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Techniques of Water-Resources Investigations
of the United States Geological Survey

Chapter A6

QUALITY ASSURANCE PRACTICES FOR THE CHEMICAL AND BIOLOGICAL ANALYSES OF WATER AND FLUVIAL SEDIMENTS

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Book 5

Laboratory Analysis

Conductivity Meters

1. Application or scope

1.1 This practice details procedures to follow in using conductivity meters. Conductivity meters are of relatively uncomplicated design and produce excellent results with simple quality control measures.

1.2 Conductivity meters consist essentially of a source of alternating current, a wheatstone bridge, a null indicator, and a conductivity cell. Conductivity cells usually consist of two thin plates of platinized metal, rigidly supported with a very precise parallel spacing. Pure platinum electrodes and circular carbon rings imbedded in an epoxy-type plastic cell are also used.

2. Practice

2.1 Basic operational procedure

2.1.1 At regular intervals, visually check the cell to insure that the platinized electrode surfaces are in good condition, that the electrodes are not bent, distorted, or fouled, and that the lead wires are properly separated and shielded to prevent electrolytic and capacitive current.

2.1.2 Clean and replatinize electrodes whenever the readings become erratic or inspection shows that any platinum black has flaked off. New electrodes must also undergo these cleaning and platinizing steps.

2.2 Calibration procedure

2.2.1 Allow the conductance instrument to electronically stabilize.

2.2.2 Prepare the KCl standard with care (NOTE 1). Compare the conductivity of a newly prepared standard with a previously prepared standard in order to ensure that the standard is correct.

NOTE 1. To prepare a 0.00702*N* potassium chloride solution, dissolve 0.5234 g KCl, dried at 180°C for 1 hour, in demineralized water and dilute to 1,000 mL (Skougstad and others, 1979); this solution has a specific conductance of 1,000 μmho/cm at 25°C. For potassium chloride solutions which will have other specific conductances, see Standard Methods (American Public Health Association and others, 1976).

2.2.3 Carefully measure the temperature of the standard solution (NOTE 2).

NOTE 2. Temperature significantly affects conductance measurements since conductance increases about 2 percent per degree Celsius. In the U.S. Geological Survey, specific conductance measurements are routinely reported at 25°C.

2.2.4 For direct-reading instruments with temperature compensation, measure the temperature of a 1,000 μmho/cm KCl standard, set the temperature control, and adjust the instrument to read 1,000. If another scale is used, check the calibration with another standard which is known to be in the range of the new scale.

2.2.5 For direct-reading instruments that are not temperature compensated, calibrate the instrument to read the conductance value of the KCl standard solution at the measured temperature by preparing a table of the conductivity of 0.00702*N* KCl versus temperature. If another scale is used, check the calibration with another standard which is known to be in the range of the selected scale.

2.2.6 For resistance measurements made using a wheatstone bridge, determine the cell constant of a particular cell according to directions in the methods manual. Inasmuch as the cell constant can change, it is necessary to recalculate this constant periodically. The resistance of sample solutions, and consequently their specific conductance, may be determined at 25.0°C by using a 25°C bath or by allowing samples to stabilize in a constant-temperature room (Skougstad and others, 1979). However, usually it is easier to determine experimentally the resistance of a standard KCl solution at 0.1°C intervals and make a correction to obtain the corresponding conductance at 25.0°C.

2.3 Measurement procedure

2.3.1 Carefully and thoroughly rinse the cell between each sample.

2.3.2 Record the temperature of each sample solution to the nearest 0.1°C.

2.3.3 Record the reading on the meter.

2.3.4 If the conductance meter is functioning properly, it is not necessary to check the standardization more than twice a day.

2.4 *Read-out and graphical techniques*

2.4.1 For a direct-reading instrument with temperature compensation, record the specific conductance directly from the meter with the temperature compensator adjusted to the observed temperature of the sample solution.

2.4.2 For a direct-reading instrument which is not temperature compensated, obtain the reading at a certain temperature x and multiply it by the ratio of the specific conductance of KCl solution at 25.0°C to that of the same solution at temperature x. This ratio is obtained from the table which is prepared as discussed in 2.2.5. If the resistance of the sample is measured by a wheatstone bridge, determine the specific conductance of the sample by dividing the resistance of the KCl standard solution at temperature x by the resistance of the sample solution at temperature x and multiplying by 1,000.

References

American Public Health Association and others, 1976, Standard methods for the examination of water and wastewater (14th ed.): Washington, D.C., American Public Health Association, 1193 p.

