic testing of most public water supplies is stopped at the end of

the Confirmed Test. The Confirmed Test also is valuable in testing samples from the source of a water supply and from various parts of a water treatment plant.

The multiple-tube method is based on the laws of probability and is used to obtain an estimate of the number of bacteria in a sample, expressed as the Most Probable Number (MPN). For that reason, it is usually called the MPN method. It requires the initial planting into culture media of one or more measured portions of sample, and then the application of the proper qualitative cultural tests to each portion. For each sample portion, a "yes" (positive) or "no" (negative) answer is sought regarding the presence of coliform bacteria. After the laboratory procedures have been completed, a summary of all the positive and negative results is made and correlated with the initial sample volumes planted. The MPN value is finally determined by referring to a table of Most Probable Numbers rather than by making a separate computation for each sample.

Several different sample inoculation plans are offered in *Standard Methods*. Two are described here (laboratory application of the other plans is usually similar): Plan A involves the initial planting of five 10 ml portions and is recommended for testing water of drinking water quality. For a more sensitive test, this plan can be applied to the planting of five 100 ml portions of sample, preferred in many water treatment plants. Plan B involves the planting of five 10 ml portions, five 1 ml portions, and five 0.1 ml portions and is recommended for testing waters of poorer than drinking water quality. This inoculation plan is used when coliform bacteria are known to be present, and the purpose of the test is to determine the number.

When different test sensitivities are needed, the sample volumes for either of these plans can be varied. For example, the inoculation pattern of Plan B can be modified so that five 1.0 ml, five 0.1 ml, and five 0.01 ml sample portions are planted instead.

Quantitative results can be achieved only when the original sample-planting volumes are selected so that positive results are obtained from some sample portions and negative results are obtained from others in a series of tubes of culture medium planted with measured sample volumes. With five sample portions each of 10 ml, 1 ml, and 0.1 ml, respectively, quantitative results ranging from 2 to 542 coliform bacteria per 100 ml can be obtained. To extend the range to higher coliform bacteria densities, smaller sample fractions must be added to the series. When coliform densities are consistently high, one or more of the larger sample increments may be omitted, as indicated below:

| Expected Coliform Density per 100 ml | Decimal Series (5 Tubes Each) |
|--------------------------------------|-------------------------------|
| 2-542 | 10; 1, 0.1 ml |
| 20-5,420 | 1, 0.1, 0.01 ml |
| 200-54,200 | 0.1, 0.01, 0.001 ml |
| 2,000-542,000 | 0.01, 0.001, 0.0001 ml |

With some sample sources, the apparent ease of selection of one of the above series of three decimal increments is deceptive. Great fluctuations in coliform density may necessitate initial plantings of four or even five decimal increments in

order to secure laboratory results in which some tubes give positive results and some give negative results. Experience with the water being tested will indicate the need for planting more than three sample increments.

2. Warning

This Manual presents an abridged version of the multiple-tube fermentation method given in *Standard Methods*. Since *Standard Methods* allows a considerable range of personal choice in equipment, culture media, and testing procedures, the latest edition should be consulted for a complete account of all the permissible options and their application.

3. Apparatus

3.1. Sterilizing equipment:

(a) A sterilizing oven. See Membrane Filter Method, Sec. 3.1(a).

(b) An autoclave. See Membrane Filter Method, Sec. 3.1(b). The total exposure of the culture media to heat should not exceed 60 minutes, because excessive heat exposure breaks down the lactose in such media as lauryl tryptose broth and brilliant green lactose bile broth.

3.2. An incubator. See Membrane Filter Method, Sec. 3.2.

3.3. A balance. See Membrane Filter Method, Sec. 3.3.

3.4. Water distillation or deionizing apparatus. See Membrane Filter Method, Sec. 3.4.

3.5. Sample bottles. See Membrane Filter Method, Sec. 3.5.

3.6. Dilution bottles. See Membrane Filter Method, Sec. 3.6.

3.7. Measuring pipets. See Membrane Filter Method, Sec. 3.7.

3.8. Media preparation equipment. See Membrane Filter Method, Sec 3.8.

3.9. Culture tubes containing inverted fermentation vials, for use with lauryl tryptose broth and brilliant green lactose bile broth (Fig. 15). Many workers prefer 20 x 150 mm tubes with 10 x 75 mm vials to contain 10 ml portions of culture media. Culture tubes of 25 x 150 mm size are used with the 10 x 75 mm fermentation vials for 20 ml portions of media. It is suggested that all culture tube closures be metal caps; these fit down about 1 inch over the upper end of the tube. These caps should be loose enough for easy removal, but should not be easily shaken off. Screw-capped tubes are not advised for fermentation media.

3.10. Petri dishes, 100 mm in diameter, 15 mm deep. Plastic dishes are acceptable, if known to be free from soluble antibacterial matter.

3.11. Microscope with oil immersion objective lens for examining Gram stain slides.

3.12. Culture tube racks. The openings should be large enough to accept the largest culture tubes used. One of the most versatile types is rectangular in shape (ten openings by five openings) and accommodates 50 culture tubes.

3.13. An inoculation loop and needle made of 3 inch or 4 inch lengths of 24 B & S gage or 26 B & S gage wire. Nichrome wire is acceptable, but platinum-iridium is better. The wire lengths are set in handles of metal or glass about the diameter of a pencil. To form the inoculation loop, the wire is bent in a circle 3 - 4 mm in diameter. For the inoculation needle, the wire may be straight, or the end 1/8-inch portion may be bent about 10 deg.

3.14. A gas burner, bunsen or similar type.

4. Culture Media and Solutions

Commercially available dehydrated media simplify the preparation of culture media and are, therefore, advised for laboratory work. Difco Laboratories (Detroit, Mich.), Baltimore Biological Laboratories (Baltimore, Md.), and other suppliers produce such media in a powder form which can be easily weighed, dissolved in distilled water, and distributed into tubes or other culture containers prior to sterilization.

4.1. Buffered dilution water. See Membrane Filter Method, Sec. 4.1.

4.2. Lauryl tryptose broth, for Presumptive and Completed Tests. Use this broth as the primary planting medium for the Presumptive Test and to determine the fermentation of pure culture isolates in the Completed Test. Prepare the medium as follows:

(a) Dissolve a quantity of dehydrated culture medium in distilled water so as to produce a broth of the strength appropriate for the amount of sample being planted. For example, to produce single-strength (1x) broth, dissolve 35.6 g of dehydrated medium per liter of distilled water. The following table is useful:

| Volume of Sample per Tube <i>ml</i> | Strength of Medium | Amount of Dehydrated Medium per Liter <i>g</i> | Volume of Medium per Tube <i>ml</i> | Tube Size <i>mm</i> |
|--|--------------------|---|--|------------------------|
| 1 | 1x | 35.6 | 10 | 20x150 |
| 10 | 1.5x | 53.4 | 20 | 25x150 |
| 100 | 4x | 142.4 | 35 | dilution bottle |

(b) After dissolving the weighed medium, dispense the predetermined volumes into culture tubes with inverted fermentation vials, place the caps on the culture containers, and sterilize in the autoclave for 15 minutes at 121°C.

(c) Store the sterilized culture medium at room temperature (approximately 25°C) for periods up to a maximum of one week. Prolonged storage at room temperature will result in changes in concentration through evaporation. Storage in the refrigerator is usually undesirable because of the likelihood of air entering the medium. Such dissolved air is released during the incubation, resulting in false positives.

4.3. Brilliant green lactose bile broth, for Confirmed Test:

(a) Dissolve 40 g of the commercially available dehydrated medium per liter of distilled water, and dispense in approximately 10 ml portions into culture tubes with inverted fermentation vials. Sterilize for 15 minutes in the autoclave at 121°C. If possible, check the medium after sterilization with a pH meter to see that the pH is between 7.1 and 7.4.

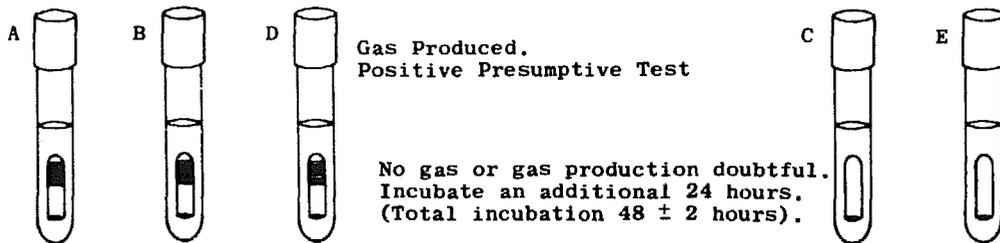
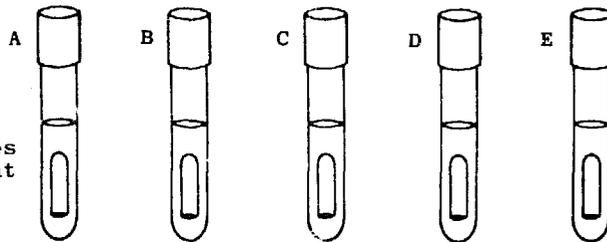
(b) Follow the storage requirements given for lauryl tryptose broth, Sec. 4.2

(c). In addition, protect the medium from light during storage to avoid undesirable changes in the dye component.

4.4. Eosin methylene blue agar, for Completed Test. Use this medium for the isolation of pure cultures as a preliminary stage in the Completed Test. Prepare as

A. PRESUMPTIVE TEST

Inoculate 10 ml of water sample to each of 5 Lauryl Tryptose Broth culture tubes and incubate 24 ± 2 hours at $35 \pm 0.5^\circ\text{C}$.



B. CONFIRMED TEST - Transfer a loopful of inoculum to:

Brilliant Green Lactose Bile Broth.
Incubate 48 ± 2 hours at $35 \pm 0.5^\circ\text{C}$

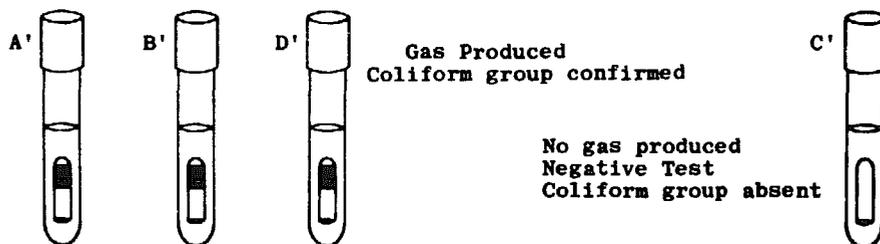
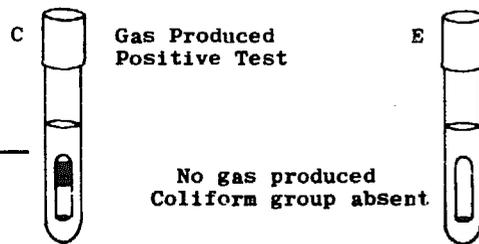
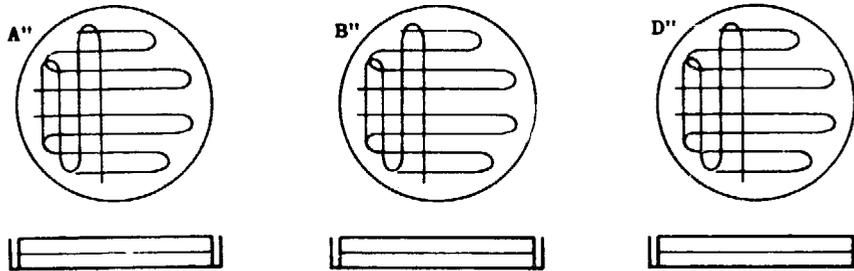


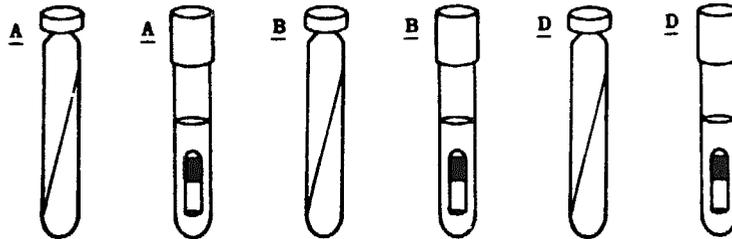
Fig. 14 (A). Schematic Diagram for Presumptive and Confirmed Tests

II. Bacteriologic Examination

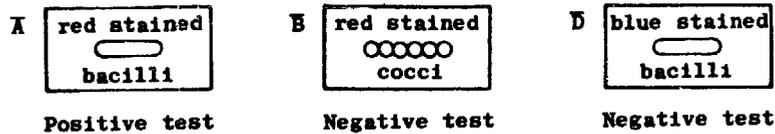
C. COMPLETED TEST - Streak inocula from tubes A', B', and D' on Eosin Methylene Blue Agar Plate. Incubate 24 ± 2 hours at $35 \pm 0.5^\circ\text{C}$.



Transfer a small amount of growth from a typical coliform colony found on EMB plate to a Nutrient Agar Slant and Lauryl Tryptose Broth. Incubate Lauryl Tryptose Broth for 24 or 48 ± 2 hours at $35 \pm 0.5^\circ\text{C}$. Incubate agar slants 18-24 hours.



Prepare a Gram Stain with a small amount of bacterial growth from each Nutrient Agar Slant.



Positive Completed Test: Gas produced, Gram negative (red stained), nonspore forming, rod-shaped bacteria demonstrated.

Negative Completed Test: Failure to meet any one or all of the above positive Completed Test requirements.

Fig. 14 (B). Schematic Diagram for Completed Test

(a) Dissolve 37.5 g of the commercial dehydrated medium per liter of distilled water. Heat to boiling in order to melt and dissolve the agar in the dehydrated medium. While the medium is still hot, dispense it into tubes or bottles. Many workers prefer to dispense the medium in 100 ml portions in dilution water bottles.

(b) Sterilize the medium in the autoclave for 15 minutes at 121°C. After sterilization, the pH should be between 7.3 and 7.5. When the medium is to be used, melt it by suspending the bottle in boiling water. Then pour into sterile petri dishes in portions of approximately 15 ml each. Cool until solidified; the medium is then ready for use.

(c) For prolonged storage periods, keep the sterile medium in tightly closed bottles, out of the light. Once the medium has been poured into petri dishes, refrigerate it and use within one or two days.

4.5. Agar slants, to grow pure cultures for preparation of Gram stain when performing Completed Test:

(a) Dissolve 18.5 g of the commercially available Plate Count Agar per liter of distilled water. Use heat as needed to melt and dissolve the agar. Dispense the hot medium in portions of 5-10 ml into culture tubes. Sterilize at 121°C for 15 minutes in the autoclave.

(b) After removal from the autoclave, while the medium is still hot and in a fluid state, place the tubes in a slanted position. Keep them in that position until the medium has solidified. Tightly closed, screw-capped culture tubes are recommended as they allow the medium to be stored almost indefinitely.

4.6. Gram stain solutions, for staining bacterial smears as part of Completed Test:

(a) Crystal violet solution. Dissolve 2 g crystal violet dye in 20 ml of 95 per cent ethyl alcohol, and filter through coarse filter paper or cheesecloth. Dissolve 0.2 g ammonium oxalate, $(\text{NH}_4)_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$, in 20 ml distilled water. Mix these solutions in equal portions. Difficulties encountered with the Gram stain method frequently can be resolved by reducing the amount of crystal violet in this solution to as low as 10 per cent of the amount shown above.

(b) Lugol's iodine solution. Dissolve 2 g potassium iodide, KI, in 5 ml distilled water. Add 1 g iodine crystals to the potassium iodide solution and stir until the iodine is dissolved. Add 295 ml distilled water and mix.

(c) Safranin solution:

(1) Safranin stock solution. Dissolve 2.5 g safranin dye in 100 ml of 95 per cent ethyl alcohol.

(2) Counterstain solution. Mix 10 ml safranin stock solution with 90 ml distilled water.

(d) Decolorizer. Use 95 per cent ethyl alcohol, alone or mixed in equal parts with acetone.

5. Sampling Procedure

See Membrane Filter Method, Sec. 5.

6. Laboratory Procedure — First Day

The first-day operations apply equally to the examination of samples involved in the Presumptive, the Confirmed, or the Completed Test.

6.1. Prepare the tubes of lauryl tryptose broth required for the Presumptive Test, and arrange them in a culture tube rack in an orderly manner. For planting sample portions of 10 ml or more, use the correct special concentration of lauryl tryptose broth (see Sec. 4.2).

6.2. Label the culture tubes, showing the assigned laboratory number for the laboratory record sheet and the sample planting volume selected. A simple labeling code is illustrated below for a series of decimal increments of sample consisting of five tubes each:

| | Tube 1 | Tube 2 | Tube 3 | Tube 4 | Tube 5 | Explanation |
|--------------------|--------|--------|--------|--------|--------|------------------------------|
| Laboratory No. | 471 | 471 | 471 | 471 | 471 | Tubes with 10 ml of sample |
| Volume & Tube Code | 10a | 10b | 10c | 10d | 10e | |
| Laboratory No. | 471 | 471 | 471 | 471 | 471 | Tubes with 1 ml of sample |
| Volume & Tube Code | 1a | 1b | 1c | 1d | 1e | |
| Laboratory No. | 471 | 471 | 471 | 471 | 471 | Tubes with 0.1 ml of sample |
| Volume & Tube Code | -1a | -1b | -1c | -1d | -1e | |
| Laboratory No. | 471 | 471 | 471 | 471 | 471 | Tubes with 0.01 ml of sample |
| Volume & Tube Code | -2a | -2b | -2c | -2d | -2e | |

The work of identifying culture tubes can be minimized by labeling only the first tube for each series of like sample volumes. For example, label only the tubes in the "Tube 1" column of the above table for initial sample plantings; the identity of the remaining tubes is indicated by their relative positions in the rack. But fully identify all later subcultures from the original sample plantings.

6.3. Shake the sample vigorously, approximately 25 times, using an up-and-down motion.

6.4. Measure the selected sample volumes into the labeled tubes of lauryl tryptose broth, taking care not to introduce any bacteria into the culture medium except those from the sample itself. Use a sterile 100 ml graduated cylinder for 100 ml sample portions, a sterile 10 ml pipet for 10 ml sample portions, and a sterile 1 ml pipet for portions of 1 ml or less.

Handle the pipet only near the mouthpiece; do not allow any of the pipet near the delivery end to touch anything except the sample itself and the inside of the culture tube. When using the pipet to withdraw sample portions, do not dip the pipet more than $\frac{1}{2}$ inch into the sample; otherwise, sample water running down the outside of the pipet will get into the culture medium, making the sample measurement inaccurate.

Deliver sample portions of 1 ml or less down into the culture tube near the surface of the culture medium. Do not deliver small sample volumes at the top of the culture tube or allow them to run down inside the tube; otherwise, too much sample will fail to reach the culture medium.

Prepare sample volumes of 0.01 ml or less by dilution as follows:

(a) 0.01 ml and 0.001 ml sample volumes. Pipet 1 ml of sample into a bottle containing 99 ml of sterile water. Cap the bottle and shake vigorously. Pipet 1 ml portions of this diluted sample into the tubes of culture medium to get 0.01 ml of sample; pipet 0.1 ml portions to get 0.01 of sample.

(b) 0.0001 ml and 0.00001 ml sample volumes. Pipet 1 ml of the first dilution

(0.01 ml of sample) prepared in Step 6.4(a) into a second bottle containing 99 ml of sterile dilution water. Cap the bottle and shake vigorously. Pipet 1 ml portions of this second dilution into the tubes of culture medium to get 0.0001 ml of sample; pipet 0.1 ml portions to get 0.00001 ml of sample.

Always plant the diluted sample in the culture medium within 30 minutes after preparing the dilution. Longer delay may result in unpredictable changes in bacterial densities.

6.5. After measuring all portions of the sample into the respective tubes of culture medium, shake the rack of inoculated tubes gently to insure good mixing of the sample with the culture medium. Avoid vigorous shaking because air bubbles may be shaken into the fermentation vials and ruin the test.

6.6. Incubate the inoculated tubes of lauryl tryptose broth at $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 24 hours \pm 2 hours.

7. Laboratory Procedure - Second Day

7.1. After incubation as prescribed in Sec. 6.6 remove the rack of culture tubes from the incubator and shake the rack gently. If gas is about to appear in the tubes, shaking will speed the process.

7.2. Examine each tube carefully. Report as a positive test each tube showing gas in the inverted fermentation vial. Report each tube not showing gas as a negative test. Gas in any quantity is a positive test.

7.3. Record the results in the space provided on the laboratory data sheet for 24 hour Presumptive Test results. If the examination is limited to the Presumptive Test, carry out Step 7.4. If a Confirmed or Completed Test is to be performed, skip Step 7.4 and proceed to Step 7.5.



Fig. 15. Culture Tube With Inverted Fermentation Vial

7.4. Discard all the gas-positive tubes. Return all the gas-negative tubes in the rack to the 35°C incubator for an additional 24 hours \pm 2 hours. Then proceed with Sec. 8.

7.5. Selection of tubes for Confirmed Test or Completed Test:

(a) If the original sample inoculation pattern for the Presumptive Test consisted

only of five tubes of lauryl tryptose broth, each inoculated with 10 ml or 100 ml of sample, apply Confirmed Test operations to all cultures showing gas.

(b) If the original sample inoculation pattern consisted of five tubes of lauryl tryptose broth for each of three or more sample volumes, Confirmed Test procedures need not always be applied to all gas-positive cultures from the Presumptive Test. If all five cultures for two or more consecutive sample volumes are gas-positive, select the set of five cultures representing the smallest volume of sample in which all tubes are gas-positive. Apply Confirmed Test operations to all of these cultures, and to all other gas-positive cultures representing smaller volumes of sample.