Reilley, C. N., and Sawyer, D. T., 1961, Experiments for instrumental methods: New York, McGraw-Hill, p. 11.

Skougstad, M. W., Fishman, M. J., Friedman, L. C., Erdmann, D. E., and Duncan, S. S., eds., 1979, Methods for determination of inorganic substances in water and fluvial sediments: U.S. Geological Survey Techniques of Water-Resources Investigations, Book 5, Chapter A1, 626 p.

U.S. Environmental Protection Agency, 1979, Handbook for analytical quality control in water and wastewater laboratories: U.S. Environmental Protection Agency EPA–600/4–79–019, Cincinnati, p. 3–1—3–15.

Gas Chromatographs

1. Application or scope

1.1 This practice details procedures to be followed in using gas chromatographs to analyze water samples, and particularly to determine pesticides.

1.2 Although all gas chromatographs require similar operational optimization and calibration procedures, some variation in instrumental quality control may be necessary since commercially available instruments vary somewhat and since different columns and detectors must be used depending on analytical methodology.

2. Practice

2.1 Basic operational procedure

2.1.1 Unless there is sufficient reason for an exception, use column tubes of glass or Teflon. Copper and stainless steel may cause decomposition of certain compounds in the column (Sherma, 1979).

2.1.2 Use columns of either 2 mm or 4 mm ID for pesticide (NOTE 1). For electron-capture detectors, columns of about 2 mm are usually preferred (Goerlitz and Brown, 1972).

NOTE 1. Sample injection volumes should not exceed 10 μL for the 2 mm and 80 μL for the 4 mm ID columns.

2.1.3 Acid-wash each new column with dilute HCl, rinse thoroughly with distilled water, methanol, and hexane, and treat with Glass Treat or similar silylating reagent. Allow to dry completely.

2.1.4 When packing a column, use vibration and pressure and (or) vacuum to settle the material and small plugs of "silanized" glass wool to hold the packing in place. Sherma (1979) recommends hand vibration for high quality columns.

2.1.5 After installing the column in the gas chromatograph, but before connecting to the detector, condition it as follows (Goerlitz and Brown, 1972):

2.1.5a Purge the columns for 30 minutes with inert carrier gas.

2.1.5b Turn off carrier gas flow and heat columns to 250°C for 2 hours (NOTE 2).

NOTE 2. Do not exceed the manufacturer's maximum usable temperature during the conditioning procedure.

2.1.5c Reduce the temperature to 210°C and allow it to equilibrate for 30 minutes.

2.1.5d Turn on carrier gas to about 30 mL/mm and continue heating at 210°C for about 12 hours (NOTE 3).

NOTE 3. Between 24 and 72 hours may be required for fluorinated or other high-bleed liquids, especially when using the electron-capture detector.

2.1.6 Tightly cap columns not in use and recondition them before reuse if they have been out of the instrument for more than a few days.

2.1.7 After conditioning, inject an appropriate solution (see 2.1.7a for pesticides), calculate the theoretical plate value, and compare to published results to determine performance, retention-time characteristics, and efficiency of each column. Use the following formula (NOTE 4):

$$n = 16\left(\frac{tr}{\Delta t}\right)^2 \qquad (17)$$

where

n = number of theoretical plates,
tr = uncorrected retention time of peak, and
Δt = peak retention width (length of baseline cut by the two tangents of the peak at the half-height point).

NOTE 4. Within the Geological Survey Central Laboratory System, calculations are being performed by a computer program using the chromatographic peak data and the formula:

$$p = \left(\frac{tr}{w_i}\right)^2 5.54 \qquad (18)$$

where

p = number of theoretical plates,
tr = uncorrected retention time, and
w_i = peak width at half height.

2.1.7a Inject a solution of DDE, dieldrin, and DDT to test for organochlorine insecticides; inject parathion to test for organophosphorus insecticides, and inject 2,4,5–T to test for chlorinated phenoxy acid herbicides (NOTE 5).

NOTE 5. Using the computer program indicated in NOTE 4, the plate numbers for more pesticides can be easily calculated.

2.1.7b For example, if p,p'–DDT is used as a standard, a 1.8-m column should have an efficiency of more than 1,500 theoretical plates in order to be acceptable for pesticide analysis. A column must be replaced as soon as deterioration is observed as indicated by changes in elution pattern, relative retention time, relative proportion of peaks, and peak geometry.