Example: Assume that five 10 ml portions, five 1 ml portions, five 0.1 ml portions, and five 0.01 ml portions of sample were planted in Presumptive Test Medium. Assume further that after 24 hours of incubation gas was observed in all five of the 10 ml sample portions, in all five of the 1 ml portions, in three of the 0.1 ml portions, and in one of the 0.01 ml portions. Using the plan described above, apply Confirmed Test operations to all the cultures representing 1 ml of sample, the three gas-positive tubes for the 0.1 ml portion, and the one gas-positive culture representing 0.01 ml of sample. This plan assumes that all of the 10 ml sample portions would give positive results if subjected to Confirmed Test operations.

7.6. Label one tube of brilliant green lactose bile broth to correspond with each gas-positive Presumptive Test culture selected for Confirmed Test procedures.

7.7. Gently shake the rack of Presumptive Test cultures. With an inoculation loop, transfer one drop from each gas-positive Presumptive Test culture to the corresponding tube of brilliant green lactose bile broth. Sterilize the inoculation loop in a gas flame and cool just prior to making each transfer.

7.8. After each transfer, place the inoculated tube of brilliant green lactose bile broth in the culture rack in the position formerly occupied by the gas-positive Presumptive Test culture. Discard the original gas-positive Presumptive Test culture. After the transfers, the rack should contain a mixture of tubes of 24 hours-old gas-negative Presumptive Test cultures and the newly inoculated Confirmed Test cultures.

7.9. Return the cultures to the 35° C incubator for an additional 24 hours ± 2 hours.

8. Laboratory Procedure - Third Day

8.1. Remove the rack of culture tubes from the incubator and shake the rack gently.

8.2. Examine each culture tube and report as a positive test each culture showing gas in any quantity in the fermentation vial; report as a negative test culture in which no gas is visible. If the examination is limited to the Presumptive Test carry out Step 8.3. If a Confirmed or Completed Test is to be performed, skip Step 8.3 and proceed to Step 8.4.

8.3. Record the results in the space provided on the laboratory record sheet for 48 hour Presumptive Test results. Then discard all Presumptive Test cultures and proceed with Sec. 12.

8.4. Record the results of the lauryl tryptose broth cultures in the space provided on the laboratory sheet for 48 hour Presumptive Test results. Record the re-

sults of the brilliant green lactose bile broth cultures in the space for 24 hour Confirmed Test results.

8.5. In the event that no additional Presumptive Test cultures become gas-positive in the second 24 hour incubation period and all the 24 hour Confirmed Test cultures are gas-positive, further culturing is unnecessary if the examination is limited to the Confirmed Test; in that case, proceed with Sec. 12. If any of the above conditions do not apply, continue with Step 8.6.

8.6. Transfer all gas-positive 48 hour Presumptive Test cultures to brilliant green lactose bile broth as described in Sec. 7.6-7.9. Discard all gas-negative 48 hour Presumptive Test cultures.

8.7. Consolidate the 24 hour gas-negative Confirmed Test cultures with the newly inoculated Confirmed Test cultures planted in Step 8.6. If the examination of the sample is limited to the Confirmed Test, discard all gas-positive Confirmed Test cultures. If the examination is to be continued to the Completed Test, save the gas-positive Confirmed Test cultures and transfer them as described in Sec. 8.9.

8.8. Incubate the 24 hour gas-negative cultures and the newly inoculated Confirmed Test cultures for 24 hours \pm 2 hours at 35° C. If the examination is limited to the Confirmed Test, proceed with Sec. 9. If the Completed Test is to be performed, carry out Step 8.9.

8.9. Streak-plate inoculation for Completed Test (the following operations apply to all Confirmed Test positive cultures):

(a) Label a petri dish preparation of eosin methylene blue agar to correspond with each gas-positive Confirmed Test culture. Mark the bottom of the petri dish off in fourths, considered as numbered 1 through 4, for identification in making the inoculation.

(b) Flame sterilize and cool an inoculation needle whose end 1/8 inch is bent slightly. Dip the needle about 1/2 inch into the gas-positive Confirmed Test culture being transferred. In streaking the plate, avoid tearing the surface of the culture medium with the needle. Make the inoculation by lightly touching the side of the end 1/8 inch of the needle to the surface of the medium, taking care not to gouge it with the point. Gently draw the needle back and forth over the entire area of two adjacent quarters of the eosin methylene blue agar surface. Resterilize and cool the inoculation needle. Then draw it back and forth over one of the two inoculated quarters of the medium surface and over the third (uninoculated) quarter. Finally resterilize and cool the needle again and draw it back and forth between the third and fourth quarters of these surfaces of the medium. This procedure isolates some of the individual cells on the third or fourth quarter. Individual colonies will develop, each from one of the cells isolated during the inoculation.

(c) Cover the petri dish and incubate for 24 hours \pm 2 hours at 35° C in an inverted position (inoculated surface of the medium directed downward).

9. Laboratory Procedure - Fourth Day

9.1. Remove the rack of culture tubes from the incubator and shake the rack gently.

9.2. Examine all culture tubes and record as a positive test each one showing gas in any quantity in the fermentation vial; record as a negative test any tube showing no gas. Since some of the Confirmed Test cultures are 24 hours old and others are

48 hours old, be sure to identify each culture and record the result in the proper space (24 hour or 48 hour Confirmed Test) on the laboratory record sheet.

9.3. If any 24 hour Confirmed Test cultures are gas-negative, incubate them for a second period of 24 hours \pm 2 hours at 35°C. If the examination is limited to the Confirmed Test, carry out Step 9.4. If the Completed Test is to be performed, skip Step 9.4 and proceed with Steps 9.5-9.8.

9.4. Discard all 48 hour Confirmed Test cultures. Omit Steps 9.5 - 9.8 and proceed with Sec. 10 after incubation of the cultures in Step 9.3. If all 24 hour Confirmed Test cultures were gas-positive, discard the tubes because further culturing is not required for the Confirmed Test; proceed directly with Sec. 12.

9.5. Discard all gas-negative 48 hour Confirmed Test cultures.

9.6. Prepare streak-plate inoculations for colony isolation in eosin methylene blue agar from all gas-positive Confirmed Test cultures as described in Sec. 8.9.

9.7. Remove the eosin methylene blue agar plates prepared in Sec. 8.9 from the incubator and inspect them for typical coliform bacteria colonies well isolated from each other. Typical coliform bacteria colonies (termed nucleated colonies) have a dark center when viewed through the bottom of the petri dish. When viewed from the upper surface, the colonies may or may not have a green-gold sheen, this is not the basis for recognition of coliform bacteria colonies.

9.8. From each eosin methylene blue agar plate having typical coliform bacteric colonies, select one such colony which is at least 0.5 centimeters from any other colony. With a flame-sterilized inoculation needle, transfer a small amount of the growth as a streak inoculation on the surface of an agar slant (Sec. 4.5) and also inoculate a tube of lauryl tryptose broth. If typical coliform bacteria colonies are not present, select two of the atypical coliform bacteria colonies (pink) and transfer each to a tube of lauryl tryptose broth and to an agar slant. If neither typical (nucleated) nor atypical (pink) coliform bacteria colonies appear to be present, select two of the most representative colony types observed and transfer each of these to a tube of lauryl tryptose broth and to an agar slant. Proceed with the test as with typical colonies.

9.9. Discard the eosin methylene blue agar plate cultures after making the transfers. Incubate the newly inoculated lauryl tryptose broth cultures for 24 hours \pm 2 hours at 35°C. Incubate the agar slant cultures for at least 18 *but not more than 24 hours*; longer incubation may result in irregular staining reactions when the Gram Test (Sec. 10.5 - 10.7) is applied.

10. Laboratory Procedure - Fifth Day

10.1. Remove any remaining Confirmed Test cultures from the incubator, examine the tubes, and record the results as described in Sec. 9.2. If the examination is limited to the Confirmed Test, discard the remaining cultures and proceed with Sec. 12. If the Completed Test is to be performed, continue as follows:

10.2. Prepare streak-plate inoculations for colony isolation on eosin methylene blue agar from all gas-positive Confirmed Test cultures as described in Sec. 8.9.

10.3. Remove the eosin methylene blue agar plate cultures prepared in Sec. 9.6 from the incubator, examine for typical coliform bacteria colonies, and make transfers to lauryl tryptose broth and to agar slants as described in Sec. 9.7 - 9.9.

10.4. Inspect the 24 hour cultures in lauryl tryptose broth prepared in Sec. 9.8

TABLE 7
*MPN for Five 10-ml Tubes Planted**

| No. of Tubes Giving Positive Reaction out of Five 10 ml Tubes | MPN per 100 ml |
|--|----------------------|
| 0 | 0 |
| 1 | 2.2 |
| 2 | 5.1 |
| 3 | 9.2 |
| 4 | 16.0 |
| 5 | indeterminate |

* If a volume other than 10 ml was used, apply the correction described in Sec. 12.2(c).

and record the presense or absence of visible gas in the fermentation vial. Return the gas-negative cultures to the 35° C incubator for another 24 hours. Discard the gas-positive cultures.

10.5. Remove all agar slant cultures prepared in Sec. 9.8 from the incubator no later than 24 hours after incubation. Prepare a bacterial smear from each agar slant culture as follows:

- (a) Thoroughly clean a glass slide to free it of any trace of oily film.
- (b) Place one drop of distilled water on the slide.
- (c) With an inoculation needle, suspend a tiny amount of growth from the nutrient agar slant culture in the drop of water.
- (d) Mix the thin suspension with the tip of the inoculation needle. Then allow the water to evaporate.
- (e) Fix a smear by gently warming the slide over a flame.

10.6. Stain each smear corresponding with a gas-positive culture in Step 10.4. (Set aside any smears representing lauryl tryptose broth cultures which were gas-negative after 24 hours' incubation, and stain them only if gas appears in the second 24 hour incubation period.) The staining procedure follows:

- (a) Flood the smear for 1 minute with crystal violet solution.
- (b) Flush the excess crystal violet off the slide by washing it gently in running water.
- (c) Flood the smear with Lugol's iodine solution for 1 minute.
- (d) Wash the slide in running water, and blot dry with filter paper or other clean absorbent paper.
- (e) Decolorize the smear using 95 per cent alcohol solution with gentle agitation for 30 seconds, blot and dry.
- (f) Counterstain for 10 seconds with safranin solution. Then wash in running water, and blot dry.

10.7. Examine the slide under the microscope, using the oil immersion objective. Coliform bacteria are nonspore-forming, rod-shaped cells, occurring singly, in pairs, or, rarely, in short chains; they are Gram-negative - that is, they will be stained red by the staining procedure. If Gram-positive cells are present, they will

be stained blue. If spore-forming bacteria are found, it is necessary to conduct further tests to ascertain whether coliform bacteria are mixed with the culture; consult the latest edition of *Standard Methods* for instructions on repurifying cultures and applying additional differential test.

10.8. Report each culture as a positive Completed Test if it is shown to be gas-positive and if microscopic examination of the stained smear demonstrates Gram-negative, nonspore-forming, rod-shaped bacteria.

11. Laboratory Procedure - Sixth Day and Later

For most water examinations, only a few "straggler" cultures will remain at this time. The routine for examining all types of subcultures from the original sample now has been described. The method for concluding the laboratory work on the few remaining cultures, therefore is presented in greatly compressed fashion.

11.1. Examine the eosin methylene blue agar plate cultures and transfer representative well-separated colonies to agar slants and to lauryl tryptose broth as described in Sec. 9.7 - 9.9. Incubate at 35° C and inspect the broth tubes for gas production after 24 hours, and, if necessary, after 48 hours. Stain and examine the Gram-stained smears from agar slant cultures representing 24 hour gas-negative and 48 hour gas-positive cultures as described in Sec. 10.6 and 10.7. Proceed to Sec. 12 for the calculation and interpretation of results.

11.2. Examine the cultures in lauryl tryptose broth for gas production. If gas is produced after 24 hours or after 48 hours, prepare and examine a Gram-stained smear from a 24 hour agar slant culture. Terminate all laboratory operations if no gas is evident after 48 hours of incubation, and consult Sec. 12 for the calculation and interpretation of MPN results.

12. Interpretation of Results

12.1. In answering the question, "Is a water bacteriologically safe to drink?," the limits established in the US Public Health Service Drinking Water Standards are a good guide. These limits are based on the initial planting of five 10 ml portions or five 100 ml portions of a standard sample.

(a) When 10 ml portions are examined, not more than 10 per cent in any month may show the presence of the coliform group. The presence of the coliform group in three or more 10 ml portions of a standard sample must not occur:

- (1) In two consecutive samples; or
- (2) In more than one sample per month when less than 20 are examined per month; or
- (3) In more than 5 percent of the samples when 20 or more are examined per month.

(b) When 100 ml standard portions are examined, not more than 60 per cent in any month may show the presence of the coliform group. The presence of the coliform group in all five 100 ml portions of a standard sample must not occur:

- (1) In two consecutive samples;
- (2) In more than one sample per month when less than five are examined per month; or
- (3) In more than 20 per cent of the samples when five or more are examined per month.

(c) When organisms of the coliform group occur in three or more of the 10 ml

TABLE 8

MPN for Five 10-ml, Five 1-ml, and Five 0.1-ml Tubes Planted

| No. of Tubes Giving Positive Reaction out of: | | | | No. of Tubes Giving Positive Reaction out of: | | | |
|---|--------------------|----------------------|-----------|---|--------------------|----------------------|-----------|
| Five 10 ml Portions | Five 1 ml Portions | Five 0.1 ml Portions | MPN Index | Five 10 ml Portions | Five 1 ml Portions | Five 0.1 ml Portions | MPN Index |
| 0 | 0 | 1 | 2 | 3 | 5 | 0 | 25 |
| 0 | 0 | 2 | 4 | 4 | 0 | 0 | 13 |
| 0 | 1 | 0 | 2 | 4 | 0 | 1 | 17 |
| 0 | 1 | 1 | 4 | 4 | 0 | 2 | 21 |
| 0 | 1 | 2 | 6 | 4 | 0 | 3 | 25 |
| 0 | 2 | 0 | 4 | 4 | 1 | 0 | 17 |
| 0 | 2 | 1 | 6 | 4 | 1 | 1 | 21 |
| 0 | 3 | 0 | 6 | 4 | 1 | 2 | 26 |
| 1 | 0 | 0 | 2 | 4 | 2 | 0 | 22 |
| 1 | 0 | 1 | 4 | 4 | 2 | 1 | 26 |
| 1 | 0 | 2 | 6 | 4 | 2 | 2 | 32 |
| 1 | 0 | 3 | 8 | 4 | 3 | 0 | 27 |
| 1 | 1 | 0 | 4 | 4 | 3 | 1 | 33 |
| 1 | 1 | 1 | 6 | 4 | 3 | 2 | 39 |
| 1 | 1 | 2 | 8 | 4 | 4 | 0 | 34 |
| 1 | 2 | 0 | 6 | 4 | 4 | 1 | 40 |
| 1 | 2 | 1 | 8 | 4 | 5 | 0 | 41 |
| 1 | 2 | 2 | 10 | 4 | 5 | 1 | 48 |
| 1 | 3 | 0 | 8 | 5 | 0 | 0 | 23 |
| 1 | 3 | 1 | 10 | 5 | 0 | 1 | 31 |
| 1 | 4 | 0 | 11 | 5 | 0 | 2 | 43 |
| 2 | 0 | 0 | 5 | 5 | 0 | 3 | 58 |
| 2 | 0 | 1 | 7 | 5 | 0 | 4 | 76 |
| 2 | 0 | 2 | 9 | 5 | 1 | 0 | 33 |
| 2 | 0 | 3 | 12 | 5 | 1 | 1 | 46 |
| 2 | 1 | 0 | 7 | 5 | 1 | 2 | 63 |
| 2 | 1 | 1 | 9 | 5 | 1 | 3 | 84 |
| 2 | 1 | 2 | 12 | 5 | 2 | 0 | 49 |
| 2 | 2 | 0 | 9 | 5 | 2 | 1 | 70 |
| 2 | 2 | 1 | 12 | 5 | 2 | 2 | 94 |
| 2 | 2 | 2 | 14 | 5 | 2 | 3 | 120 |
| 2 | 3 | 0 | 12 | 5 | 2 | 4 | 148 |
| 2 | 3 | 1 | 14 | 5 | 2 | 5 | 177 |
| 2 | 4 | 0 | 15 | 5 | 3 | 0 | 79 |
| 3 | 0 | 0 | 8 | 5 | 3 | 1 | 109 |
| 3 | 0 | 1 | 11 | 5 | 3 | 2 | 141 |
| 3 | 0 | 2 | 13 | 5 | 3 | 3 | 175 |
| 3 | 1 | 0 | 11 | 5 | 3 | 4 | 212 |
| 3 | 1 | 1 | 14 | 5 | 3 | 5 | 253 |
| 3 | 1 | 2 | 17 | 5 | 4 | 0 | 130 |
| 3 | 1 | 3 | 20 | 5 | 4 | 1 | 172 |
| 3 | 2 | 0 | 14 | 5 | 4 | 2 | 221 |
| 3 | 2 | 1 | 17 | 5 | 4 | 3 | 278 |
| 3 | 2 | 2 | 20 | 5 | 4 | 4 | 345 |
| 3 | 3 | 0 | 17 | 5 | 4 | 5 | 426 |
| 3 | 3 | 1 | 21 | 5 | 5 | 0 | 240 |
| 3 | 4 | 0 | 21 | 5 | 5 | 1 | 348 |
| 3 | 4 | 1 | 24 | 5 | 5 | 2 | 542 |

portions (or in all five of the 100 ml portions) of a single standard sample, daily samples from the same sampling point must be collected promptly and examined until the results from at least two consecutive samples show the water to be of satisfactory quality.

(d) Results should be reported in terms of the stage to which the test was carried. For example, report the "Completed Test: Coliform-positive in 3 out of 5 100 ml portions."

12.2. When a quantitative rather than a qualitative interpretation is desired, the Most Probable Number (MPN) of coliform bacteria can be calculated by the procedure outlined below. The procedure assumes that five portions of each of three sample volumes (say, 10 ml, 1 ml, and 0.1 ml) were planted initially. If only one sample volume was used (for example, if only five 10 ml portions were planted initially), the MPN can be found by referring to Table 7, but the figure obtained will be much less accurate than with the three-volume planting. With the three-volume planting, the MPN can be found from Table 8, in the following way:

(a) Codify the test results. For example, if five 10 ml portions, five 1 ml portions, and five 0.1 ml portions were planted initially, and positive tests for coliform bacteria were obtained from all five of the 10 ml portions, from three of the 1 ml portions, and from none of the 0.1 ml portions, the coded result of the test would be 5-3-0.

Note: If some other three-volume series is used, the coding procedure will be the same, but a correction must be made later - see Step (c) below. If a four-volume or five-volume series is used, the coding procedure becomes more difficult - see Table 9 for examples; a later correction may or may not be required, as explained in Step (c).

(b) Find the MPN in Table 8 associated with the coded test results from Step (a). For example, if the coded results are 5-3-0, locate the horizontal line of print in which 5 appears in the column headed "five 10 ml portions," 3 appears in the column headed "five 1 ml portions," and 0 appears in the column headed "five 0.1 ml portions." Follow the line to the right, and read 79 in the column headed "MPN Index." The same principle is used in looking up the MPN Index for any other code. Note that, up to this point, it does not matter whether the sample volumes represented by the results are the same as those in column headings.

(c) Calculate any necessary correction of the MPN value found in Step (b). Table is based on the assumption that the first number of the codified laboratory result represents five 10 ml sample portions, and the second and third numbers of the code represent 1 ml portions and 0.1 ml portions, respectively. When that is the case, no correction of the MPN value found is needed; it is the MPN of coliform organisms per 100 ml of the sample. When the first number of the code represents a volume other than 10 ml of sample, apply the following correction to obtain the MPN per 100 ml: Multiply the MPN Index found in Step (b) by 10 and divide the result by the sample volume represented by the first number of the code.

Example: Assume that the code 5-3-0 represents a sample in which the code 5 corresponds to 0.01 ml of sample (the 3 and the 0 representing 0.001 and 0.0001 ml of sample, respectively). Then:

$$\text{MPN per 100 ml} = 79 \times 10 \div 0.01 = 79,000.$$

TABLE 9

Examples of Coding Procedure for Four-Volume or Five-Volume Plantings

| No. of Tubes Giving Positive Reactions out of: | | | | | Code | See Explanatory Note |
|--|--------------------|----------------------|-----------------------|------------------------|-------|----------------------|
| Five 10-ml Portions | Five 1-ml Portions | Five 0.1-ml Portions | Five 0.01-ml Portions | Five 0.001-ml Portions | | |
| 5 | 4 | 1 | 0 | 0 | 5-4-1 | |
| 5 | 5 | 4 | 0 | 0 | 5-4-0 | (a) |
| 5 | 4 | 1 | 1 | 0 | 5-4-2 | (b) |
| 5 | 5 | 5 | 2 | | 5-5-2 | (c) |
| 5 | 5 | 5 | 5 | | 5-5-5 | (d) |
| 0 | 0 | 0 | 0 | | 0-0-0 | (e) |
| 0 | 1 | 0 | 0 | | 0-1-0 | (f) |

Explanation-

(a) When all the inoculated tubes of more than one of the decimal series give positive results, select the smallest sample volume (in this case, 1 ml) in which all tubes give positive results, and let this be the first number of the code series, provided enough sample volumes were planted to give a total of three numbers for the code. In this example, if portions of 0.01 ml had not been planted, the code would have to be written 5-5-4.

(b) In this example, positive results appear in four different sample volumes when the first number of the code is considered (properly so) to be a 5. Table 8 is designed for use with only three numbers in the code series. In this case, the number of positive tubes from the smallest sample volume is added to the number of positive tubes in the next smallest sample volume, so that the last number in the code becomes 2.

(c) If portions of 0.0001 ml had been planted originally, with zero positive results, the code would have been 5-2-0. However, it is not permissible to assume results that might have occurred if the tubes had been planted.

(d) This is an indeterminate test result. Many MPN tables do not give a value for such results. If the table does not show an MPN for a particular code sequence, find the code for the next highest number of positive tubes for which an MPN is shown (5-5-2 in this case) and report the result as "greater than" the MPN shown for that code.

(e) Like (d) above, this is an indeterminate result. Since the code does not appear in the table, look up the MPN for code 1-0-0 and report the result as "less than" the value shown for 1-0-0.

(f) Follow the code pattern shown when this sort of unusual laboratory result occurs.

12.3. The MPN should be reported in terms of the stage to which the test was carried. For example, report "Coliform bacteria 79,000 per 100 ml according to the MPN Confirmed Test."

It is important to remember that the MPN value found is merely the "most probable" number. The actual number of coliform organisms in the sample may be lower or higher than this figure. The tables in the latest edition of *Standard Methods* give the variation which may be expected for each MPN.

III. Biologic Examination

Introduction

Plankton is composed of small animals and plants (algae) that float, or drift, dispersed in the open water. When they are abundant, they may cause the water to look cloudy or may float on a surface as a scum. Such a heavy growth is called a "bloom". Plankton blooms cause serious problems for the water utility operator, such as tastes or odors, shortened filter runs, or changes in pH. Since these effects may also result from causes other than plankton, it is important to ascertain the real trouble before attempting to make corrections. The procedures outlined in this Manual, if carefully followed, will show whether or not plankton organisms are present and, if present, in what quantities and kinds. Small numbers of some types may have little importance, whereas equal numbers of others may well spell trouble.

Plankton counting and identification may be done very simply, or it may be developed into a highly technical operation, depending on individual skill and the time available. The beginner should use great caution in applying the results of his identifications until he has gained considerable experience. Study under the guidance of a specialist in classifying organisms is strongly advised. As in all such fields, the greater the skill applied, the greater will be the returns.

This Manual is intended to give only a basic introduction to plankton microscopy. When more information is desired, the latest edition of *Standard Methods for the Examination of Water and Wastewater* should be consulted. Other useful references are listed at the end of this Introduction.

In this part of the Manual, certain sections are marked with an asterick (*). These describe fundamental procedures and equipment. Sections not so marked describe additional equipment and give more advanced procedures and information which may be considered optional. Some of the more specialized procedures should be used only by persons with adequate experience and training.

Use of the microscope is not covered here. Individuals unfamiliar with this instrument should consult the instruction manual which comes with most microscopes or seek the assistance of some informed person in the community, such as a teacher or a hospital or laboratory worker. While many good books on microscopy

may be found in technical libraries, most of them are for the advanced worker or specialist rather than for the beginner. An excellent 16-mm sound motion picture for the beginner, entitled "The Compound Microscope," is available free of charge from Bausch and Lomb, Inc., Rochester, N.Y. A good brief description of the technique of the microscope is also given in Chapter V of Ref. 3 below.

References and Bibliography

Some of the publications listed below are referred to in the text. Others are included for those who want more details or require information on topics not covered in this Manual. Particular attention is called to the general discussions offered in Ref. 1, 2, and 3, and to the list of additional publications given in Ref. 4. References 5 through 10 are to a group of papers dealing with plankton problems in specific parts of the country. Reference 11 is a good general reference covering the basic nature of the interrelationships among the physical, chemical, and biologic aspects of reservoirs and other waters.

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20. Prescott, G. W. *How to Know the Fresh-Water Algae*. W. C. Brown Co., Dubuque, Iowa (1954). Line drawings and detailed keys for identification.
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A. Apparatus and Reagents

*1. Essential Equipment

1.1. Compound microscope (Fig. 16) with 10x eyepiece and 10x objective (also called "low power" or "16mm"). Modern instruments with binocular eyepieces are convenient, but any instrument with a good lens system, in which the working mechanism is in good condition, can be used.

1.2. Standard 1 x 3 inch microscope slides and coverglasses (Fig. 17). Coverglasses of No. 1 thickness are very thin and delicate to handle. Those of No. 2 thickness are a little sturdier and are recommended for general use. Coverglasses may be in the form of squares (22 mm or 7/8 inch squares are suggested) or circles, which many prefer.

1.3. Common medicine droppers with bulbs which will draw in slightly over 1 ml, or 1 ml pipets with large apertures. Polyethylene is best, as some plankton tends to stick to glass.

1.4. Lens paper for cleaning microscope lenses. Lens paper or other tissue dampened with a solvent such as xylene is excellent for removing traces of immersion oil or other stubborn dirt.

1.5. Soft, lint-free toweling for wiping slides, coverglasses, and other equipment.

1.6. Field collecting bottles. Clean (but not necessarily sterile) 500 ml reagent bottles are recommended. Smaller sizes should be avoided in the interest of obtaining as representative a sample as possible. Larger sizes, such as gallon jars, are perfectly acceptable. Polyethylene or similar material is excellent, as plankton will not stick to it.

The following equipment is necessary for making counts:

1.7. A standard Sedgewick-Rafter plankton counting cell and coverglass (Fig. 17b).

1.8. Whipple plankton-counting disk (Fig. 18), also called reticule or micrometer. It should be installed in the eyepiece of the microscope (ordinarily the right one in a binocular scope, as the left eyepiece is usually adjustable and hence does not have a constant magnification). Carefully unscrew the upper portion of the eyepiece and drop the Whipple disk on to the shelf or platform which will be seen about halfway down inside (Fig. 19). Replace the top lens. Point the eyepiece toward a piece of white paper on the table top and note whether or not the lines on the disk appear sharp and clear. If not, unscrew the top lens again and turn the disk over. If there is no improvement, this is the best that can be done. The sharpness of these lines is of secondary importance, because the eye is actually focused on the microscope field below, and the Whipple field is primarily a guide.

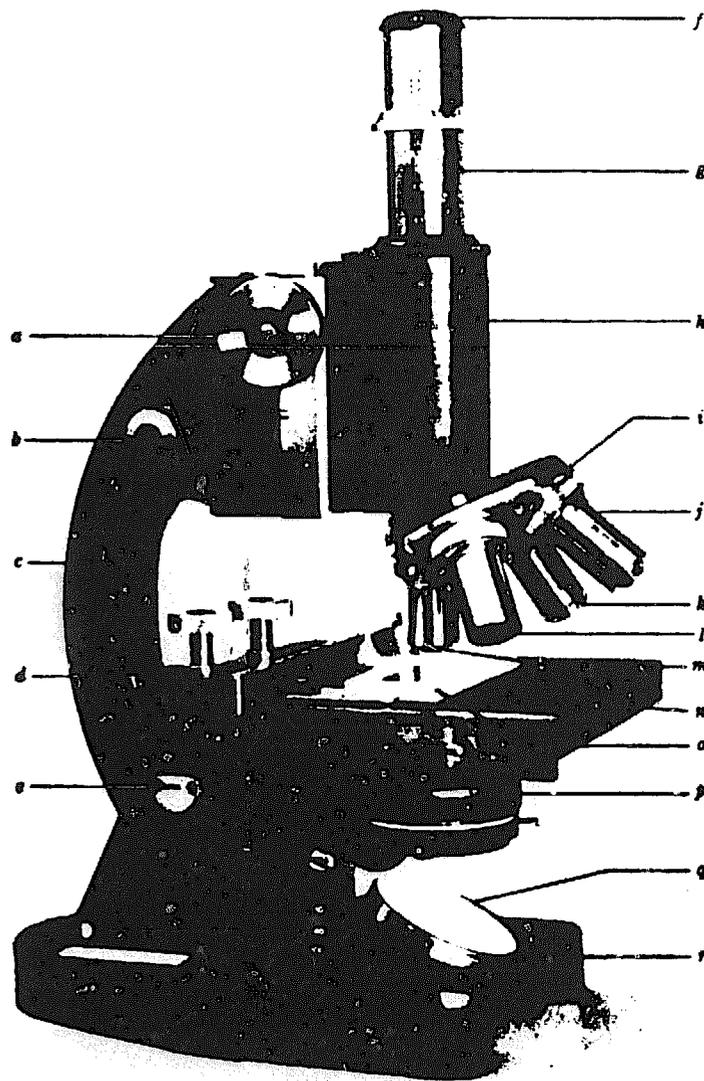


Fig. 16. Compound Microscope

Key: (a) coarse adjustment; (b) fine adjustment; (c) arm; (d) mechanical stage (movable); (e) pivot; (f) eyepiece; (g) drawtube; (h) body tube; (i) revolving nosepiece; (j, k, l, m) objective lenses (40x, 100x, 20x, 10x); (n) fixed stage; (o) Sedgwick - Rafter cell in place; (p) substage condenser; (q) mirror; (r) base.

2. Optional Equipment

The following additional equipment will be found useful:

2.1. Microscope accessories:

(a) Objectives (magnifications approximate): 20x (8 mm, or intermediate objective); 40x (4 mm, or high-power dry objective); 90x (1.8 mm, or oil immersion objective).

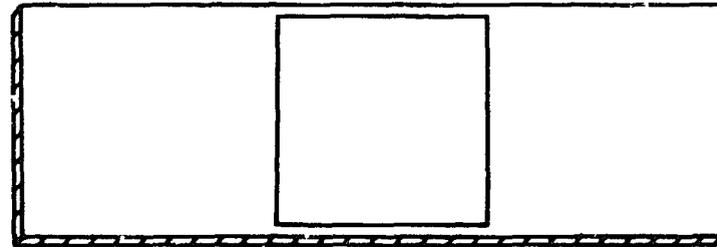
(b) Mechanical stage. This may be of the simplest nongraduated type, although

graduations are often helpful in relocating a given area on a slide.

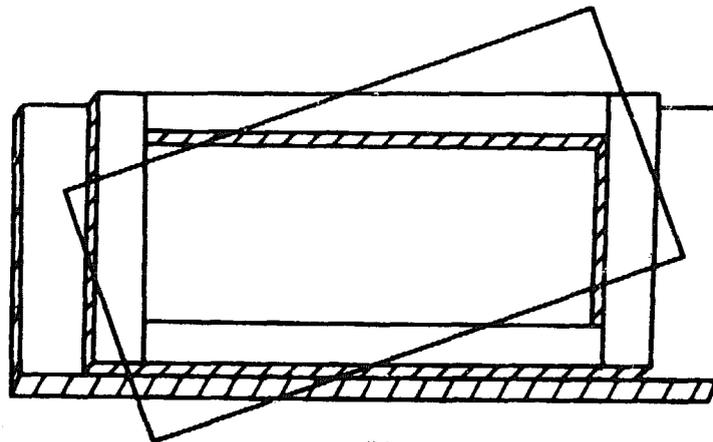
(c) Substage condenser with diaphragm to control light.

(d) Microscope lamp. Much might be said about light sources for microscope work, but, in general, almost any light that can be reflected in the substage mirror - even room light if bright enough - can be adjusted to give good results.

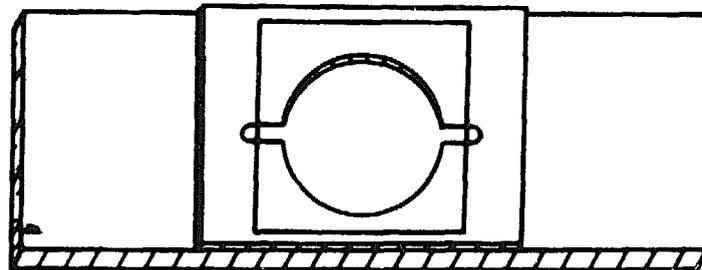
(e) Stage micrometer for calibration of the Whipple disk (Fig. 20).



(a)



(b)



(c)

Fig. 17. Microscope Slides and Cells

Key: (a) standard slide (actual size) with coverglass; (b) Sedgwick-Rafter cell with coverglass in position for filling; (c) nanoplankton or Palmer-Maloney cell.

2.2. Wide-field dissecting microscope. Specifications for lens combinations for this instrument are not exacting. Provision should be made for total magnifications ranging from approximately 5x or 10x to 50x or more. This instrument is useful for observing the larger organisms and for counting larger plankton forms where the entire cell will need to be surveyed quickly.

2.3. Nannoplankton slide (Fig. 17c), also called Palmer or Palmer-Maloney slide or cell. This slide is designed for the study of very small species which require a magnification greater than 200x, but where the use of oil immersion is not practicable. The nannoplankton slide is 0.4 mm deep and hence permits the use of a 40x objective.

2.4. Thermometer for use in the field, approximate range 0°C to 50°C or 32°F to 100°F. Specifically constructed field thermometers can be purchased, or a laboratory thermometer can be supported inside a cylinder of hardware cloth by sections cut from a one-hole rubber stopper.

2.5. Kemmerer type water sampler (Fig. 21). the 1 l size is usually adequate. A built-in thermometer can frequently be obtained if desired. (Many other plank-

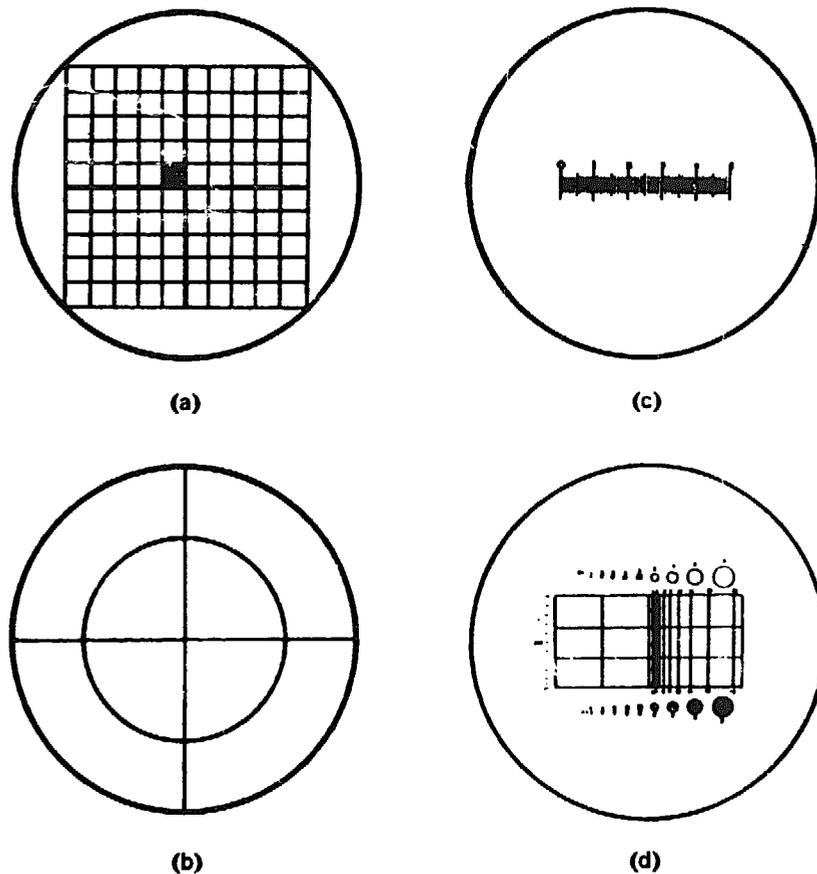


Fig. 18. Eyepiece Micrometers

Key: (a) Whipple square; (b) quadrant ruling; (c) linear scale; (d) Porton reticule for estimating particle size.

ton collecting devices are available. A less expensive one which is meeting with wide acceptance is the Frautschy bottle, sold by Hytech Corporation, "G" Street Pier, San Diego, Calif.)



Fig. 19. Method of Mounting Whipple Disk

The Kemmerer sampler should be equipped with enough 1/4 inch or 3/16 inch line to somewhat more than reach the bottom of the body of water being sampled. Other associated items include a "messenger" (a hollow brass cylinder which slides on the line), a 6 inch piece of rubber tubing to fit on the release valve at the bottom of the sampler, and a means for measuring the depth of the sample. Paints or dyes may be used to mark fabric lines, while special measuring devices are needed for metal cables. Metal cables, reels, and winches are good investments if an extensive sampling program is anticipated. Brass chains with small metal disks to mark the depth can be purchased but are expensive, hard on the hands, and subject to

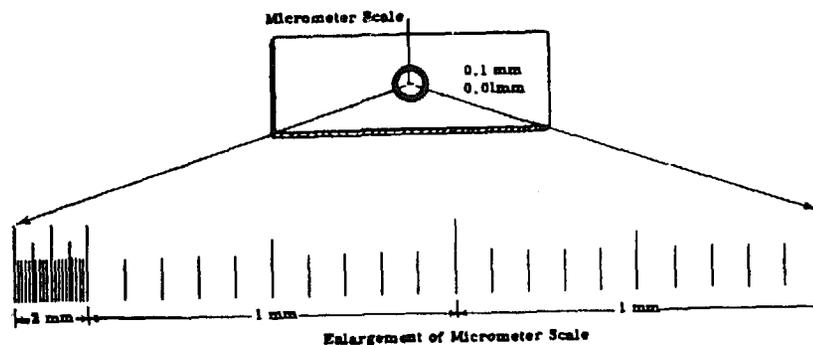


Fig. 20. Stage Micrometer

breaking if a single link is weakened. If available, a twisted sash cord is excellent for hand sampling, as the roughness of the strands makes it possible to hold the lines easily, even when wet. Braided line is often very slippery.

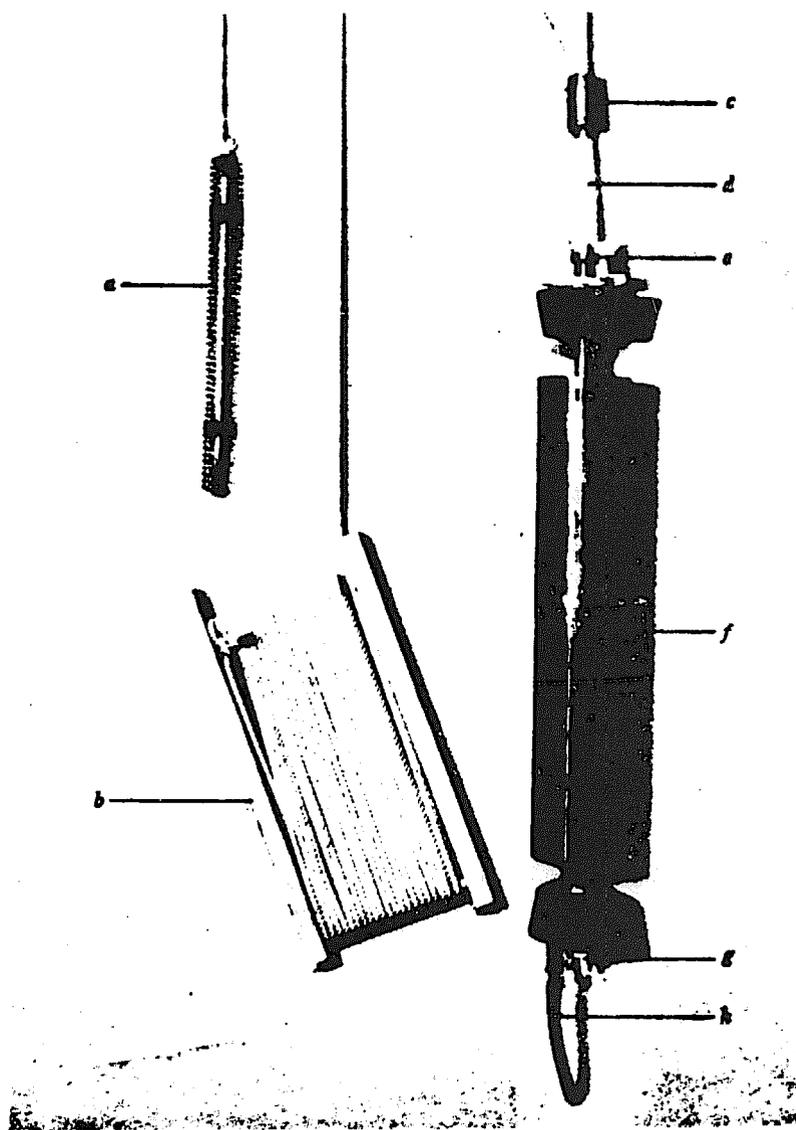


Fig. 21. Kemmerer Sampler

Key: (a) thermometer rigged in hardware cloth cylinder, held in place by sections cut from one-hole rubber stoppers; (b) reel of twisted 1/4 inch line; (c) "messenger" held by string for safety; (d) suspension line; (e) catch; (f) body of sampler; (g) knot at bottom of line; (h) rubber tubing on sample release valve.

2.6. Conventional concentration devices. The use of plankton nets, centrifuges, and sedimentation funnels is not covered in this Manual. *Standard Methods* should be consulted for the techniques used in the examination of plankton-poor or plankton-rich waters which require concentration, dilution, or other special procedures.

2.7. Membrane filter equipment. Many laboratories are already equipped with membrane filter equipment for the bacteriologic examination of water. If available, this equipment can be very helpful in certain types of plankton examination.

Note: Sources of Kemmerer samplers and other types of special collecting equipment are listed in a publication of the American Society of Limnology and Oceanography entitled *Sources of Limnological and Oceanographic Apparatus and Supplies*, Special Publication No. 1, available from the Society at Ann Arbor, Mich.

3. Reagents

*3.1. Commercial formalin. Although commercial formalin contains only about 40 per cent formaldehyde gas, it is customary to consider it a pure reagent in mixing stated dilutions. Thus, when a 5 per cent solution is called for, 5 ml of commercial formalin is mixed with 95 ml of water. The strength of formalin is not appreciably altered by the formation of solid white flakes on standing or freezing.