2.1.8 Calculate the resolution:

$$R = \frac{2d}{W_1 W_2} \tag{19}$$

where

R = resolution
d = the distance between the apex of each peak, and
W_1 and W_2 = width of baselines at the point where tangents to each peak intersect.

At least one of the columns to be used should give an R value of at least 1.0 for the compound of interest. Separation of p,p'–DDE and dieldrin on the mixed phase column, for example, should give a resolution of at least 1.0.

2.1.9 Check resolution and theoretical plate values at least monthly. Record data in a notebook. Include identification number of the column, material in it, mesh size, percent coating, and date prepared. Include a sample chromatograph in the book to which graphs from subsequent analyses can be compared to detect gradual decomposition of the column.

2.1.10 Identify each column by attaching a metal tag with an assigned number, material in it, mesh size, percent coating and date prepared.

2.1.11 Precise column temperature control (±0.5°C) is mandatory if reproducible analyses are to be obtained. The column-oven temperature is generally set between 175° to 200°C. To monitor the temperature, insert a mercury thermometer through an unused injection port or insert the stem of a precalibrated dial thermometer through the oven door.

2.1.12 Column temperature may be checked by computing the relative retention ratio for two pesticides (for example p,p'–DDT compared to aldrin) as specified in Sherma, 1979, p. 108.

2.1.13 The inlet temperature is usually maintained at 25° to 50°C above the maximum column-oven temperature. Change the septum at the end of each day; avoid handling the septum with the fingers.

2.1.14 The quality of compressed gas needed depends on the type of detector. Use only the grade of gas recommended by the vendor for the particular instrument being used. "Specialty" grade, or equivalent, is generally specified for electron-capture detectors.

2.1.15 Change gas cylinders when tank pressure reaches 200 lb/in^2 to avoid potential fouling of system and detector. Replace gas cylinders immediately if the gas produces excessive baseline noise or poor sensitivity.

2.1.16 Gas cylinders should be equipped with dual stage regulators. The gas chromatograph must be equipped with accurate needle-valve gas-flow controls. If these controls were not previously calibrated, this can be accomplished by using a soap-bubble flow meter and a stopwatch. The optimum flow rates of gases used with various detectors can be found either in the manufacturer's manual or in the procedure for a particular determination. Shut off purge gas and gas to columns not in use (but going to the same detector) when measuring the flow rate of the column.

2.1.17 Choose a detector suited to the compounds being determined and the sensitivity desired. For example, the electron-capture detector is extremely sensitive to electronegative functional groups and therefore to constituents such as: halogens, conjugated carbonyls, nitrates, nitrites, and organometals. The selective sensitivity to halides makes this detector of particular value for the determination of

many pesticides. Although the sensitivity of the microcoulometric detector is not outstanding, it is specific for halogen-, sulfur-, or nitrogen-containing compounds. Other commonly used detectors include the flame-ionization, flame-photometric, electrolytic-conductivity, and alkali-flame detectors. The detector temperature is generally maintained at between 25° to 50°C above the maximum column-oven temperature.

2.1.18 Check the condition of nickel-63 detectors at least monthly, and more often if noise, low response, nonlinearity, or other symptoms indicate the possibility of a dirty detector. Use an electrometer amplifier to give a detector profile as shown in the instrument manuals. Usually when a poor profile is recorded, the detector is not usable and must be returned to manufacturer for cleaning and replacement or cleaning of the source, gaskets and electrodes. Spare detectors are necessary to eliminate lost time while a detector is being cleaned and repaired.

2.1.19 Help prevent detector contamination from a carrier gas containing grease or water vapor by using a molecular sieve filter drier; check or regenerate the trap at least monthly. If a detector becomes contaminated from this or other sources, follow the manufacturer's cleaning instructions.

2.1.20 A recorder with an adequate input voltage range and pen response is used to record the chromatogram. In addition, the actual peak area values must be determined by a digital integrator when peak separation warrants, or by a compensatory polar planimeter reading to the nearest 0.01 in^2 if a digital integrator is not suitable because of a lack of peak separation (NOTE 6).

NOTE 6. Baseline noise should be less than 1 percent of full scale. Proper adjustment of the recorder gain control is important. If gain is lowered too much, to compensate for excessive baseline noise, peaks are jagged or flat instead of pointed.

2.1.21 Because of the stabilization time needed for the proper operation of a gas chromatograph, it is common practice to keep the instrument turned "on" continuously. When not actually in use, keep a low carrier flow (about 25 mL/min) through the column and a purge of 25 to 30 mL through the detector.