3.2. Thimerosal stabilizer. This recently developed short-term preservative is proving to be very useful for the temporary stabilization of plankton with a minimum of distortion and decomposition. Prepare as follows:

(a) Thimerosal stock solution. Dissolve approximately 1 g sodium borate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$) and approximately 1 g thimerosal (also called merthiolate) in 1 l distilled water. Shake and dissolve carefully before use. Store in a refrigerator. A precipitate may form on cooling. The amounts of sodium borate and thimerosal can be varied to adjust to different conditions. The proportions given above are for use in warm weather with normal potable water supplies. In cooler weather lesser amounts may be used.

(b) Lugol's stock solution. Make a saturated solution of potassium iodide, KI (approximately 12 g) in 95 per cent ethanol (100 ml), leaving a precipitate in the bottom. Since this is likely to be a slow process, a well-stoppered bottle should be used. Add iodine crystals to this solution to saturation (approximately 1 g), maintaining excess crystals in the bottom.

(c) Preparation of preservative. Slowly add approximately 1.7 ml of the Lugol's stock solution to 1 l of thimerosal stock solution, keeping all precipitates dissolved. At temperatures normally encountered this mixture is stable for five weeks in summer or six weeks in winter.

3.3. Methyl cellulose solution. Dissolve 1 g methyl cellulose (viscosity rating of 15 centipoises) in 9 ml distilled water. Avoid boiling. Discard and prepare fresh when contamination with mold or bacteria becomes evident.

B. Collection of Samples

*1. Sampling Program

1.1. Plankton sampling should be carried out according to a carefully planned and consistently followed program, with reference to both the frequency and the

location of sampling. As most plankton organisms are small and have relatively short lives, their numbers may vary from day to day and from place to place, in response to changing weather and seasonal conditions.

The size of the plankton sampling program must be determined by the economics and needs of the plant. It might range from a sample from each major part of the reservoir taken daily, or even more often, to a sample a month from the plant intake. A minimum suggested program is once a week, with increased frequency as the need develops.

Every effort should be made to maintain a consistent series of identical observations, once the program has been firmly launched. Units, techniques, and sampling stations should be standardized as far as possible so that observations of one year can be compared directly with those of preceding years. On the other hand, new observations that could benefit the program should not be ignored, nor should some station or procedure that is obviously unprofitable be continued. Before adopting a proposed program, discuss it with someone who has had experience with a plankton counting operation.

1.2. If only one sample is taken per day, it should always be taken at the same time, as many plankton organisms move up and down at different times during the day. If possible, day and night samples should be taken, especially if the samples are taken at the surface.

1.3. Sampling stations or points should be as widely distributed as possible. A minimum program would include only samples taken from the raw-water line coming into the plant. Additional sampling stations might be set up: just ahead of the plant intake; out in the center of the broad area ahead of the intake; at the upper end of the reservoir; off the mouth of all major bays and shallow areas; in the plant before coagulation, between coagulation and filtration, and after filtration, and elsewhere, depending on local conditions.

1.4. Plankton samples may be of two types - "surface" or "depth." Surface samples are usually taken 6 to 12 inches below the surface. The exact manner of collecting the samples should be clearly stated at the outset of the sampling program since, if all samples at a given location are not taken in the same manner, the results from one time to the next cannot be compared and much of the value of the program is lost. The surface film should not be included unless it is always sampled in the same way each time.

Some kinds of "plankters" (plankton organisms) are regularly found at depths rather than near the surface. The specific depths sampled depend on the total depth of the reservoir and other factors. If the total depth is 30 feet, samples might be taken at the surface, at 10 feet, and at 25 feet. If the total depth is 100 feet, samples might be taken at the surface and at 15, 50, and 80 feet. Samples every 10 feet would be better.

2. Surface Sampling

To collect a surface sample, simply push the bottles down beneath the surface and allow it to fill at hand depth. If preservative is added to the sample bottle in advance, take the sample in a second container and immediately transfer it to the first bottle.

3. Depth Sampling

For depth samples, use the Kemmerer bottle (Fig. 21) in the following manner:

- 3.1. Mark off the line into feet or yards in some manner that will not interfere with the passage of the messenger. Use a code of paint or dye with a fabric line.
- 3.2. Tie a string through the hole in the messenger.
- 3.3. Thread the line through the messenger and the sampler, and knot it below the sampler.
- 3.4. Slip the 6 inch length of rubber tubing over the release valve on the bottom of the sampler.
- 3.5. Tie the messenger with a single bow knot to some object on the boat or wharf.
- 3.6. Slip enough line through the messenger to suspend the sampler.
- 3.7. Separate the two rubber stoppers and set the catch.
- 3.8. Lower the sampler to the desired depth, slipping the line through the messenger all the while (split messengers can be obtained which can be removed during this lowering operation).
- 3.9. Untie the messenger and release it down the line. A slight jerk will be felt as the messenger trips the catch and the sampler closes.
- 3.10. Pull the sampler to the surface and fill the sample bottle through the rubber tube on the release valve. Be careful not to lay the sampler down until all necessary samples have been obtained.
- 3.11. By working quickly, an approximate temperature reading may be made on the sample water at this time. These samples can also be used for certain chemical determinations.

4. Sample Preservation

It is not practicable to count living plankton samples when active forms are present. But many of these, such as certain protozoa, rotifers, and flagellates, are much more readily identified while alive. It is thus best, whenever possible, to preserve one portion of the sample immediately on collection, and to bring another portion into the laboratory fresh.

4.1. Unpreserved samples should be brought into the laboratory and studied as quickly as possible. Not over 30 to 60 minutes should be permitted to elapse in warm weather if possible, nor over 2 or 3 hours in cool weather. Such samples should be cooled in warm weather and kept from freezing in cold weather. In cooling plankton samples, the sample bottle should be well stoppered and packed in ice or other coolant; under no circumstances should ice be placed in the sample water.

4.2. The sample preservation procedures outlined below will not kill absolutely all organisms, but the samples will nevertheless be well enough stabilized for plankton counting. Formalin is irritating to the eyes and nose and tends to shrink and fade specimens, but is relatively permanent, cheap, and easy to obtain and use. Thimerosal is much milder on the operator and also produces much better specimens. It should not be relied upon, however, for over a month in warm weather, or six weeks in winter, unless stored in the dark.

It is usually convenient to put the preservative in the sample bottle before leaving the laboratory. In the field, the bottle should then be filled to some predetermined level such as, for example, 1 l. In counting, no correction is usually made for the volume of the preservative, as this small amount of error is considered to be inconsequential. To preserve 1 l samples, add 50 ml formalin or 35 ml thimerosal

solution to the sample bottle before removing it from the laboratory.

5. Composite Samples

Plankton samples may be combined or composited as are other types of samples - subject, of course, to the same restrictions on interpretation.

*6. Field Notes

The specimens are not the only useful product of field collections. Almost equally important are the notes that the sample taker makes about conditions at the time of sampling. This information often contributes greatly to the final interpretation of data. All available chemical and physical data are important, as well as the date, time of day, relative cloudiness, air and water temperatures, wind, color of the water, and any other factors that appear to be significant.

C. Examination of Samples

1. General

*1.1. Potable water supplies seldom need extensive treatment or preparation before microscopic observation. Organisms can be counted with reasonable accuracy in a Sedgwick-Rafter cell when present in numbers ranging from 100 to many thousands per milliliter. The simplicity of this treatment has advantages, since manipulations introduce errors. If concentration procedures or any other step can be eliminated, the resulting data are likely to be more reliable. Whenever practicable, therefore, it is recommended that the Sedgwick-Rafter slide be filled directly with the raw, untreated (or simply preserved) water.

If not more than 100 small forms can be found in the entire slide, this usually indicates the absence of a biologic cause for filter clogging or other problems. Even a few larger forms, however, may be of great significance.

1.2. If the quantity of some larger organism, such as water fleas or *Synura* colonies, is so low that many of the Sedgwick-Rafter slides prepared show none at all, it may be necessary to concentrate a larger volume of water in order to obtain enough to count. Concentration by the conventional Sedgwick-Rafter technique is described in *Standard Methods*. For a concentration procedure using the membrane filter, see Sec. 2 below.

1.3. Increasing importance is being ascribed to the smaller forms, especially diatoms, many of which pass through the Sedgwick-Rafter filtration. These forms can be identified only under oil immersion and are best concentrated by sedimentation procedures (Ref. 13).

2. Membrane Filter Procedures

In using the membrane filter, either of two procedures can be followed: The filtrate can be washed off and examined on a microscope slide; or the membrane can be made transparent and the plankton examined directly on the membrane.

The membrane filter technique is most useful with clear or finished waters. Since only a small volume of turbid or rich waters can be passed through the filter before it becomes clogged, it is better to examine the fresh, untreated sample in a Sedgwick-Rafter cell.

2.1. Washed-off filtrate method:

(a) Pass a known volume of water - 1 l or more, as appropriate - through the same type of filter used for bacteriologic purposes (see Part II of this Manual).

(b) Remove the filter from the apparatus and, holding it carefully by one side, wash the filtrate into a watch glass using a polyethylene medicine dropper and a small quantity of water. For more thorough washing, suck up some of the water from the watch glass and reuse several times.

(c) Make the volume up with distilled water to some convenient unit, such as 5 ml.

(d) Examine and count according to procedures outlined later in the Manual. When the count per milliliter of concentrate is found, the count per milliliter of original sample can be calculated as follows: multiply the count per ml concentrate by the volume of concentrate and divide by the volume of sample filtered.

Note: This procedure is suggested for rare forms only; consequently, it may be more convenient to report in terms of count per liter. In that case, multiply the count per ml sample as obtained above by 1,000.

2.2. Transparent-membrane method:

(a) Filter a known quantity of sample. The quantity selected should provide enough organisms visible in a microscope field to give a good count, yet not so many that they are piled on top of one another. The quantity must be determined separately for each water sample examined and may range from 2 or 3 ml to 100 ml or more.

(b) Dilute the quantity of sample to at least 50 ml with distilled water before filtering, in order to achieve even distribution over the membrane.

(c) Remove the filter from the apparatus and place it (or a portion of suitable size) on a 1 x 3 inch microscope slide.

(d) Cover with one or two drops of microscope immersion oil.

(e) Store in the dark at room temperature for approximately 24 hours, or until the filter becomes transparent. If kept in the dark, this preparation can be stored up to several months without fading, if desired.

(f) Cover with a No. 1 or No. 2 coverglass and place on the microscope. Examine and count according to the procedures outlined later in the Manual. (For additional information on counting and reporting, consult Ref. 22 and *Standard Methods*).

To calculate the count per milliliter of sample, proceed as follows: Divide the number of organisms counted by the volume of sample filtered. Then multiply this result by the total area of the filter and divide by the area of the filter counted. Some filters are marked off in squares, each of which is supposed to equal 1/100 of the total useful filtration area of the filter. If the organisms in one of these squares are counted, then the count per milliliter of sample equals 100 times the organisms counted divided by the volume of sample filtered.

*3. Drop and Coverglass Mounts

3.1. The drop mount is the simplest type of micro slide preparation. Place a drop of water on a clean slide and observe it under low power (10x objective and 10x eyepiece). Although it is impossible to focus such a mount clearly from top to bottom, a preliminary idea can be obtained as to whether there is anything to see in the water. (If the water does not stand in a drop, the slide is dirty or

there is too much water on the slide.)

3.2. For greater clarity, a coverglass may be needed with the drop mount in Sec. 3.1. Carefully lower the coverglass over the drop mount. If the coverglass is clean and free of fingerprints, no bubble need be trapped. A few bubbles, however, will do no harm for preliminary observations.

The water in the coverglass mount, containing the same organisms seen dimly in the drop mount, is now spread out in a very thin layer over an area the size of the coverglass. The organisms will be seen more clearly, but in much smaller numbers per field. Unless they are quite numerous, more time is needed to find them. This mount is ideal for use with the high-power dry objective lens when the organisms are small but abundant.

***4. Sedgwick-Rafter Cell or Slide**

Since the Sedgwick-Rafter cell is used for quantitative estimates, and not all parts of it may be observed, the organisms must be distributed as evenly as possible over the entire slide by filling the cell quickly before any particles have a chance to settle out at any one place. Proceed as follows:

4.1. Make sure that the slide and coverglass are perfectly clean. Place the coverglass on the slide so that opposite corners are slightly open (Fig. 17b).

4.2. Select a 1 ml pipet with a large opening at the tip so that the water will run out rapidly and the material suspended in it will not clog the pipet. A small opening may be enlarged easily by carefully grinding back the tip on an emery wheel, holding the pipet at an angle to the face of the wheel and rotating it slowly.

4.3. Shake the sample thoroughly but not violently. Avoid hard shaking, as this may break up colonies of delicate forms and thus artificially increase the count.

4.4. Quickly fill the pipet slightly over the 1 ml mark, apply the tip to an open corner of the cell, and release the water as rapidly as possible without losing control of the flow. The water should pull across underneath the coverglass by capillary attraction, expelling the air through the opening opposite the pipet.

4.5. When the slide is full, straighten the coverglass on the slide, if necessary.

4.6. If bubbles develop around the edge after prolonged observation, empty the slide and clean and refill it, being careful to mix the sample thoroughly again. Thinner coverglasses conform more closely to the surfaces of the cell walls and tend to reduce evaporation losses.

5. Nannoplankton Slide

The nannoplankton or Palmer-Maloney slide holds only 0.1 ml. It is intended only for counting the smaller forms which require the use of the high-power dry objective and which can be adequately sampled in 0.1 ml volumes. (The high-power dry objective cannot be used with the Sedgwick-Rafter cell.)

6. Special Preliminary Procedures

Routine procedure should begin with the observation of the fresh, unpreserved sample in a Sedgwick-Rafter cell. When the organisms present have been identified, the count or analysis should be completed as quickly as possible. If organisms are present which move about too rapidly or are too small for accurate identification and counting in the Sedgwick-Rafter cell, special preliminary procedures may be

necessary as described below. After these have been carried out and all of the types of organisms identified, the preserved sample (see Part B, Sec. 4) can be used for the count.

6.1. If the fast-moving organisms are small and abundant, a coverglass mount or nanoplankton cell mount may provide a close enough look so that they can be identified.

6.2. If the organisms still move too fast to see clearly, slow them down with methyl cellulose, as follows:

(a) Spread a drop of methyl cellulose over the area of a coverglass on a plain microscope slide.

(b) Place a drop of water containing the active organisms on the methyl cellulose.

(c) Cover with a coverglass and observe. At first, little difference may be noticed except for the presence of large methyl cellulose crystals. In a few minutes, however, individual organisms will slow down, making it possible to observe them easily, even with 40x magnification. A little experience will show just how much methyl cellulose to use and how best to apply it.

6.3. Large active organisms may also be slowed down for identification with methyl cellulose, or else identified in the preserved sample. The most difficult to deal with are the rotifers and certain other forms which usually fold up or collapse on preservation. Maintaining the methyl cellulose preparation for an hour or more, with plenty of water so that it does not dry up, will sometimes induce these forms to expand.

If a few large forms (water fleas, for example) are present in small numbers — less than two or three per milliliter — a portion of the sample should be concentrated and the count reported per liter or gallon. A rapid survey of the entire slide with a wide-field dissecting microscope may also be helpful.

D. Identification of Plankton

*1. General

The particular material visible under a microscope consists of mineral matter, dead vegetable matter, dead animal matter, and living vegetable and animal organisms. The plankton embrace both of the latter types of organisms, which must be differentiated from the surrounding debris. The mineral matter comprises such irregularly shaped and nontransparent particles as clay, sand, chalk, and iron corrosion.

Dead vegetable and animal matter may present some problems and demand experience to recognize. In the case of vegetable matter, a cell or fiber which has escaped complete disintegration can offer a clue to identity. The structure of dead animal matter may be more difficult to recognize. The fact that it is possible to see into and through the bodies of many organisms under a microscope helps in the detection of plankton in nonliving material.

Although there is no simple and easy way to learn to identify plankton, and first observations are likely to prove disappointing (because of the minute size of the

organisms, even under a microscope), it is not necessary to be a professional taxonomist to derive limited benefit from the microscopic examination of water. Practice will soon enable one to distinguish living particles from nonliving particles, and, at that point, plankton counting can begin.

Since accurate plankton identification calls for skilled judgments, training of some kind is strongly recommended. Formal training can be acquired at a college or university, or at a state or US Public Health Service training school; self-training can be accomplished by studying publications like those listed on pp. 125-6.

***2. Observation Technique**

2.1. Read the record of the field collector. Did the water in the reservoir show any particular color? Does the water in the sample bottle, when held up to the light, look faintly greenish, brownish, grayish, or some other color?

2.2. Set up the microscope and adjust the light or mirror to a suitable level of illumination with the 10x eyepiece and the 10x objective.

2.3. Shake or swirl the sample bottle thoroughly but not violently, and prepare a drop mount. Place it on the stage, and observe it. Since the drop of water is relatively thick, it will be necessary to focus from top to bottom with the coarse adjustments to observe all parts of the drop.

2.4. If the drop is seen to be swarming with great numbers of objects, whether they are all moving or not, stop at once, place a coverglass on the drop, and prepare for more intensive study.

2.5. If few or no floating objects can be found, moving or not, discard the drop, wipe the slide clean for the next use, and prepare a Sedgwick-Rafter cell mount. Remember always to swirl or agitate the sample bottle thoroughly, but not too violently, before removing a sample for examination.

2.6. Now examine the preparation under the microscope. Are there any particles, living or not, which resemble the color of the water in the glass? Since the color of microscopic organisms is most accurately judged in daylight, daylight or a close approximation thereof should be used for microscope illumination. Color in translucent or nearly transparent organisms under the microscope may be very faint. Not many colored particles are needed in a single microscope field to impart a distinct color to a mass of water.

Color may be due to nonliving particles of mineral or vegetable matter. The distinction between living and nonliving particles is one of the most subtle, yet one of the most basic in the whole field of plankton analysis. Regularity of shape and textural appearance in living cells are something for which practice alone can give adequate appreciation.

When no particles are found which match the color of the water, it may be due to dissolved materials, such as from decaying vegetable matter in a swamp or from industrial wastes like dyes. Certain bacteria also cause red and other water colors, but, being very minute, are not visible in the Sedgwick-Rafter cell.

***3. Water Color and Plankton Type**

3.1. Green, especially grass or yellow-green shades, is due to green algae.

3.2. Blue-green algae usually produce blue-green, and gray-green colors.

3.3. Grayish or whitish colors are uncommon except in polluted waters, where

they may be due to bacteria or protozoa.

3.4. Reddish colors, especially distinct "red clouds" in the water, may be due to microcrustacea such as water fleas (*Daphnia*, for example).

3.5. Brown colors, especially the darker shades, may be due to certain flagellated algae such as *Trachelomonas*. These are usually fairly large and easy to recognize.

3.6. Golden-brown colors may be due to diatoms or dinoflagellates.

3.7. Muddy, indistinct, and in-between colors are often due to mixtures of types, or to plankton plus suspended nonliving solids.

***4. Distinction Between Plants and Animals**

4.1. The ability to distinguish between plants and animals is of basic importance. Larger forms of both plant and animal life are relatively easy to recognize and identify. The larger floating or suspended plantlike forms usually are green and are composed of many units or "cells" readily visible to the unaided eye. They may resemble masses of tangled threads of filaments (algae), or they may have leaves and stems (in this case, however, they will not be true plankters). They cannot, of course, move under their own power. Some "higher plants" (not algae) consist only of tiny green plates of leaves 1/16 to 1/8 inch across, with tiny hanging roots, or of minute, oval green granules 1/32 to 1/16 inch long.

The larger planktonic animals have obvious animal characteristics. They may have eyes and legs and exhibit greater activity than plants. They range from colorless through various shades of red, yellow, brown, and black. Some may be wormlike, others resemble microscopic clams with legs. Though tiny, they may often be seen without a microscope.

4.2. Smaller forms, often minute, may consist of only a single unit or cell. In this group, the distinction between plants and animals is usually very simple: plant cells have color, animal cells usually do not. Movement is not a good basis for distinguishing between plants and animals among these smaller forms. Neither is the shade of color. Whether brown, yellow, red, blue-green, or green, they are "plants" if they possess the chemical chlorophyll. In the extremely small cells, however, color may be very difficult to determine.

***5. Classification Key**

Most of the published material on the identification of aquatic organisms is highly technical. The key in Table 10 is suggested to enable the beginner to decide in what general group his plankton forms belong. The key is strictly a game of "follow instructions." Many cases will arise where no clear-cut statements can be made without using technical terminology, or where descriptions are in relative terms only. In such cases, until more skill or training is acquired, record the organisms as "Undetermined No. 1," "Undetermined No. 2," etc., and pass on to the next type.