2.2 Calibration procedure

2.2.1 The calibration procedures are performed after the gas chromatograph has stabilized, thermally and electronically, and with the operating conditions adjusted as specified. Use a precision, gas-tight microliter syringe that can be accurately filled, that will deliver reproducible injections, and that may be easily cleaned.

2.2.2 Flush the syringe several times with the standard to be inserted, then overfill the syringe, withdraw it from the sample container, check it visually for bubbles, and discharge the excess solution.

2.2.3 Immediately and smoothly inject the standard. The volume injected is measured by reading the syringe both before and after injection.

2.2.4 To determine linear response for each pesticide of interest, use a suite of four to six standards, spaced at equal logarithmic intervals of the concentration range. The concentration of the pesticide in the series of standard solutions should be such to calibrate either the full range of linear detector response or the range of anticipated concentration in the sample, whichever is less.

2.2.5 Pesticide standards may be obtained from reliable sources such as gas chromatography specialty houses or from the instrument manufacturer. In some cases, additional purification may be necessary. They should be refrigerated or stored in a desiccator during prolonged storage. At least two separate sources should be used.

2.2.6 Inject a standard of a given concentration until at least three peaks have the same reading (within 5 percent) at the same attenuation.

2.2.7 If a linearized detector is part of the operating instrument, the following alternative calibration procedure may be used: Inject four calibration standards. The first is one order of magnitude above the detection limit for the pesticide of interest, the second is twice the concentration of the first; the third is between three and four times the concentration of the first, and the fourth standard is either two or three orders of magnitude greater than the first standard or is equal to the highest anticipated concentration of pesticide, whichever is less. If

the average reading of the most concentrated standard does not deviate from linearity by more than 5 percent, the calibration curve is acceptable and the linearized detector can be adjusted to give a straight line response over the entire calibration range.

2.2.8 Use one of the above calibration procedures semiannually or when response factors have changed by more than 10 percent, when a new column has been installed, or when any other major changes have been made in the system. Record all data, including date of calibration, in a notebook.

2.2.9 In addition, include at least two sets of two standards each in each day's determination. Use concentrations at the first and second order of magnitude above the detection limit.

2.2.10 Analyze one set of standards before any samples are analyzed to verify proper operation of the instrument. Operating conditions are satisfactory if a line drawn between the read-out values for these two standards is parallel to the original calibration curve and if these values do not differ by more than 10 percent from the values for the same concentrations on the original calibration curve.

2.2.11 The values obtained from these two standards are used as a basis for determining the concentrations of the samples analyzed on that particular day. The second and any subsequent sets of standards will be introduced later for a continued check of operating conditions. Inject a second set of standards if more than 2 hours have elapsed since the start of the run, if instrument conditions are changed, or if the analyst suspects (because of very concentrated or dirty samples) any change in instrument response. If the results from the standards indicate that a problem exists, prepare fresh standards and repeat the procedure. If this does not solve the problem, the difficulty exists in the instrument and must be located and corrected before proceeding.

2.2.12 Record all information pertinent to the analysis of standards or samples, such as analysis date and time, column description, operating conditions, sample number, and type of pesticide, directly on the recorder chart.

2.3 Measurement procedure

2.3.1 After calibration has been completed, the analysis of water samples may begin. Prepare sample solutions as directed in the analytical procedure.

2.3.2 If the concentration of the prepared sample does not fall within the working range of the procedure, either concentrate or dilute the sample extract or use an alternative analytical method.

2.3.3 Fill the syringe and inject the sample as described in steps 2.2.2 and 2.2.3 above.

2.3.4 After a sample extract has been injected, do not make any subsequent injections until the last compound has been eluted and the baseline has returned to normal.

2.3.5 Before every set of samples, inject a reagent blank. If baseline drift is indicated, take corrective measures before proceeding with the analysis.

2.4 Read-out and graphical techniques

2.4.1 Compare the relative retention times (the ratio of the retention time of an unknown to that of a selected standard on two or more columns) to qualitatively identify constituents. For pesticides use as the standard: aldrin for chlorinated hydrocarbon insecticides, parathion for organophosphorus insecticides, and the methyl ester of 2,4-D for chlorinated phenoxy acid herbicides.

2.4.2 A digital integrator or data system (such as the Hewlett Packard 3352) is the most accurate device for measuring chromatographic peaks and must be used whenever conditions permit. For the first calibration procedure (described in the earlier subsection on "Calibration Procedure," involving the use of four to six standards) derive a least squares equation ($y = mx + b$) from the observed values of concentrations and peak areas of the standards. Use the response factor, m, thus obtained to calculate the concentrations of the sample solutions.

2.4.3 The measurement of peaks is often complicated by the different manners in which they appear. They may appear as (a) a single peak or two or more completely separated, discrete peaks, (b) two or more discrete peaks not completely separated, (c) a small peak or shoulder on the leading or trailing edge of a relatively large peak, or (d) two or more peaks perfectly overlapping each other. Generally, the digital integrator can be used with satisfactory results for cases (a) and (b).

2.4.4 It is very difficult to isolate a shoul-

der from a larger peak in a reproducible manner. In this situation, construct a line drawn to conform with the shape of the larger peak, then measure both the area of the shoulder and the area of the larger peak with a planimeter. Average at least two independent measurements to obtain the peak area of each portion. The area under the larger peak is usually quite accurate, that of the shoulder is not. Similarly, components eluting at nearly the same time to form a single peak are easily misinterpreted. Preferably, in either of the above situations where the peak separation is poor, make changes in order to isolate compounds for quantitative determination. For example, use a different type column, different type detector, or thin-layer chromatography.

2.4.5 Because a column may not separate all pesticides present in a water sample, corroborate each pesticide detected by at least one other technique (for example, different type column or different type detector). For example, all water samples and sediments containing pesticides must be analyzed by electron-capture gas chromatography using two different types of columns for confirmation. Because lack of peak resolution increases the apparent concentration, the lower concentration from two determinations is more likely to be correct and, in general, should be reported. If peak resolution seems poor for both determinations and (or) if differences in the two results seem large, further corroboration should be used.

2.4.6 The presence of pesticides at concentrations greater than 1.0 μg/L in water or 10.0 μg/kg in sediment samples must be confirmed by conductivity gas chromatography; report the electron-capture values since conductivity detectors are inherently less sensitive and pesticide concentrations are commonly near their detection limits. The quantitative results between the specific-element and the electron-capture detectors should, however, agree within 20 percent. If the pesticide concentration is greater than 2.0 μg/L in water or 20 μg/kg in sediment, the pesticide must be confirmed by gas-chromatography–mass spectrometry.

Selected References

American Society for Testing and Materials, 1975, Annual book of ASTM standards, Part 42: Philadelphia, American Society Testing Materials, p. 174.

Budde, W. L., and Eichelberger, J. W., 1979, An EPA manual for organic analyses using gas chromatography/mass spectrometry: U.S. Environmental Protection Agency EPA–600/8–79–006, Cincinnati, 142 p.

Goerlitz, D. F., and Brown, E., Methods for analysis of organic substances in water: U.S. Geological Survey Techniques of Water-Resources Investigations, Book 5, Chapter A3, 40 p.

Goerlitz, D. F., and Lamar, W. L., 1967, Determination of pheonoxy acid herbicides in water by electron-capture and microcoulometric gas chromatography: U.S. Geological Survey Water-Supply Paper 1817–C, 21 p.

Sherma, Joseph, 1979, Manual of analytical quality control for pesticides and related compounds in human and environmental media: U.S. Environmental Protection Agency EPA–600/1–79–008, Research Triangle Park, North Carolina, 401 p.

U.S. Environmental Protection Agency, 1979, Handbook for analytical quality control in water and wastewater laboratories: U.S. Environmental Protection Agency EPA–600/4–79–019, Cincinnati, p. 8–1 - 8–10.

U.S. Office of the Federal Register, 1979, Guidelines establishing test procedures for the analysis of pollutants; proposed regulations: Federal Register, v. 44, no. 233, p. 69464–69575.

Potentiometers

1. Application and scope

1.1 This practice details procedures to be followed in using potentiometric meters. The design and operation of the meters are quite uncomplicated and most of the problems with this type of instrumentation are associated with the electrodes.

1.2 Ion-selective electrode procedures employ a potentiometer in conjunction with a reference electrode and an ion-selective electrode that is responsive to the ion of interest (NOTE 1).

NOTE 1. In the last few years, some ion-selective electrode determinations have been automated. The care of the involved electrodes is identical to that for manual procedures, although the manufacturer's manual should be consulted for additional information on the operation of the potentiometer.

2. Practice

2.1 Basic operational procedure

2.1.1 Electrodes must be conditioned according to the manufacturer's directions before they are ready for initial use. Ion-selective electrodes tend to deteriorate with use. Periodically replace or, if possible, recondition them.