The key consists of a series of statements arranged in "couplets" (groups of two) to help the observer make a series of decisions about a particular specimen. Begin with couplet No. 1; decide which statement (1a or 1b) applies to the specimen under study. Then go on to the couplet indicated by the number printed at the right of the statement that applies. For example, if the specimen is microscopic — that is, too small to be seen with the naked eye — go to couplet No. 3; but if the

TABLE 10
Key to Plankton Types

| | Next Couplet | | Next Couplet |
|---|-----------------|--|-----------------|
| 1a. Organisms microscopic, visible only with a microscope (less than approximately 1 mm in length) | 3 | 6b. Plants golden or golden brown in color, with tendency to sharply angular borders. Cell walls rigid, often with regular patterns visible. May be round, boat-shaped, or thread-like. Some boat-shaped or elongated types may exhibit jerky back-and-forth or side-to-side movement. Others may cling side by side to form ribbons; others end to end to form other shapes of clusters: <i>Diatoms</i> . | |
| 1b. Organisms larger, over 1 mm in length. | 2 | | |
| 2a. Color green, structure usually filamentous or stringy | 6 | 7a. Plant consists of thread or filament, or of mass of filaments or filament-like structures: <i>Filamentous Green Algae</i> . | |
| 2b. Structure buglike or wormlike, or with two clamlike shells. Eyes, legs, hairs often visible | 9 | 7b. Plant consists of unit of one cell or several cells in compact, non-filamentous mass or group: <i>Coccid Green Algae</i> . | |
| 3a. Colored or pigmented (color will tend to fade in time in preserved samples). Descriptions below refer to live or freshly preserved material . . . | 4 | 8a. Body composed of single unit or cell. May be enclosed in sheath or case. May exhibit beating of tiny hairlike cilia when alive. Tends to shrivel and become unrecognizable when preserved, except for cases or shells: <i>Protozoa</i> . | |
| 3b. Colorless or transparent (colored food particles may sometimes be seen inside the body) | 8 | 8b. Body composed of many cells, structure relatively complex (but no legs). Crowns of hair-like cilia beat actively in head region in life, draw in and lose shape on preservation: <i>Rotifers</i> . | |
| 4a. Cells containing internal structures such as special color-carrying bodies (chloroplasts), nuclei, etc. | 5 | 9a. Eye(s) and legs visible, or eyes and elongated transparent body | 10 |
| 4b. Cells with no internal structure evident. Pigment dispersed throughout. Generally bluish or blue-green in color. Color may be very faint under microscope. Single cells may be very small. Some filamentous forms motile: <i>Blue-Green Algae</i> . | | 9b. Body enclosed in two minute clamlike shells, within which all legs can be drawn. Jointed legs extend from between shells for swimming. Shells usually opaque. Microcrustaceans: <i>Ostracods</i> . | |
| 5a. Plant consists of single cells or groups of cells, free to move about as units. Red eyespot present. One or more hairlike flagellæ per cell give strong mobility to single cells, rolling motion to clumps. Some types may change shape while swimming; some have long spines: <i>Flagellates</i> . | | | |
| 5b. Not as described in 5a | 6 | | |
| 6a. Plants green, any size or shape, but lacking flagellæ. | 7 | 10a. Single dark eye, often with many lenses attached | 11 |

TABLE 10 (Cont.)

Key to Plankton Types

| | |
|--|--|
| 10b. Two eyes; elongated, segmented, transparent body up to 1/2 inch in length. Midge fly larva: <i>Chaoborus</i> . | 11b. Body relatively transparent, more cylindrical, no shell extending down over legs. Two large front appendages or legs (actually antennae) used for locomotion, remaining appendages shorter. Young smaller in size, body more rounded, three pairs of conspicuous appendages: <i>Nauplius larvae</i> . Two reddish or orange egg masses often trailed by female: microcrustacean: <i>Copepod</i> . |
| 11a. Two shelllike covers come down on either side of the body. Legs extend from between shells. Eggs often present in large pouch inside upper back part of shell. Beak extends below eye. Microcrustacean: <i>Branchiopod</i> or <i>Water Flea</i> . | |

specimen is large enough to see (not to study) without a microscope, go to couplet No. 2. Continue in this way, looking at the specimen frequently to refresh your memory, until you come to a true statement that is followed by a name instead of a reference to another couplet. This is the name of the group to which the specimen belongs.

The broad, general groups named in the key may be acceptable for preliminary surveys or as general indications of conditions. More detailed identification is required for more conclusive work. For this kind of work, use a more technical and complete key. However, until you have acquired skill in more detailed identification, have your conclusions checked by a specialist.

If the key does not seem to work for a given specimen, assume that it is an exceptional form that requires a more technical treatment. Set it aside as undetermined and proceed to the next specimen. Not included in the key are forms that normally grow attached to the bottom, things that grow on land, duckweed, pollen grains, and eggs or young of various animals.

For illustrations of the organisms described in the key, see *Standard Methods* and the references listed on pages 125-6, especially Ref. 2.

E. Counting Procedures

The so-called counting of plankton includes two aspects: the enumeration of the various kinds (qualitative) and the counting of the numbers of individuals (quantitative).

1. Procedure for Qualitative Study

A qualitative study should always be made with a higher-power magnification

before starting a Sedgwick-Rafter count, unless the local plankton are already well known to the operator. In making a qualitative study, proceed as follows:

1.1. Prepare a coverglass mount.

1.2. Scan the slide with 100x magnification (or lower, if available) to identify the larger forms or those already familiar.

1.3. Use the high-power dry objective (400x) to identify small species not recognizable under lower powers.

1.4. Use the oil immersion objective to identify very minute forms, especially diatoms. Have very little water underneath the coverglass when using oil with fresh preparations. Diatoms are best observed in permanent-type mounts (Ref. 13). The beginner should not attempt to classify diatoms as to genus and species, except for a few distinctive forms.

Note: The nanoplankton cell may also be used for qualitative studies at 400x magnification (see Sec. 10 below).

1.5. Compile a list of all organisms identified. Group these according to major types. Number the forms not identified, and attach rough sketches to the list so that, if they are identified at some later date, the list can be completed. This list is useful by itself, but its usefulness will be greatly increased in connection with many of the procedures outlined below.

2. Proportional Counts

If, in addition to listing species or kinds, a record is made of relative numbers — for example, “60% Species A, 25% Species B, 10% Species C, etc.” — the study becomes a proportional count. This very useful type of count materially increases the value of the study.

3. Differential Counts

A differential count is a proportional count which has been extended, by the techniques described below, to counts per milliliter. It has the greatest potential usefulness of any type of count, since it tells the operator not only what kinds of organisms are present, but how many and in what proportion. It is also the most difficult to count to make because it requires the ability to recognize each species or kind in the Sedgwick-Rafter cell on sight.

*4. Quantitative Counts

Plankton quantities are commonly reported in units per milliliter. Units may be individual cells or organisms, “clump units,” or “standard units.” The distinction between cells or organisms and clump units is based on whether the individual cells of colonies, filaments, or other such associations are counted separately, or whether the entire clump of cells, colony, or filament is counted as one clump unit. The following sections refer to the clump unit count (or “clump count”). (For a discussion of standard units and other methods, see *Standard Methods* and Ref. 3 or 14.)

While some of the following procedures can be carried out without a Whipple or plankton counting disk in the eyepiece, all are greatly aided by it; it is therefore assumed that the disk has been installed.

*5. Calibration of Microscope

For preliminary estimations or in the beginning stages of a plankton analysis program, it is assumed that magnifications are exactly as stated. A 10x ocular with a 10x objective, for example, gives a theoretical magnification of 100x. Slight variations in the grinding and mounting of lenses or differences in manufacturing processes may, however, result in significant variations in actual magnification. Changes in the interpupillary setting of binocular microscopes or in the drawtube setting of a monocular microscope also change the magnification. In the range of 200x magnifications, some manufacturers provide 20x objectives to be used with 10x oculars, and others provide 21x objectives. For accurate work, each microscope should be calibrated against an absolute standard (a stage micrometer) for each lens combination and for each tube or interpupillary setting. (For instructions, see *Standard Methods* or Ref. 19; also note Sec. 8.3, p. 145.)

*6. Use of Sedgwick-Rafter Cell

The Sedgwick-Rafter cell is 1 mm deep, 20 mm wide, and 50 mm long. It thus contains 1,000 cubic millimeters or 1 ml. Because of its depth, it is necessary to

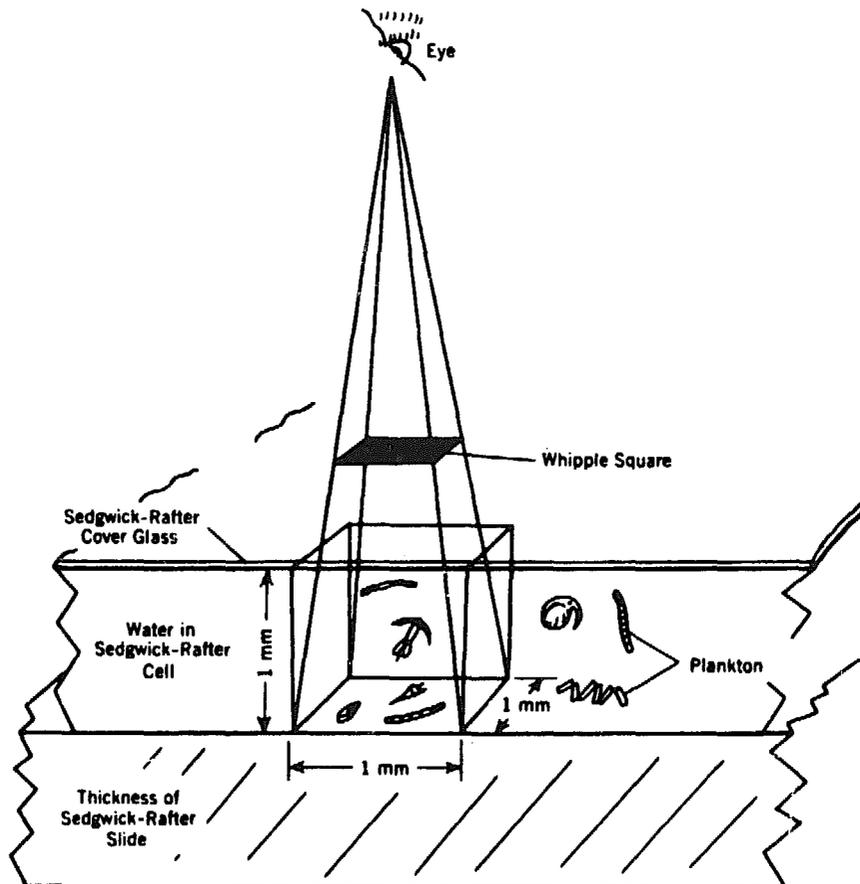


Fig. 22. Cube of Water as Seen Through Whipple Square

Only organisms lying on bottom of Sedgwick - Rafter cell are in focus; focus must be shifted in order to study entire cube.

move the focus from top to bottom for each field in order to find both the plankton types that sink to the bottom and those that float (see Fig. 22).

***7. Survey Counts**

The survey count is a count of all individuals of some particular kind in the entire cell. It is usually used only for the larger organisms, such as water fleas or *Cyclops*, which cannot be adequately sampled in smaller volumes. A stereoscopic microscope or a 5x eyepiece on a compound microscope is usually used.

***8. Strip Counts**

The strip count (see Fig. 23) is the easiest count to make in a Sedgwick-Rafter cell using a 100x (10x objective and 10x ocular) or a 200x (20x objective and 10x ocular) magnification. (The 20x objective is the highest power that can be used with a Sedgwick-Rafter cell; it should be obtained whenever possible.) A mechanical stage is required. Proceed as follows:

8.1. Count a strip the width of the Whipple field and the entire length of the Sedgwick-Rafter cell.

8.2. Multiply the number of cells counted by 20 when using a 100x magnification, or by 40 when using a 200x magnification. The result gives an estimate of the number of cells per milliliter.

8.3. To obtain a more accurate multiplier, "step off" the width of the Whipple field onto the width of the Sedgwick-Rafter cell under the microscope (see Ref. 19).

***9. Separate Field Counts**

The separate field count may be used with a mechanical stage. If no mechanical stage is available, then it is the only method that can be used for quantitative counting with the Sedgwick-Rafter Cell. Proceed as follows:

9.1. Count the organisms in ten widely separated Whipple fields. Avoid counting fields too near the ends or sides of the cell; a distance about one-third out from the side is considered best. For organisms that fall on the line, one method is to count those that are more than half inside, and to ignore those that are less than half inside. Another method is to count all organisms touching the top and right sides of the square from any direction, and to ignore all those touching the left side and bottom.

9.2. To obtain the count per milliliter, multiply the count obtained by 100, if working at 100x, or 400 if working at 200x magnification.

***10. Use of Nannoplankton Cell**

To obtain magnifications greater than 200x when making quantitative counts, the 40x objective may be used with the nannoplankton cell. This cell has a depth of only 0.4 mm, which usually permits its use even with a No. 2 thickness cover-glass. Owing to the different dimensions of the cell, however, particular attention is required for calibration and interpretation (consult *Standard Methods* or Ref. 19). This cell can be very useful for special situations. For example, since the cell volume is 0.1 ml, the entire slide may be surveyed for large or rare forms and the result multiplied by 10 to give a count per milliliter. Heavy plankton concentrations,

such as in waste stabilization ponds or activated sludge tanks, may also be quantitatively estimated by the use of this cell. The Sedgwick-Rafter cell would provide entirely too thick a film to see through.

***11. Records and Forms**

No single format for keeping plankton records can satisfy all needs. Excellent plans are offered in Ref. 2 and 18. Other plans are available in Ref. 3, 14, and *Standard Methods*, as well as from water plants which perform plankton analyses. What is most important is the establishment of a planned record-keeping program which will permit comparative evaluations.

F. Significance and Application of Results

Algae are normal and constant inhabitants of nearly all natural surface waters. Wherever algae grow, there, too, will be found bacteria, fungi, and various animals. These different organisms interact in the open waters to carry on a chain of life. The algae, as green plants, use the dissolved solids (nutrient minerals), water, and carbon dioxide to build themselves up. Bacteria and animals feed on the dead of live organic algae material. The bacteria in turn die, and their bodies are broken down by other bacteria, thus eventually returning the elements to the mineral condition. If any part of this cycle were to be eliminated, the resulting water might contain materials that would cause tastes and odors or be otherwise undesirable. The living creatures serve to stabilize the water and to degrade or decompose foreign material such as pollution that may get in. Plankton are thus not always altogether bad. (A more complete discussion of the various effects of plankton will be found in Ref. 2.)

The more frequently observations are made, the greater the likelihood of noting the beginning of an upswing in abundance of plankton. Counts may drift along at a few hundred per milliliter for an extended period, then suddenly jump to thousands within two or three days. This increase may result from sheer growth of the plankton population stimulated by a change in the weather or by the spring or fall overturn of a stratified reservoir, or it may result from a mass of plankton drifting out of some fertile backwater or tributary. Such occurrences again emphasize the importance of field measurements to determine water temperatures and other associated factors at various depths, and the day-to-day distribution of plankton in the various parts of the reservoir.

***1. Effect on Plant Operation**

Excessive numbers of one or another of the plankton types may cause no end of trouble in water treatment plants — shortened filter runs, tastes and odors, difficulties with flocculation and sedimentation. It is thus important for the plant operator to know what is in the water.

No specific rules can be set down here which will account for all local circumstances. It would be entirely misleading to cite a series of plankton counts and imply that the consequences of each would always be the same. In general, it has been observed that counts of a few hundred organisms per milliliter are seldom

associated with serious plant troubles, whereas counts or blooms of more than 2,000 or 3,000 per milliliter are often significant. Even in relatively small numbers, however, certain types of plankton can cause problems. The flagellate *Synura*, for example, is reported to cause a detectable odor in numbers of 20 to 30 colonies or less per milliliter, and concentrations of 100 or more can lead to serious troubles. Diatoms such as *Synedra*, *Tabellaria*, or *Melosira* will appreciably reduce filter runs in concentrations of a few hundred per milliliter.

*2. Diagnosis of Water Quality

Microscopic examination of a water enables the determination of the real nature of the turbidity and color of the water. It is important to know whether a turbid condition is due to inert suspended material which may be expected to settle out in a few days, or to plankton that may grow into a nuisance.

A great deal can be learned about the quality of a water by an examination of the plankton it contains. Although information is far from complete on this score, certain generalizations can be made:

Severely polluted, warm, or hard waters tend to encourage blue-green algae.

Cold and clean waters generally favor diatoms.

Green algae tend to be more abundant in spring and late fall.

Blooms of animal plankton are often associated with partially stabilized pollution.

Although exceptions can be found in nearly every group of plankton, this type of information can nevertheless be very helpful in managing a body of water. For a more extensive discussion of this topic, see Ref. 2.

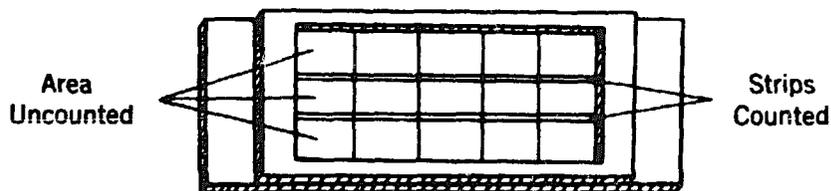


Fig. 23. Strip Counting

"Strips" are not marked on slide but simply represent area counted.

*3. Importance of Field Observations

The field notes should always be studied to ascertain whether or not high winds, a cloudy day, unseasonable temperatures, or some other factors may have affected the collection. These factors should likewise be taken into account in the application of any corrective measures that may seem to be indicated.

*4. Prediction of Blooms

The specific use to which plankton counts are put will differ from plant to plant, as implied above. It should be emphasized, however, that sudden plankton growths or blooms tend to occur year after year at about the same time. Thus, one of the great benefits that can result from a systematic program of plankton counting is

the ability to forecast expected blooms. This ability makes it possible to stock activated carbon, chlorine, or some other critical material in advance of a sudden demand. If the raw-water supply permits the application of control measures, the first appearance of a known troublemaker at a certain time of the year may be the signal for an application of copper sulfate, thus preventing a difficult problem from developing.

***5. Plankton Control**

Two general approaches are available for the control of plankton in surface waters: reduction of fertility (pollution control) or chemical control.

5.1. Pollution control is usually an area-wide problem which is often beyond the jurisdiction of water utility personnel. Information on the occurrence of pollutional plankton in the water supply, however, can frequently be helpful to local pollution control agencies. Pollution control and flow regulation are among the few techniques available for the control of plankton in rivers.

5.2. No satisfactory substitute has so far been found for copper sulfate in the chemical control of plankton in lakes and reservoirs. Dosages of copper sulfate for plankton control are well established in most localities. (Reference 2 gives a table showing the relative toxicity of copper sulfate to various species of algae; this might enable an operator to save significant quantities of chemical by taking cognizance of the kind of algae present.)

***6. Selection of Proper Level for Drawoff**

If the plant has the capacity to control the depth of the reservoir drawoff, a knowledge of the plankton and other water characteristics at the various depths will enable the operator to select the most favorable level. This is not always a deeper level, as some plankton forms may concentrate at 15 to 20 feet and be virtually absent at the surface. This situation may change from night to day, as animal forms may move upward at night and certain algae may move downward. There is a general tendency for plankton to be more uniformly distributed vertically at night than in the daytime.

***7. In-Plant Treatment Control**

Many operators make use of in-plant plankton analyses to determine the effectiveness of treatment. Samples analyzed before and after one or more of the various treatment processes will show their efficiency in the physical removal of plankton and other particulate material.

***8. Management of Reservoir Systems**

Because of the simplicity of the analyses as well as the importance of plankton, plankton analyses and associated observations are often useful in the management of water supply systems involving two or more reservoirs or other bodies of water. Such observations may serve to signal the approach of a turnover or warn of the existence of a slug of undesirable water or other dangerous condition. This information may permit the affected reservoir to be taken out of service before the undesirable water contaminates the system. Plankton analyses also are helpful in determining when such a reservoir is again ready for use.

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Supplement to Simplified Procedures for Water Examination— Laboratory Manual

I-a Instrumental Methods

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Foreword

Instruments are becoming established in many phases of daily activity and the water-treatment plant laboratory is no exception. Short term and lengthy training courses are emphasizing the importance of instruments for precise and accurate analyses. Recognition and promotion of this trend is provided in this supplement to *Simplified Procedures for Water Examination*. Attention is focused on the common instruments that can benefit the plant operator rather than the sophisticated or specialized analyst. In keeping with the objective of the original manual, M12, the procedures are intended to help improve the operation of a water-treatment plant.

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I-a Instrumental Methods

Instruments for chemical analysis are commonly used in most water treatment plants. Several reasons account for this development. There is a saving in analysis time coupled with an improvement in the sensitivity and accuracy with which the determination can be performed. Instruments can eliminate human bias, can often overcome the color and turbidity interferences that beset visual estimations, and in many cases minimize the number of standards that must be prepared for colorimetric work.

Instruments are no cure-all. They must be used properly and checked frequently to avoid the possible false values that can emanate from defective equipment and/or improper operation.

Most of the colorimetric methods described in the chapter, Chemical Examination, can be completed by an instrumental finish as well as a visual color comparison. In addition to photometric methods, this supplement will touch briefly on the common instrumental determinations for residual chlorine, turbidity, pH, fluoride, and conductivity—all of which are on the routine schedule of quality water-treatment plant laboratories. Emphasis has been directed to the inexpensive instruments priced at less than \$1000 rather than to the sophisticated digital and automatic models.

Significant Figures. A reported analytical result should contain only the numbers that are warranted by the test. Except for the last number, all the designated digits should be known with certainty.

Laboratory measurements are conducted with varying care and precision. Samples can be measured with nessler tubes, graduated cylinders, or pipets, depending on the purpose of the analysis and the results expected. Volumetric pipets are normally reserved for the most exact operations. Solid chemical reagents can be weighed to less than a milligram on many analytical balances. Titrations can be performed with burets bearing 0.1 or 0.05-ml markings. Solutions for chemical analysis can be prepared with the desired degrees of correctness corresponding with the overall needs.

In general, the instruments described in this supplement can be read with certainty to two figures and a third figure can be estimated; hence, the reported result will normally contain three or fewer figures. However, constant attention should be paid to the fact that the end result will be controlled by the measurement made with the least precision—the weakest link in the chain. The final reported value should be rounded out to reflect the least precise (worst) rather than the most precise (best) measurement.

Photometric Methods

1. Purpose of Test

The photometer has become a valuable fixture in modern laboratories because of its precision and accuracy as well as its saving of working time. The instrument can measure small differences in color intensities not easily seen by the human eye, thereby eliminating the variable human factor in visual color estimation. Other advantages include freedom from changing natural light conditions, dissimilar lamp spectra, and analyst color blindness—all of which can adversely affect visual estimations.

The improved precision and accuracy that is possible with the photometric approach provides a simple means of evaluating the applicability of an analytical method to a water sample of unknown composition. The procedure called *standard addition* or *spiking* involves the introduction of known amounts of the constituent to be measured into separate but equal portions of the sample; and then observing the completeness of the recovery. A sufficient amount of the constituent must be added to overcome the limits of experimental error.

2. Warning

Photometers yield the best results in the middle of their operating range represented by a 20 per cent transmittance or 0.70 absorbance on the lower end of the scale and an 80 per cent transmittance or 0.10 absorbance at the upper end. At low transmittance or high absorbance, 10 per cent or 1.0 respectively, the instrument responds insensitively to appreciable color and concentration changes. At high transmittance or low absorbance, 95 - 100 per cent or 0.02 respectively, slight construction differences in the cuvettes (sample containers or cells) and their placement in the sample compartment can materially affect the readings. Errors can also result from the presence of condensed moisture, dust, or fingerprints on the cuvette surfaces, or bubbles within the solution. Under such circumstances, visual comparison of very faint colors in nessler tubes may be preferred to photometric measurements in the 90 - 100 per cent transmittance or 0.04 absorbance zone.

The analyst should be alert continuously to the emergence of off-colors and turbidity in the samples and standards that can easily pass unnoticed during photometric measurements. Since the instrument provides a reading, albeit a questionable one, *every* unusual value should be verified to remove suspicion about its validity.

On the physical side, heed should be paid to the possibility of fatigue and loss of sensitivity in the photocells, and fluctuations in the line voltage and electrical and mechanical failure in the instrument.

Sound practice calls for the preparation of a reagent blank along with every set of samples and at least one standard in the upper end of the optimum concentration range in order to confirm the reliability of the calibration curve, reagents, instrument, and technique of the analyst. When instruments are used for hourly readings, they should be checked once or twice during every shift.

3. Apparatus

3.1 A photometer consists of five basic components: (1) a light source, (2) a wavelength control unit, (3) a detector, (4) a meter, and (5) a sample compartment. A bulb produces white light that is a mixture of several colors. When white light is passed through a color filter, grating, or prism, a desired color may be isolated. The selected color is generally complementary to that in the developed sample. The selected light pass-

es through the treated sample and the extent of absorption (blackness) is registered on a detector connected to a meter graduated in per cent of transmittance or logarithmic absorbance units. The sample compartment may accept a fairly broad range of vessels from 10-mm diameter test tubes and vials to special cells of 100-mm light path or more. Longer light paths enable the estimation of lower concentrations. For the most part, the dimensions of the sample containers called cuvettes fall into the 25-mm or less class. The final selling price governs the refinements to be included in the particular instrument.

3.2 Operating precautions:

Several precautions are advisable in the operation of all instruments. Each instrument must be zeroed and standardized according to the manufacturer's directions. The cover on the sample compartment must be kept tightly closed during instrument measurements in order to minimize light leakage. The sample compartment should be kept clean and dry at all times to prevent impairment and corrosion of the vital units. The external surfaces of the cuvette must be wiped clean with a soft cloth or cleansing tissue to remove dust, liquid, and smudge marks that can cause incorrect readings. Cuvettes must be rinsed free of remains from previous tests to ensure reliable readings of the standards and samples under examination. Distilled-water rinses may be necessary to achieve the desired removal. Since instruments vary in their readout systems, the proper eye position is important when making an accurate reading.

3.3 Check of accessories:

Upon delivery, the instrument and the supplied apparatus should be examined for operating condition and adequacy. The cuvettes should be inspected for the presence of surface nicks and scratches that could adversely affect the instrumental readings.

The placement of the cuvettes in the sample compartment deserves special attention, and the evaluation can be conducted in the following manner.

a. After the instrument has been turned on and allowed to warm up for several minutes, set the wavelength control at 510 nanometers or insert a blue-color filter in the proper slot.

b. Fill each cleaned cuvette to the same level with distilled water, and wipe the fingerprints, dust, and smudges off the exterior surfaces with a soft, lint-free cloth or cleansing tissue.

c. Introduce the first cuvette into the sample compartment and adjust the meter to a 95.0 per cent transmittance or 0.020 absorbance reading.

d. Withdraw the first cuvette and replace in turn by the other cuvettes, recording the transmittance or absorbance readings of each individual cuvette.

e. If necessary, rotate the tubes or vials in the sample compartment so that all readings are the same (95.0 per cent or 0.020 absorbance) using the remaining tubes or vials in the purchased set. (A difference of 0.5 per cent in transmittance or 0.002 absorbance readings between individual tubes may be tolerated.)

f. Permanently mark each tube to indicate the proper placement in the tube holder in order to achieve the 0.5 per cent or 0.002 absorbance tolerance.

g. Repeat this procedure by replacing the distilled water with the potassium chromate solution described in paragraph 3.4b in order to identify the cuvettes and tubes that have matching internal path lengths and thus produce identical results. In the latter case, make the transmittance or absorbance readings at the 60 per cent or 0.222 absorbance point on the meter scale rather than the 95.0 per cent or 0.020 absorbance point used with distilled water.

3.4 Check of instrument function:

a. Although several instrument checks are possible, make at least one routine operating check when the instrument is used only occasionally. When used hourly make checks once or twice a shift.

b. Dissolve sufficient potassium chromate (K_2CrO_4) crystals in distilled water to form

a solution that will yield a transmittance in the 35 - 60 per cent or 0.450 - 0.220 absorbance range.

c. Record for future reference the exact transmittance or absorbance reading of the potassium chromate solution when the instrument is functioning properly. Initiate prompt corrective measures when routine check readings depart from this figure.

d. Since the potassium chromate solution is relatively stable, prepare a minimum of 1-liter solution for use over an extended period of time. Discard the small quantity needed for a routine check rather than risk the contamination that might occur by returning the potassium chromate test solution to the original storage bottle.

4. General Procedure for Preparing Calibration Curve

4.1 Prepare the calibration curves for the colorimetric methods described in this manual by observing the suggested guidelines for the wavelength, color filter, light path, and concentrations presented in the following table.

| Constituent | Colorimetric Method | Wavelength nanometer | Color Filter | Light Path cm | Concentration Range mg/l |
|----------------------|--|-------------------------|--------------|------------------|---|
| Aluminum | Aluminon | 525 | Green | 1 | 0.05 - 1 |
| Ammonia Nitrogen | Nessler | 400 - 425 | Violet | 5 1 | 0.05 - 1 0.4 - 5 |
| Chlorine Residual | <i>N, N-diethyl-p-phenylenediamine</i> (DPD) Orthotolidine-arsenite | 515 | Green | 1 | 0.05 - 4 |
| | | 400 - 450 | Violet | 5 1 | 0.02 - 0.3 0.10 - 1.5 |
| | | | Blue-green | 1 | 0.5 - 7 |
| Chlorine Dioxide | <i>N, N-diethyl-p-phenylenediamine</i> (DPD) | 515 | Green | 1 | 0.05 - 4 as chlorine 0.03 - 1.5 as chlorine dioxide |
| Copper | Cuprethol | 435 | Violet | 1 | 0.05 - 1 |
| Iron | Phenanthroline | 510 | Green | 5 1 | 0.01 - 0.08 0.10 - 0.4 |
| Manganese | Persulfate | 525 | Green | 5 1 | 0.02 - 0.4 0.10 - 1.5 |
| Phosphate | Molybdenum Blue | 650 - 690 | Red | 5 1 | 0.04 - 0.5 0.3 - 2 |
| Silica | Molybdosilicate | 410 | Violet | 5 | 0.08 - 5 |
| | | | | 1 | 4 - 25 |

4.2 Since each colorimetric method has a well defined concentration range, narrow or enlarge the concentration range to a certain extent by varying the wavelength or color filter and the dimensions of the light path. Beware of interfering colors that could nullify such a step. Use a wavelength or color filter that preferably yields a straight calibration line rather than a curved line. A curved line can be used if the curvature is not severe.

4.3 Using distilled water* and a standard solution of the constituent under examination, prepare at least five standards encompassing the lower and upper concentration range and three equally spaced concentrations in between plus a reagent blank.

4.4 Add the reagents in the required sequence to develop the color in each standard.

4.5 Follow the exact steps that are performed in the analysis of the samples. Pay special attention to proper spacing and timing of the standards and samples when color development time is important for a correct result.

4.6 Pour an adequate volume of each developed standard into separate, clean, and matched sample tubes, vials, or cuvettes that fit into the photometric instrument that is available.

4.7 Adjust the instrument to the correct wavelength or insert the most suitable color filter into the designated slot.

4.8 After sufficient warm-up time, zero the instrument with distilled water or a reagent blank prepared as specified for the constituent of interest. In the case of line-operated laboratory models, allow the instrument to warm up prior to this stage and maintain on a standby basis until ready to make measurements.

4.9 Note the absorbance or per cent of transmittance meter reading for each of the standards. Adopt the correct eye position while making the reading.

4.10 Make sure that the readings are made with the sample compartment tightly closed.

4.11 Plot the instrumental readings versus concentration in one of two ways to obtain the calibration curve:

a. Plot the absorbance versus concentration on ordinary coordinate graph paper, or
b. Plot the per cent of transmittance on the logarithmic scale versus the concentration on the linear scale of semilogarithmic graph paper.

c. Draw a smooth curve connecting the points. A straight line starting from the zero point on the graph indicates an ideal color system (conforming with Beer's law) for photometric use. Such a curve is illustrated in the figure on page S-109. Although calibration curves tend to deviate from straight lines at high concentrations of the sought constituent, other cause of deviation may originate in such instrumental factors as a broad band width of the color filter, stray light due to light leaks, optical imperfections, or improper optical alignment or maintenance.

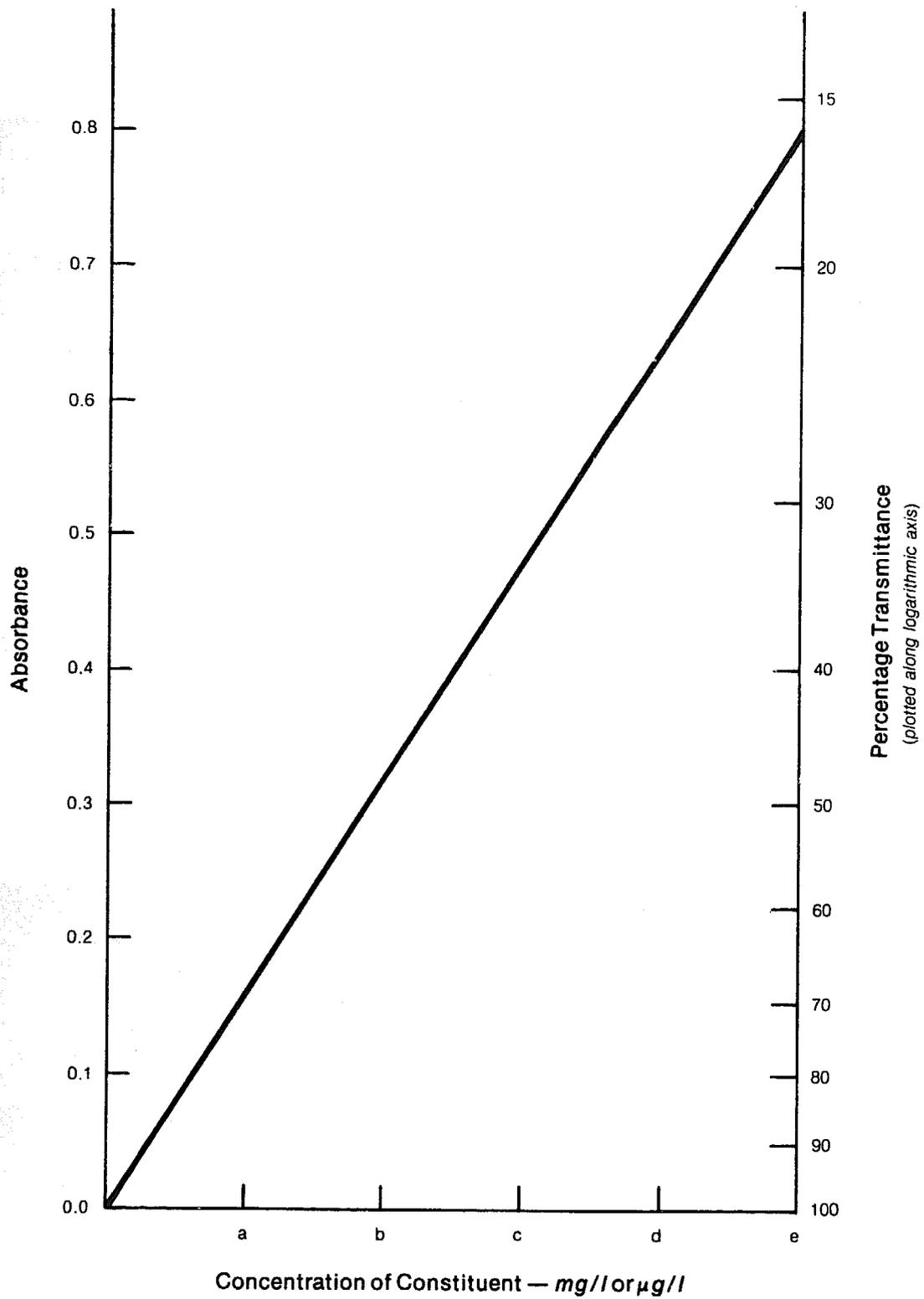
The table on page S-110 permits the conversion of per cent of transmittance to absorbance readings in the event that semilogarithmic graph paper is unavailable.

5. General Procedure for Unknown Water Samples

5.1 Handle unknown water samples by zeroing the instrument with one of the two following blanks as circumstances dictate:

a. Use distilled water as the blank when the water sample is clear and colorless.
b. Take for a blank a portion of the original untreated sample that contains natural color or turbidity. Such a blank will often nullify the false photometric reading contributed by the natural color or turbidity.

*Filter distilled water through a 0.45- μ m membrane filter when interfering levels of turbidity (0.5 - 3.0 or more nephelometric turbidity units) are present.



Typical Photometric Calibration Curve

| Transmittance <i>per cent</i> | Absorbance |
|----------------------------------|------------|----------------------------------|------------|----------------------------------|------------|----------------------------------|------------|
| 1.0 | 2.000 | 26.0 | .585 | 51.0 | .292 | 76.0 | .119 |
| 1.5 | 1.824 | 26.5 | .577 | 51.5 | .288 | 76.5 | .116 |
| 2.0 | 1.699 | 27.0 | .569 | 52.0 | .284 | 77.0 | .114 |
| 2.5 | 1.602 | 27.5 | .561 | 52.5 | .280 | 77.5 | .111 |
| 3.0 | 1.523 | 28.0 | .553 | 53.0 | .276 | 78.0 | .108 |
| 3.5 | 1.456 | 28.5 | .545 | 53.5 | .272 | 78.5 | .105 |
| 4.0 | 1.398 | 29.0 | .538 | 54.0 | .268 | 79.0 | .102 |
| 4.5 | 1.347 | 29.5 | .530 | 54.5 | .264 | 79.5 | .100 |
| 5.0 | 1.301 | 30.0 | .523 | 55.0 | .260 | 80.0 | .097 |
| 5.5 | 1.260 | 30.5 | .516 | 55.5 | .256 | 80.5 | .094 |
| 6.0 | 1.222 | 31.0 | .509 | 56.0 | .252 | 81.0 | .092 |
| 6.5 | 1.187 | 31.5 | .502 | 56.5 | .248 | 81.5 | .089 |
| 7.0 | 1.155 | 32.0 | .495 | 57.0 | .244 | 82.0 | .086 |
| 7.5 | 1.126 | 32.5 | .488 | 57.5 | .240 | 82.5 | .084 |
| 8.0 | 1.097 | 33.0 | .482 | 58.0 | .237 | 83.0 | .081 |
| 8.5 | 1.071 | 33.5 | .475 | 58.5 | .233 | 83.5 | .078 |
| 9.0 | 1.046 | 34.0 | .469 | 59.0 | .229 | 84.0 | .076 |
| 9.5 | 1.022 | 34.5 | .462 | 59.5 | .226 | 84.5 | .073 |
| 10.0 | 1.000 | 35.0 | .456 | 60.0 | .222 | 85.0 | .071 |
| 10.5 | .979 | 35.5 | .450 | 60.5 | .218 | 85.5 | .068 |
| 11.0 | .959 | 36.0 | .444 | 61.0 | .215 | 86.0 | .066 |
| 11.5 | .939 | 36.5 | .438 | 61.5 | .211 | 86.5 | .063 |
| 12.0 | .921 | 37.0 | .432 | 62.0 | .208 | 87.0 | .061 |
| 12.5 | .903 | 37.5 | .426 | 62.5 | .204 | 87.5 | .058 |
| 13.0 | .886 | 38.0 | .420 | 63.0 | .201 | 88.0 | .056 |
| 13.5 | .870 | 38.5 | .414 | 63.5 | .197 | 88.5 | .053 |
| 14.0 | .854 | 39.0 | .409 | 64.0 | .194 | 89.0 | .051 |
| 14.5 | .838 | 39.5 | .403 | 64.5 | .191 | 89.5 | .048 |
| 15.0 | .824 | 40.0 | .398 | 65.0 | .187 | 90.0 | .046 |
| 15.5 | .810 | 40.5 | .392 | 65.5 | .184 | 90.5 | .043 |
| 16.0 | .796 | 41.0 | .387 | 66.0 | .181 | 91.0 | .041 |
| 16.5 | .782 | 41.5 | .382 | 66.5 | .177 | 91.5 | .039 |
| 17.0 | .770 | 42.0 | .377 | 67.0 | .174 | 92.0 | .036 |
| 17.5 | .757 | 42.5 | .372 | 67.5 | .171 | 92.5 | .034 |
| 18.0 | .745 | 43.0 | .367 | 68.0 | .168 | 93.0 | .032 |
| 18.5 | .733 | 43.5 | .362 | 68.5 | .164 | 93.5 | .029 |
| 19.0 | .721 | 44.0 | .357 | 69.0 | .161 | 94.0 | .027 |
| 19.5 | .710 | 44.5 | .352 | 69.5 | .158 | 94.5 | .025 |
| 20.0 | .699 | 45.0 | .347 | 70.0 | .155 | 95.0 | .022 |
| 20.5 | .688 | 45.5 | .342 | 70.5 | .152 | 95.5 | .020 |
| 21.0 | .678 | 46.0 | .337 | 71.0 | .149 | 96.0 | .018 |
| 21.5 | .668 | 46.5 | .332 | 71.5 | .146 | 96.5 | .016 |
| 22.0 | .658 | 47.0 | .328 | 72.0 | .143 | 97.0 | .013 |
| 22.5 | .648 | 47.5 | .323 | 72.5 | .140 | 97.5 | .011 |
| 23.0 | .638 | 48.0 | .319 | 73.0 | .139 | 98.0 | .009 |
| 23.5 | .629 | 48.5 | .314 | 73.5 | .134 | 98.5 | .007 |
| 24.0 | .620 | 49.0 | .310 | 74.0 | .131 | 99.0 | .004 |
| 24.5 | .611 | 49.5 | .305 | 74.5 | .128 | 99.5 | .002 |
| 25.0 | .602 | 50.0 | .301 | 75.0 | .125 | 100.0 | .000 |
| 25.5 | .594 | 50.5 | .297 | 75.5 | .122 | | |

5.2 When the chemical reagents impart color or turbidity, treat a parallel volume of distilled water in the same manner as the water sample. Instrumentally determine the amount of color produced versus an untreated distilled water blank, convert the photometric reading into mg/l or $\mu\text{g/l}$ by reference to the calibration curve, and subtract the mg/l or $\mu\text{g/l}$ result from the value obtained on each water sample. Determine such a reagent blank when the reagents are first prepared and used, and at weekly intervals, unless subsequent contamination or reagent deterioration justifies greater frequency.

6. General Procedure for Standard Addition Confirmatory Check

The standard addition confirmatory check affords a means of eliminating suspicion about the presence of interference and ascertaining the positive or negative nature of the interference in infrequent or new samples on which the history is relatively unknown.

6.1 Measure three equal portions of the unknown water sample.

6.2 To the first sample portion add a known amount of standard solution containing the constituent being sought.

6.3 To the second sample portion add a known but larger amount of standard solution.

6.4 Leave the third sample portion unspiked.

6.5 Dilute all portions to the same volume with the unknown water sample.

6.6 Develop the color in all three portions as called for in the prescribed procedure.

6.7 If the values on the two spiked sample portions are higher by the amount that was artificially added, then the result on the original unspiked sample can be assumed to be correct. If the recoveries exceed or fall short of the calculated amount by more than experimental error, the trouble may be attributed to an interference present in the unknown sample itself.

7. Instrument Kits

Several manufacturers supply a kit that consists of the photometric instrument, pre-calibrated transmittance or absorbance curves, and reagents for the determination of an assortment of constituents. The instruments can operate from a battery or an alternating current source. The kits are tailored to the capabilities of the particular instrument.

Careful attention should be paid to the chemical reagents supplied with the instrumental kit. Despite each manufacturer's effort to offer stable reagent formulations, chemical preparations may deteriorate with time. Periodic checks of the reagents are advisable for verification of one or more points on the supplied calibration curves. Departures from the norm warrant thorough investigation since they may be caused by any one or more of the following factors: (1) an improperly performed procedure, (2) a deteriorated reagent or standard solution, or (3) such instrumental defects as stray light leaks, optical imperfections, or faulty optical alignment or maintenance.

Obviously, defective reagents and standard solutions should be discarded and fresh stocks ordered or prepared. Exposure to sunlight or extreme heat or moisture may also curtail the shelf life of reagents and standards, necessitating protective measures against these destructive elements.

Standards are commercially available for various analyses as an aid to the analyst who does not possess the facilities or necessary chemicals or who desires a quality control check on the accuracy of his own work.

Conductivity

1. Purpose of Test

Since the conductivity of drinking waters is often related to the concentration of the dissolved mineral salts or filtrable residue, departures from the norm may signal changes in the mineral composition of the raw water, seasonal variations in reservoirs, daily chemical fluctuations in polluted rivers, or the intrusion of industrial wastes. However, only experience with a given water supply will confirm this relevance of conductivity measurements. Conductivity can also offer a valuable clue to the approximate size of an unknown water sample that can be taken for a common chemical analysis. Most drinking waters in the US exhibit a conductivity from about 50 to 1000 micromhos/cm at 25C with highly mineralized water exceeding the latter figure. The custom of reporting conductivity values in micromhos/cm at 25C requires the accurate determination of each sample's temperature at the time of conductivity measurement.

2. Warning

Platinum electrodes should be cleaned and replatinized whenever instrumental readings become erratic or indistinct, or when inspection reveals that some of the platinum black has flaked off. Conductivity increases with temperature at a rate of approximately 2 per cent per degree Celsius, placing great importance on accurate temperature measurement. Water containing substantial suspended matter is best settled before a conductivity measurement is attempted in order to minimize cell and electrode fouling. Oils, greases, and fats can also coat the electrodes and affect the accuracy of the readings—a problem that can be overcome by immersing the electrodes as needed in detergent solutions.

3. Apparatus

3.1 Self-contained conductivity instruments consist of a source of alternating current, a Wheatstone bridge, a null indicator, and a conductivity cell. The null point is disclosed by an alternating current galvanometer or a cathode-ray tube. Some instruments give readings in ohms while others read directly in conductivity or conductance units of micromhos/cm. Low-drain, solid-state circuitry has resulted in compact, battery-operated, direct-reading conductivity meters suitable for field applications. Quality laboratory and field instruments are equipped for operation over a wide conductivity range.

3.2 The conductivity cell makes up one arm of the Wheatstone bridge, a device for measuring electrical resistance. The cell contains a pair of rigidly mounted electrodes, the design, shape, size, and position of which influence the numerical value of the cell constant. The cell constant is determined by measuring the resistance of a standard 0.0100 *M* potassium chloride solution at 25C. A cell constant in the 0.1 to 2.0 range is satisfactory for the measurement of most drinking waters. The cell with a constant of 0.1 will yield best results in the 1 to 400 micromhos/cm range while the cell with a constant of 2.0 will function best in the 200 to 10 000 micromhos/cm range. Conductivity cells containing platinized electrodes are available in either the pipet or the immersion form, suitable for laboratory measurements. Electrodes constructed of durable common metals (stainless steel among others) are widely used for continuous monitoring and field studies. For optimum performance, conductivity cells must be kept clean at all times and immersed in distilled water when out of service for daily and weekly periods.

3.3 Thermometer covering the range of 23C to 27C and readable or estimable to the nearest 0.1C. A 0 - 50C thermometer will serve the purpose.

4. Reagents

4.1 Conductivity water:

Use this water for the preparation of the standard potassium chloride solution and all sample dilutions. Prepare in one of the following two ways.

a. Pass distilled water through a mixed-bed deionizer, discarding the first 1000 ml of flow, to produce a water of less than 1 micromho/cm conductivity. Such a deionizer can be purchased or constructed of a 25-cm column of glass tubing (1 - 2.5 cm in diameter) that has been charged with 2 parts by volume of a strongly basic anion-exchange resin in the hydroxyl form and 1 part by volume of a strongly acidic cation-exchange resin in the hydrogen form. Use ion-exchange resins of analytical grade quality.

b. Re-distill distilled water in an all-heat-resistant distillation unit. Boil the re-distilled water and cool to room temperature just before the preparation of the standard potassium chloride solution. Prepare as described in the section Carbon Dioxide (Free), paragraph 4.1, page 31.

4.2 Standard 0.0100 M potassium chloride solution:

a. Place approximately 2 g potassium chloride (KCl) in a weighing bottle or dish. Insert in a drying oven operating in the temperature range of 110-130C, and dry for 2 hr or overnight. Transfer the weighing bottle or dish to a desiccator and allow to cool to room temperature.

b. On an analytical balance carefully weigh 0.7456 g potassium chloride (KCl). Carefully transfer the weighed chemical to a 250-ml beaker and dissolve in 100 ml conductivity water.

c. Transfer the solution to a 1-liter volumetric flask, rinsing the beaker with three 100-ml portions of conductivity water.

d. Dilute further to the 1-liter mark with a conductivity water. Stopper and mix thoroughly. Store in a glass-stopped heat-resistant bottle. Use this standard reference solution which has a conductivity of 1413 micromhos/cm at 25C to determine cell constants between 0.1 and 2.0.

5. Determination of Cell Constant

5.1 Wash out the conductivity cell with at least three portions of standard 0.0100 M potassium chloride solution.

5.2 Adjust the temperature of a fourth portion to 25C \pm 0.1C by immersion in a carefully controlled water bath or other means available in the laboratory.

5.3 Slowly and carefully submerge the electrodes in a sufficient volume of standard potassium chloride solution so that the liquid level rises above the vent holes of the conductivity cell and no air bubbles form or cling to the vital measuring surfaces.

5.4 Observe and record the temperature of the standard potassium chloride solution to the nearest 0.1C.

5.5 Follow the manufacturer's instructions in the operation of the instrument and the measurement of the resistance.

5.6 Compute the cell constant by means of the following equation:

$$\text{Cell constant} = \frac{\text{Resistance reading of standard potassium chloride solution} \times 0.001413}{[(\text{Temperature of standard potassium chloride solution} - 25) \times 0.02] + 1}$$

5.7 Determine the cell constant daily with standard potassium chloride solution before undertaking sample measurements. Repeat during the course of the day if extended sample measurements are necessary. Record for future reference. When treated with care, the cell constant should not change.

6. Measurement of Sample Conductivity

6.1 Thoroughly rinse the conductivity cell with one or more portions of the water sample. Use extra rinses to minimize solution or sample carry-over when the sample conductivity differs by a factor of 5 or more from the conductivity of the standard potassium chloride solution or preceding water sample.

6.2 Adjust the temperature to $25\text{C} \pm 0.1\text{C}$ of the sample portion to be tested.

6.3 Slowly and carefully submerge the electrodes in a sufficient volume of water sample so that the water level rises above the vent holes of the conductivity cell and no air bubbles form or cling to the vital measuring surfaces.

6.4 Observe and record the temperature of the water sample to the nearest 0.1C.

6.5 Follow the manufacturer's instructions in the operation of the instrument and the measurement of the resistance. Adopt the correct eye position while making the reading. For best results, rinse, read, record, resample, read, and record so that two similar readings are obtained.

6.6 Compute the sample conductivity by means of the following equation:

Sample conductivity
in micromhos/cm
at 25C =

Cell constant X 1 000 000

Resistance reading of sample X
[0.02 X (Sample temperature in degrees Celsius - 25) +]

6.7 If the conductivity of the sample exceeds the range of the instrument, dilute the sample with conductivity water and repeat Steps 6.1 - 6.6.

6.8 Report conductivity values below 1000 micromhos/cm in whole numbers and above 1000 to three significant figures. When dilution is necessary, report the dilution factor and the reading on the diluted sample as well.

Electrometric Method for pH

1. Purpose of Test

The pH of most natural waters falls within the 6.0 to 8.5 range. The electrometric method of pH measurement excels the colorimetric indicator method in both accuracy and freedom from such sample interferences as color, turbidity, chlorine, organic and colloidal matter, and oxidizing and reducing agents. Solid state circuitry enables modern pH meters to attain an accuracy of ± 0.5 pH unit with the inexpensive models and better than ± 0.005 pH unit on the more accurate models. An accuracy of ± 0.05 pH unit and report of values to the first decimal are sufficient in most situations.

2. Warning

Measurement errors can ensue from mechanical or electrical failure involving weak batteries, cracked and aging glass electrodes, a plugged liquid junction in the reference electrode, improperly filled reference electrodes, and fouling of the electrodes with oily,

greasy, or precipitated materials. Damage can result from cleaning or wiping the glass electrode with an abrasive or dirty tissue or cloth. Errors can occur when the temperature of the buffer solutions and samples varies by more than 5C. Aeration and agitation should be minimized during sample measurement in order to reduce carbon dioxide loss. A change of 1 mg/l carbon dioxide can significantly alter the pH of a low-residue water.

3. Apparatus

3.1 Instrument types available:

pH meters are marketed in simple and expanded-scale models, the latter being suited for the measurement of pH and such additional ions as fluoride for which ion-selective electrodes have been developed. The scales and circuitry of direct-reading ion-selective meters similarly enable the measurement of both pH and supplementary ions. Features present in quality line-operated pH meters include voltage regulation, calibration stability, temperature compensation, and adaptability for titrimetric operations. Compact battery-operated instruments are available for field determination of pH.

3.2 Component parts:

The major components of a pH meter consist of a sensing electrode and a reference electrode which are connected to a high-impedance voltmeter capable of registering the voltage (electromotive force) of the high-resistance electrodes. The sensing electrode is usually constructed of a special glass whose voltage fluctuates with the pH of the water sample. The standard calomel reference electrode provides a stable and constant (+ 0.246) voltage against which the hydrogen-ion-selective glass electrode voltage can be compared. The meter scale is graduated in pH units, with the more versatile models additionally incorporating a millivolt scale. Direct-reading ion-selective meters also possess logarithmic scales suitable for ion-selective measurements, necessitating care in reading the proper scale.

3.3 Electrodes:

a. *Glass:* Several hours of soak time of a new glass electrode or one that has dried after prolonged standing may be needed to restore it to stable and reliable function. The tip of the glass electrode should be immersed in water when out of service for daily or weekly intervals. Manufacturers' instructions should be followed in these matters. The normal pH electrode performs best in the pH range of 1 through 9. The glass electrode exhibits a slight error below a pH of 1.0. When the ordinary glass electrode must be used in the alkaline range, a correction for the sodium error is made in the final result by reference to a chart or calculations supplied by the manufacturer of the electrode. Special individual electrodes are normally recommended in the pH range above 10 where sodium ions contribute a serious error, and for measurements at temperatures above 60C. The tips of the glass electrodes should be inspected regularly for scratches or signs of abrasion that could affect the response and linearity.

b. *Reference:* A sleeve-type standard calomel reference electrode can serve for both the pH and fluoride measurements. When not in use, the tip of the electrode should be protected against damage with a rubber cap designed for this purpose. The proper liquid level in the electrode should be maintained by adding saturated potassium chloride solution to a point ¼ in. below the filling hole. If necessary, the electrode should be shaken vigorously to break up any crystals that may have compacted at the bottom of the electrode. The rubber sleeve covering the filling hole should be removed before making any pH measurements.

c. *Combined:* Conventionally, the sensing and reference electrodes are offered in separate distinctive units. However, the two electrodes have been combined into a single common body for handling ease, ruggedness, the measurement of small sample volumes, and titrations.

3.4 Temperature effect and compensation:

Temperature affects pH measurements in two important ways: (1) the electrodes themselves vary in potential, and (2) the ionization in the sample may increase or decrease. In the first case the voltage change of the sensing electrode varies linearly with pH. At room temperature, a change of one pH unit causes a voltage change of approximately 60 millivolts (mV). At 0C, the freezing point of water, the unit pH-voltage change declines to 54 mV, and rises to 70 mV at 100C, the boiling point of water. The temperature dial on a pH meter corrects for this variable voltage response. The more expensive pH instruments also embody an electrode slope control for correcting the nontheoretical response, matching the sensitivity of the meter to the voltage from the electrodes. The second effect due to ionization is inherent in the sample and is denoted by setting down both the temperature and the pH of each sample.

3.5 Operating precautions:

A pH meter must be standardized with a standard solution specifically prepared for the purpose. Best results prevail when the standard buffer solution has a pH value approximating that of the water sample. In the case of potable water samples that normally fall within a neutral range, the phosphate standard solution (pH 6.9) offers a satisfactory starting selection. A second and third buffer may be used to check the pH meter's linearity of response over the desired sample range. The pH 4 phthalate buffer and the pH 9 borate buffer are satisfactory for this purpose. Careful rinsing of the electrodes to remove all traces of the buffer is essential before the introduction of succeeding buffers and subsequent sample measurement.

4. Reagents

4.1 Precautions in the preparation of standard buffer solutions:

- a. Rely on chemicals that meet American Chemical Society specifications, or equal or superior quality.
- b. Dissolve all of the solid salts in distilled water during the preparation of the buffer solutions.
- c. Prepare the buffer solutions, which are used infrequently as the need occurs, to prevent deterioration evidenced by mold growth or change in pH.
- d. Store the buffer solutions in polyethylene bottles preferentially, or heat-resistant glassware.
- e. For convenience, use commercially available buffer tablets, powders, or concentrates of tested quality that can be dissolved in distilled water and diluted to a specified volume, often 100 ml; or the prepared standard buffer solutions stocked by reliable chemical supply houses.
- f. Discard a buffer solution when mold growth becomes visible.

4.2 Distilled water for the preparation of standard buffer solutions:

Boil a sufficient volume of distilled water for 15 min and cool to room temperature shortly in advance of the preparation of the following standard buffer solutions. Prepare as described in the section, Carbon Dioxide (Free), paragraph 4.1, page 31.

4.3 Standard buffer solution, pH 6.86 at 25C:

- a. Place 4 g each of potassium dihydrogen phosphate (also called potassium monobasic phosphate), $[KH_2PO_4]$, and disodium hydrogen phosphate (also called sodium dibasic phosphate) $[Na_2HPO_4]$, in separate weighing bottles or dishes. Insert in a drying oven operating in the temperature range of 110 - 130C, and dry the salts for 2 hr. Transfer the weighing bottles or dishes to a desiccator and allow the salts to cool to room temperature.

b. On an analytical balance separately weigh 3.388 g potassium dihydrogen phosphate and 3.533 g disodium hydrogen phosphate. Transfer the weighed chemicals to a 500-ml beaker and dissolve in 300 ml distilled water.

c. Transfer the solution to a 1-liter volumetric flask with three 200-ml portions of distilled water, dilute to the 1-liter mark with distilled water, stopper, and mix thoroughly.

4.4 Standard buffer solution, pH 4.01 at 25C:

a. On an analytical balance, weigh 10.12 g potassium biphthalate ($\text{KHC}_8\text{H}_4\text{O}_4$). Transfer the weighed chemical to a 500-ml beaker and dissolve in 300 ml distilled water.

b. Transfer the solution to a 1-liter volumetric flask with three 200-ml portions of distilled water, dilute to the 1-liter mark with distilled water, stopper, and mix thoroughly.

4.5 Standard buffer solution, pH 9.18 at 25C:

On an analytical balance, weigh 3.80 g sodium borate decahydrate (also called borax) [$\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$]. Transfer the weighed chemical to a 500-ml beaker and dissolve in 300 ml distilled water.

b. Transfer the solution to a 1-liter volumetric flask with three 200-ml portions of distilled water, dilute to the 1-liter mark with distilled water, stopper, and mix thoroughly.

5. Standardization of pH Meter

5.1 Differences in the many makes and models of pH meters make it impossible to provide detailed instructions for the correct operation of every instrument. Therefore, follow the manufacturer's instructions on the care and operation of the instrument and accessories on hand. Observe the ensuing general precautions that apply in most instances and that merit attention. Wet the new glass electrode and the calomel reference electrode thoroughly and prepare for use in accordance with the manufacturer's directions.

5.2 Rinse the pH and reference electrodes with distilled or deionized water.

5.3 Blot the wet electrodes dry. Use a soft cleansing-type tissue paper that will not scratch or mar the glass surfaces of the electrodes.

5.4 Fill the beaker with the proper volume of pH 6.9 phosphate buffer solution.

5.5 Lower the electrodes into the solution so that the bulbs or sensing area of the electrodes are completely submerged and free of entrapped and adhering air bubbles.

5.6 Read the temperature of the buffer solution with a thermometer.

5.7 Adjust the temperature dial of the pH meter to the temperature of the buffer solution.

5.8 Turn on the pH meter. Allow the instrument to warm up for a minute or more. In the case of line-operated laboratory models allow the instrument to warm up prior to this stage, and maintain on a "standby" basis until ready to make readings.

5.9 With the proper control knob adjust the instrument to the pH value of the standard buffer solution recorded in the table on page S-118. Be certain that the needle drift ceases and that your eyes are properly focused on the scale before making the final setting.

5.10 Turn the instrument to "standby."

5.11 Raise the electrodes clear of the standard buffer solution, and replace the beaker of standard buffer solution with a waste beaker.

5.12 Rinse the electrodes with distilled water, and blot dry as described in paragraphs 5.2, 5.3. Remove the waste beaker.

5.13 Proceed to paragraph 5.14 or 5.6 depending on the accuracy desired, the stability of the instrument, or the time available.

5.14 If time allows, check the linearity of electrode response on a second minimally, and even a third, buffer solution. Choose the pH 4 phthalate buffer if the water samples will fall below pH 7 or the pH 9 borate buffer if the samples will rise above pH 9.

| <i>Variation of pH Values of Standard Buffer Solutions with Temperature</i> | | | |
|---|---------------------------------------|--|------------------|
| Temperature C | pH Value of Standard Buffer Solutions | | |
| | Potassium biphtalate | Potassium Dihydrogen Phosphate plus Disodium Hydrogen Phosphate | Sodium Borate |
| 0 | 4.003 | 6.984 | 9.464 |
| 5 | 3.999 | 6.951 | 9.395 |
| 10 | 3.998 | 6.923 | 9.332 |
| 15 | 3.999 | 6.900 | 9.276 |
| 20 | 4.002 | 6.881 | 9.225 |
| 25 | 4.008 | 6.865 | 9.180 |
| 30 | 4.015 | 6.853 | 9.139 |
| 35 | 4.024 | 6.844 | 9.102 |
| 38 | 4.030 | 6.840 | 9.081 |
| 40 | 4.035 | 6.838 | 9.068 |
| 45 | 4.047 | 6.834 | 9.038 |
| 50 | 4.060 | 6.833 | 9.011 |
| 55 | 4.075 | 6.834 | 8.985 |
| 60 | 4.091 | 6.836 | 8.962 |
| 70 | 4.126 | 6.845 | 8.921 |
| 80 | 4.164 | 6.859 | 8.885 |
| 90 | 4.205 | 6.877 | 8.850 |
| 95 | 4.227 | 6.886 | 8.833 |

5.15 Repeat the steps in paragraphs 5.4 - 5.12. Regard as acceptable a pH reading on the second buffer solution which differs less than ± 0.05 pH unit from the designated value for the stated temperature in the table on page S-118. Enlarge the variance to 0.1 pH unit if a lesser accuracy is satisfactory, or replace the pH electrode. Repeat the standardization from paragraph 5.2 onward if the variation is unacceptable. Proceed to step 6.1 when instrument function is satisfactory.

5.16 Recheck the calibration during extended periods of sample measurements.

6. Determination of Sample

6.1 Turn on the instrument to "standby."

6.2 Raise the electrodes from the standard buffer solution and replace the beaker of buffer solution with a waste beaker.

6.3 Rinse the electrodes with distilled water, and blot dry. Also rinse with a small amount of water sample, if available, in sufficient quantity. Remove the waste beaker.

6.4 Fill the clean beaker with the proper volume of water sample.

6.5 Lower the electrodes into the water sample so that the electrode sensing areas are completely immersed and free of entrapped and adhering air bubbles.

6.6 Read the temperature of the water sample.

6.7 Adjust the temperature dial to the temperature of the water sample.

6.8 Turn on the pH meter.

6.9 Read the pH of the water sample on the scale directly. Be sure that the needle drift ceases and the eyes are properly focused on the scale before making the final reading.

6.10 Turn on the instrument to "standby."

6.11 Repeat the steps in paragraphs 6.2 - 6.10 when additional water samples must be determined.

Electrode Method for Fluoride

1. Explanation of Test

A sensitive pH meter equipped with an expanded scale, or a direct-reading ion-selective meter, provide a rapid means for determining fluoride through potential measurements. The sensing unit consists of a solid-state fluoride electrode containing a lanthanum fluoride crystal. The fluoride electrode paired with a sleeve-type standard calomel reference electrode are immersed in a water sample suitably buffered to pH 5.0 - 5.5 and stirring initiated until a steady measurement is obtained on the appropriately calibrated logarithmic scale of a direct-reading instrument. In the case of a pH meter, a series of fluoride solutions encompassing the range of interest (0.1 or 0.2 - 2.0 mg/l) must be prepared for the purpose of plotting a millivolt-vs-concentration calibration curve. Measurements on samples can be performed with a precision of ± 0.05 mg/l in the fluoride range of 1 mg/l.

2. Warning

The temperature dependence of the electrode makes it imperative to calibrate at a temperature as close as possible to that which will prevail with the samples. The electrode responds to fluoride ion and is inert to the bound or complexed forms. Fluoride electrodes differ in their behavior and sensitivity. Some may detect fluoride concentrations as low as 0.1 mg/l or less while others may respond only to the 0.2 mg/l level. Experience alone will reveal the minimum fluoride concentration that can be determined confidently with the electrode on hand.

3. Apparatus

3.1 An expanded scale pH meter or direct-reading ion-selective meter equipped with a fluoride electrode and a sleeve-type standard calomel reference electrode. Check the instructions supplied with the fluoride electrode for the type of reference electrode recommended since such a selection can be important.

3.2 Magnetic stirrer with a TFE-coated stirring bar.

3.3 Timer.

4. Reagents

4.1 Stock and standard fluoride solutions: Prepare as described in the section on Fluoride, paragraphs 4.2 and 4.3 page 60.

4.2 Buffer solution:

a. Measure 500 ml distilled water into a 1-liter beaker.

b. With a 100-ml graduated cylinder, measure 57 ml concentrated acetic acid (also called glacial acetic acid) and add to the 500 ml of distilled water. Mix thoroughly.

c. On a rough balance weigh 58 g sodium chloride (NaCl), and dissolve in the acetic acid solution.

d. On an analytical balance weigh 4.0 g (1, 2-cyclohexylenedinitrilo)-tetraacetic acid, also called 1, 2-cyclohexanediaminotetraacetic acid or CDTA, and dissolve in solution (c). Alternatively, due to the cost of the CDTA, substitute if desired 12.0 g sodium citrate dihydrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$), and dissolve in solution (c).

- e. Prepare a 6-N sodium hydroxide solution by weighing 48 g NaOH pellets on a rough balance and carefully dissolving in 150 ml distilled water. Cool this solution to room temperature in a cold-tap water bath. Then transfer to a 200-ml graduated mixing cylinder or volumetric flask, dilute to the mark with distilled water, stopper, and mix thoroughly.
- f. Place the beaker containing solution (d) in a cold-tap water bath and add slowly, while stirring, about 120 ml 6-N sodium hydroxide.
- g. Determine the pH of a small portion of the neutralized solution with a pH meter.
- h. Repeat the pH determination after small successive additions of sodium hydroxide until the final pH falls into the desired 5.0 - 5.5 range. Approximately 125 ml sodium hydroxide may be needed.
- i. Transfer the resulting solution to a 1-liter volumetric flask, dilute to the mark with distilled water, stopper, and mix thoroughly.

5. Procedure

- 5.1 Prepare three fluoride standards containing 0.5, 1.0, and 2.0 mg/l by adding 2.5, 5.0, and 10.0 ml standard fluoride solution (1.00 ml = 10.0 μ g F) to separate 100-ml volumetric flasks.
- 5.2 Pipet 50 ml buffer solution into each flask, dilute to the 100-ml mark with distilled water, stopper, and mix well.
- 5.3 Pipet 50 ml sample into a 100-ml volumetric flask, dilute to the mark with buffer solution, stopper, and mix well.
- 5.4 Bring the standards and the sample to the same temperature, preferably room temperature.
- 5.5 Transfer each standard and sample to separate 150-ml beakers.
- 5.6 If a direct-reading ion-selective meter is available, follow the manufacturer's instructions for the operation and calibration of the instrument.
- 5.7 If a pH meter is available, set for expanded scale measurement. Where applicable, adjust the calibration control on some models so that the 1.0-mg/l fluoride standard reads at the center zero (100 millivolts).
- 5.8 Immerse the fluoride and calomel electrodes into each beaker and activate the magnetic stirrer at medium speed. Do not begin the stirring process before the electrode immersion because entrapped air around the sensor crystal at the bottom can produce erroneous readings or needle fluctuations.
- 5.9 Record the first measurement when the instrument reading becomes steady. Leave the electrodes in the solution, and after 3 min take a final positive millivolt reading. Some electrodes may require 5 min or more to reach a stable reading with fluoride concentrations below 0.5 mg/l. At higher fluoride levels the electrode response is usually faster.
- 5.10 Rinse the electrodes with distilled water and blot dry after each sample and standard. Use a soft cleansing-type tissue paper that will not scratch or mar the glass surface of the electrode.
- 5.11 Check the instrument reading of the 1.00-mg/l fluoride standard frequently and adjust the calibration control, if necessary, to restore the previous reading. Confirm the reading after each unknown and also after each standard when preparing the standard curve.
- 5.12 Prepare a fluoride calibration curve on two-cycle semilogarithmic graph paper by plotting the millivolt readings on the vertical coordinate axis versus milligrams/liter fluoride on the logarithmic axis. Convert the millivolt reading for each sample into fluoride concentration by reference to the calibration curve.

Amperometric Method for Residual Chlorine

1. Purpose of Test

The amperometric method is unaffected by the sample color and turbidity that might invalidate a colorimetric determination. The titration is designed primarily for laboratory rather than field use, and requires more skill and care than the colorimetric methods. Differentiation between free and combined available chlorine is possibly by pH adjustment and the presence or absence of potassium iodide. Free chlorine can be determined at a pH between 6.5 and 7.5 while the combined chlorine is titrated in the presence of the proper amount of potassium iodide in the pH range of 3.5 - 4.5. The intermediate addition of a small amount of potassium iodide in the neutral pH range permits the estimation of monochloramine content.

2. Warning

Smaller sample volumes or dilution are desirable for the measurement of residual chlorine in excess of 2 mg/l. Control of pH is important for correct results. Above pH 7.5 the reaction with free chlorine becomes sluggish, below 6.5 some combined chlorine may react even in the absence of iodide. At a pH less than 3.5 oxidized manganese reacts with the titrant, while above 4.5 the titration for chlorine fails to reach completion. Analysis must be undertaken immediately after sample collection, and performed away from sunlight and without undue stirring to minimize chlorine loss.

3. Apparatus

3.1 Amperometric titrator:

The heart of the typical amperometric titrator consists of a two-electrode cell connected to a microammeter and an adjustable potentiometer. Embodied in the electrical circuit are a noble-metal electrode, a reference electrode of silver-silver chloride in a saturated sodium chloride solution, and a salt bridge. A stirrer and a buret round out the working units of the instrument.

3.2 Preparation of amperometric titrator:

For best results, observe the following housekeeping practices in the preparation and operation of the apparatus.

- a. If necessary, gently scrub all deposits and film off the noble-metal electrode with the aid of a household scouring powder.
- b. If plugging or improper flow of salt solution occurs in the salt bridge, empty the old material from the cell and replace with fresh salt.
- c. Keep an adequate supply of solid salt in the reference electrode at all times.
- d. Remove the chlorine-consuming contaminants from the stirrer and the exposed electrode system by immersion for several minutes in water containing 1 - 2 mg/l free available residual chlorine, then add potassium iodide to the same water and continue immersion for another 5 min.
- e. Thoroughly rinse the sensitized electrodes and agitator with chlorine-demand-free water or the sample to be tested. Should the chlorine concentration of the samples approximate 0.5 mg/l, condition the electrode system further by conducting two or more titrations at the 0.5 mg/l level until the titrations become reproducible.

3.3 Glassware:

Satisfy the chlorine demand of all glassware to be used in the sampling and titration of samples by subjecting the critical surfaces to a water containing 10 mg/l or more residual chlorine for at least 3 hr and then rinse with chlorine-demand-free water to remove the residual chlorine traces.

4. Reagents

4.1 Phenylarsine oxide titrant:

Phenylarsine oxide powder is unavailable in a sufficiently purified form suitable for the direct preparation of a standard solution by dissolving a specified weight of the solid in a given volume. This situation leaves two options: (1) either purchase the standard titrant from commercial sources, or (2) prepare and standardize the titrant as described in the latest edition of *Standard Methods*. **Handle this poison with extreme caution and particularly avoid taking into the mouth.**

4.2 Phosphate buffer solution, pH 7:

a. On a rough balance separately weigh 25.4 g anhydrous potassium dihydrogen phosphate (also called potassium monobasic phosphate) [KH_2PO_4] and 34.1 g anhydrous disodium hydrogen phosphate (also called sodium dibasic phosphate) [Na_2HPO_4]. Transfer the weighed chemicals to a 500-ml beaker and dissolve in 300 ml distilled water.

b. Transfer the solution to a 1-liter glass-stoppered bottle with three 170-ml portions of distilled water, and mix thoroughly.

c. With a medicine dropper add 0.5 ml sodium hypochlorite solution containing 5 per cent available chlorine (common household bleach). Stopper and mix thoroughly.

d. Set the closed bottle in a cool dark place, away from sunlight or heat, for several days so that the chlorine can react completely with the ammonium contaminants that are usually present in the phosphate compounds.

e. Remove the chlorine in the bottle in one of the two following ways:

1. Place the bottle in sunlight, indoors or outdoors, until all the chlorine disappears as a result of photochemical activity. (The time required will vary from one day in summer to one week in winter.)

2. Add sufficient dilute sodium sulfite (Na_2SO_3) solution to reduce the chlorine content to a bare trace as shown by a very faint pink color produced with DPD reagent.

f. Transfer the contents of the bottle to a 1-liter graduated cylinder and dilute to the 1-liter mark with distilled water. Mix thoroughly by pouring back into the bottle.

g. Filter the solution if any precipitate has formed on standing.

4.3 Potassium iodide solution:

a. Place 105 ml distilled water in a 250-ml flask and boil for 7-10 min. Cap the top of the flask with a clean, small inverted beaker, and allow the water to cool to room temperature. To hasten cooling, place the flask in a bath of cold running water.

b. On a rough balance weigh 5.0 g potassium iodide, (KI). Transfer the weighed chemical to the freshly boiled-and-cooled distilled water, and mix thoroughly.

c. Transfer the solution to a brown glass-stoppered bottle. Store in a dark and cool place, preferably in a refrigerator. Discard the solution when a yellow color develops.

4.4 Acetate buffer solution, pH4:

a. Measure 400 ml distilled water into a 1500-ml beaker.

b. With a 1000-ml graduated mixing cylinder, measure 480 ml concentrated acetic acid (also called glacial acetic acid) and add to the 400 ml distilled water. Mix thoroughly.

c. On a rough balance weigh 243 g sodium acetate trihydrate ($\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$) and dissolve in the acetic acid solution.

d. Transfer the solution to the 1-liter graduated mixing cylinder, dilute to the 1-liter mark with distilled water, stopper, and mix thoroughly.

5. Procedure

5.1 Fill the buret with phenylarsine oxide titrant. Record the liquid level in the buret by reading at the bottom of the meniscus. Guard against a leaky valve or stopcock which can result in the loss of titrant on standing.

5.2 Measure the appropriate sample volume for the indicated residual chlorine range:

| Residual Chlorine Range <i>mg/l</i> | Sample Volume <i>ml</i> |
|--|----------------------------|
| 0 - 2.0 | 200 |
| 2.1 - 4.0 | 100 |
| 4.1 - 8.0 | 50 |

5.3 If the pH of the sample knowingly lies outside the range of 6.5 - 7.5, add 1 ml pH 7 phosphate buffer solution. Activate the stirrer.

5.4 Titrate with standard phenylarsine oxide titrant, watching the needle movement on the microammeter scale. At the beginning of the titration expect a considerable chlorine concentration to cause considerable needle variation. When the needle moves to the end of the scale, return to midscale with the proper knob adjustment for easier observation and greater sensitivity. As the needle activity diminishes, indicating the approach of the end point, add smaller and smaller increments of titrant. At this stage record the buret readings before each titrant addition. Take as the endpoint the addition that stops needle deflection. If necessary, deduct the final addition which is due to over-titration.

5.5 Read the new buret level at the bottom of the meniscus, and calculate the volume of titrant used by subtracting the initial buret reading (paragraph 5.1) from the present reading.

5.6 Calculate the free available chlorine by multiplying the result found in paragraph 5.5 by the appropriate factor:

| Sample Volume <i>ml</i> | Multiply ml of Titrant by |
|----------------------------|---------------------------|
| 200 | 1 |
| 100 | 2 |
| 50 | 4 |

5.7 Add exactly 1 ml potassium iodide solution to the sample. If necessary, reactivate the stirrer.

5.8 Add 1 ml acetate buffer solution to the sample.

5.9 Repeat the titration procedure described in paragraph 5.4.

5.10 Read the new buret level at the bottom of the meniscus, and record the total volume of titrant used in both the free available chlorine titration (paragraph 5.4, if carried out) and the total available chlorine titration (paragraph 5.9). Multiply this total by the appropriate factor given in paragraph 5.6.

5.11 Subtract the value in paragraph 5.6 from the value in paragraph 5.10 to obtain the combined available chlorine.

CAUTION: Wash the electrodes, stirrer, and sample container thoroughly to remove every trace of iodide from the apparatus before undertaking the next free available chlorine determination. Confirm complete iodide removal by repeating the succeeding free chlorine titration in duplicate and triplicate until acceptable results prevail.

Turbidity

1. Purpose of Test

Turbidimeters based on the nephelometric principle improve the reproducibility and comparability that can be achieved between different laboratories in the measurement of low turbidity.

Additionally, formazin polymer suspension provides a turbidity standard with more reproducible light-scattering properties than the clay or turbid natural water standards normally used for visual estimations.

2. Warning

The same handling precautions apply to turbidimeter and photometric apparatus. The sample tubes should be clean both internally and externally, and discarded when scratch and etch marks appear. Surfaces through which the light beam passes should be kept free of fingerprints and smudges. A protective case may be needed for proper handling of the tubes. Samples and standards should be thoroughly mixed prior to filling of the tubes, and the bubbles allowed to escape before a reading is attempted. Tiny air bubbles can masquerade as turbidity and contribute to a false high reading. When time is pressing, removal of the tiny gas bubbles can be hastened by connecting the flask containing the sample to a similar flask that serves as a trap and thence to a vacuum source. Air bubbles can also be removed by placing the sample tube in a small ultrasonic bath for several seconds; however, confirming evidence should be sought that the procedure is without effect on the turbidity in the particular situation.

A nephelometer fails to record activated carbon or other nonreflective particles as turbidity, unlike a transmittance photometer that responds to the same materials at adequate light-path lengths.

3. Turbidimeter

3.1 Principle of nephelometer:

Nephelometry involves the measurement of the light scattered in a specific direction such as 90 deg from the incident light path. The measurement is accomplished by passing a strong beam of light through the sample. The fine particles constituting the turbidity scatter a portion of the light beam. The light scattered at right angles (or other selected angle) to the beam reaches the sensor and is converted to an electrical impulse which actuates the meter. The intensity of light striking the sensor is proportional to the turbidity. Obviously, no light is diverted to the photocell by a turbidity-free sample.

Photometric equipment operates on the transmittance principle, enabling turbidity determinations above 10 turbidity units. A long light path enhances the sensitivity of photometric measurement in the low range. For the most part, photometric devices have been supplanted for low-level turbidity estimation by more sensitive turbidimeters based on the nephelometric principle.

3.2 Design and applicability:

The photoelectric detector in a photometer and spectrophotometer is positioned to receive the direct light beam whereas the nephelometer's sensor is located off to the side. The sensor in both cases consists of a photocell or photomultiplier tube. Some turbidimeters are equipped with precalibrated turbidity scales, others may have adjustments for

converting the scale values directly into turbidity units, while the remainder require the preparation of a calibration curve. The manufacturer's instructions for instrument operation should be observed. Turbidimeters frequently function over several turbidity ranges extending from 0.5 or less to 1000. Since progressive errors can occur on sample turbidities in excess of 40 nephelometric turbidity units (NTUs), the upper scales can provide guidance in estimating the dilution for bringing the readings within the working 40 NTU range. Best results are realized on samples devoid of coarse, rapid settling material. A desirable practice is to check the instrument's freedom from drift on all ranges before complete faith is reposed in a solid scattering standard. Although dissolved color does not ordinarily register as turbidity, intense color *can* cause low results.

3.3 Calibration:

Turbidimeters are calibrated against a formazin polymer suspension produced by the mixing of hexamethylenetetramine and hydrazine sulfate solutions. The formazin suspension is easily prepared as needed and yields reproducible and stable particle sizes and shapes.

4. Reagents

4.1 Turbidity-free water:

Remove the lint and floating debris from distilled water by passing the water through a membrane filter having a pore size no greater than $0.1 \mu\text{m}$. Discard the first 200 ml of filtrate before collecting and saving the remainder in a thoroughly clean container. If the filtration reduces the turbidity, use the filtered distilled water for the preparation of turbidity standards below 1.0. If no change is observed, use the distilled water.

4.2 Hydrazine sulfate solution:

- a. On an analytical balance, weigh 1.000 g hydrazine sulfate $[(\text{NH}_2)_2 \cdot \text{H}_2\text{SO}_4]$. Transfer the weighed chemical to a 100-ml beaker and dissolve in 50 ml distilled water.
- b. Transfer the solution to a 100-ml volumetric flask with three 15-ml portions of distilled water, dilute to the 100-ml mark with distilled water, stopper, and mix thoroughly.
- c. Prepare the solution on a monthly schedule.

4.3 Stock hexamethylenetetramine solution:

- a. On an analytical balance, weigh 10.000 g hexamethylenetetramine $[(\text{CH}_2)_6\text{N}_4]$. Transfer the weighed chemical to a 100-ml beaker and dissolve in 50 ml distilled water.
- b. Transfer the solution to a 100-ml volumetric flask with three 15-ml portions of distilled water, dilute to the 100-ml mark with distilled water, stopper, and mix thoroughly.
- c. Prepare the solution on a monthly schedule.

4.4 Stock formazin polymer suspension:

- a. With volumetric pipets, measure separately 5 ml stock hydrazine sulfate solution and 5 ml stock hexamethylenetetramine solution into a 100-ml volumetric flask. Do not add additional water.
- b. Mix the contents of the volumetric flask by swirling and allow to stand for 24 hr at room temperature ($25\text{C} \pm 3\text{C}$).
- c. Dilute the contents of the volumetric flask to the 100-ml mark with distilled water, stopper, and mix thoroughly.
- d. Prepare the suspension on a monthly schedule.

4.5 Standard turbidity suspension of 40 nephelometric turbidity units (NTUs).

- a. With a volumetric pipet, measure 10 ml of the well-mixed stock formazin polymer suspension into a 100-ml volumetric flask.
- b. Dilute with turbidity-free water to the 100-ml mark, stopper, and mix thoroughly.
- c. Prepare the suspension on a weekly schedule.

4.6 Dilute turbidity suspension of 2 nephelometric turbidity units (NTUs).

- a. With a volumetric pipet, measure 5 ml of the well-mixed standard turbidity suspension of 40 NTU (4.5) into a 100-ml volumetric flask.
- b. Dilute with turbidity-free water to the 100-ml mark, stopper, and mix thoroughly.
- c. Prepare the suspension on a daily schedule.

5. Preparation of Turbidity Standards for Turbidimeter Calibration

5.1 Use the 40-NTU suspension (4.5) for the preparation of turbidity standards in the 4 through 40 NTU range and the 2-NTU suspension (4.6) for the preparation of turbidity standards in the range below 2 NTU. Prepare the following turbidity standards in 100-ml nessler tubes or volumetric flasks.

| Turbidity Standards <i>NTU</i> | Volume of Suspension Needed in ml | | Volume of Turbidity-Free Water Needed <i>ml</i> |
|-----------------------------------|-----------------------------------|-------------------|--|
| | 2-NTU suspension | 40-NTU suspension | |
| 0.05 | 2.5 | | 97.5 |
| 0.1 | 5.0 | | 95.0 |
| 0.5 | 25.0 | | 75.0 |
| 1.0 | 50.0 | | 50.0 |
| 1.5 | 75.0 | | 25.0 |
| 2.0 | 100 | | 0 |
| 4.0 | | 10.0 | 90.0 |
| 6.0 | | 15.0 | 85.0 |
| 10 | | 25.0 | 75.0 |
| 20 | | 50.0 | 50.0 |
| 30 | | 75.0 | 25.0 |
| 40 | | 100 | 0 |

5.2 Minimize particle settling by measuring as rapidly as possible the specified volumes of well-mixed suspension with measuring pipets and/or buret into individual 100-ml nessler tubes or volumetric flasks.

5.3 Dilute with turbidity-free water to the 100-ml mark, stopper, and mix by inverting each tube four times.

5.4 Let the air bubbles disappear before pouring the resulting standard into the turbidimeter tube for the instrument reading.

5.5 Use these standards to check the accuracy of an instrument scale calibrated in nephelometric turbidity units based on the formazin polymer suspension.

6. Measurement of Sample Turbidities Less Than 50 NTU

6.1 Shake the sample container to distribute the turbidity evenly throughout the sample.

6.2 After the air bubbles disappear, pour the sample into the turbidimeter tube.

6.3 Read the turbidity directly from the calibrated instrument scale or from the prepared calibration curve.

6.4 Warm a very cold sample to room temperature to prevent condensate formation on the turbidimeter tube, and wait until all the supersaturated air bubbles have dissipated by alternate shaking and standing before measuring the turbidity of very clear waters.

7. Measurement of Turbidities Exceeding 40

7.1 Shake the sample container to mix the turbidity evenly throughout the sample.

7.2 Minimize particle settling by measuring as rapidly as possible the appropriate sample volume for the indicated turbidity range:

| Sample Volume <i>ml</i> | Turbidity Range <i>NTU</i> |
|----------------------------|-------------------------------|
| 100 | 0.05 - 40 |
| 50 | 41 - 80 |
| 25 | 85 - 160 |
| 10 | 170 - 360 |
| 5 | 370 - 800 |
| 2 | 850 - 2000 |

Place the well mixed sample in a 100-ml nessler tube.

7.3 Dilute to the 100-ml mark with distilled water. Mix by inverting the tube four times.

7.4 Let the air bubbles disappear before pouring the suspension into the turbidimeter tube. Read the turbidity directly from the calibrated instrument scale or from the prepared calibration curve.

7.5 Calculate the nephelometric turbidity units (NTUs) of the sample by multiplying the result found in paragraph 7.4 by the appropriate factor:

| Sample Volume <i>ml</i> | Multiply NTU Turbidity by |
|----------------------------|---------------------------|
| 100 | 1 |
| 50 | 2 |
| 25 | 4 |
| 10 | 10 |
| 5 | 20 |
| 2 | 50 |

7.6 If other sample volumes are taken for dilution, calculate the nephelometric turbidity units by means of the following equation:

$$\text{Turbidity in NTU} = \frac{\text{Determined turbidity in NTU of diluted sample} \times \text{Final dilution volume in milliliter}}{\text{Sample volume in milliliter taken for dilution}}$$

7.7 Record results to the nearest 0.05 in the range below 1.0; to the nearest 0.1 in the 1 - 10 range; to the nearest whole number in the 10 - 40 range; to the nearest 5 in the 40 - 100 range; to the nearest 10 in the 100 - 400 range; to the nearest 50 in the 400 - 1000 range; and to the nearest 100 above 1000. Since turbidity is not necessarily linear with dilution, report the dilution factor and reading.