2.1.2 If erratic, incorrect, or nonlinear readings are observed, check the electrode to see if it is cracked or scratched. Check also to see if the electrode is sufficiently filled, if there is a break in the shielding of the electrode leads, or if there is poor connection between the electrodes and the meter.

2.1.3 Store the electrodes carefully, following the manufacturer's instructions (NOTE 2).

NOTE 2. A glass pH electrode must be soaked for several hours if allowed to dry out until stable readings can be obtained.

2.1.4 Many potentiometers have a standby mode which maintains the electronics of the instrument in a "ready" condition and requires a change from the measuring mode into the standby mode before removing the electrodes from solution. Check the manufacturer's manual to see if this change must be made and (or) what other requirements are necessary.

2.2 Calibration

2.2.1 Begin the calibration procedure only after the potentiometer has electronically stabilized.

2.2.2 To calibrate a potentiometer to determine pH, immerse the electrodes in a buffer solution (usually pH 7.00), measure the temperature of the buffer solution, adjust the temperature control and use the standardization control to give the correct reading on the meter. Check the millivolt scale while the electrode is in the pH 7.00 buffer; if the reading is not 0 ± 10 mV, replace the electrode.

2.2.2a As noted in Skougstad and others (1979), at least three buffer solutions (pH 4.00, 7.00, and 9.00) must be available to standardize the pH instrument. Provide additional standard buffer solutions, if needed, to cover the pH range of the samples. If the electrodes are functioning correctly, a reasonably correct value ($\leq$ 0.1 pH unit) should then be obtained when the electrodes are rinsed and immersed in the second buffer solution. Some meters contain a slope adjustment feature to compensate for small amounts of asymmetric behavior in the glass electrode. The manufacturer's manual describes the function and operation of this feature. If the second buffer reading differs by more than 0.1 pH unit from the known value, the cause of this excessive deviation should be located and corrected before proceeding.

2.2.2b Prepare buffer solutions every 3 months or whenever a visible change occurs, since some of the buffer solutions deteriorate with age (NOTE 3). Discard the buffer solutions used for standardization and never mix with unused portions.

NOTE 3. Prepared buffer solutions or buffer concentrates are available from instrument and chemical manufacturers.

2.2.3 To calibrate the potentiometer for ion-selective electrode procedures other than

pH, prepare and use three standards covering the concentration range, as specified in the appropriate analytical method.

2.2.3a To calibrate the instrument, set the temperature of the meter to that of the standard solutions.

2.2.3b Both "mV" and "log" scales can be used to measure concentrations. For the "mV" scale, sequentially place the electrodes in each of the three standards, and record the readings. Plot the values as described in paragraph 2.4.2; if they are satisfactory, the instrument is ready to begin analysis. If not, correct the problem before proceeding.

2.2.3c For the "log" scale, place the electrodes in the first standard and use the standardization control to set the correct concentration on the "log" scale. Then measure the concentration of the second standard, reading the concentration directly. If the reading deviates only slightly from the actual concentration, use the slope correction to correct this value. Check the third standard. If it reads correctly, the instrument is properly calibrated; if not, correct the problem before proceeding.

2.2.4 Scale expansion features are available on many meters. If the concentration range of interest is relatively narrow, expand the scale.

2.2.5 If the meter and electrodes are functioning normally, it is not necessary to check the standardization more than twice a day. This applies to measurements made in the laboratory and does not apply to field measurements where more frequent standardization checks are usually necessary.

2.3 Measurement procedures

2.3.1 The temperature of the samples to be analyzed should not differ by more than 5°C from the temperature of the pH buffers or the standard solutions.

2.3.2 Measure the temperature of the sample and set the temperature control.

2.3.3 If there is a significant difference between sample and electrode temperatures, allow the electrodes to reach the sample temperature before making an analysis. If this occurs, use a fresh portion of sample for the measurement after temperature equilibration has been reached.

2.3.4 After the meter has been calibrated and the temperature control adjusted, thoroughly rinse the electrodes, immerse them in a sample, and record the observed value.

2.3.5 Rinse the electrodes well between samples and take adequate time to obtain accurate measurements (NOTE 4).

NOTE 4. Response of the pH glass electrode becomes much slower as solutions become more weakly buffered. This problem is magnified if a weakly buffered sample follows a strongly buffered sample as is usually the situation for the first sample following standardization. This "memory effect" varies from one brand of electrode to another; consequently, electrodes should be selected which minimize this problem.

2.3.6 Use a minimum of aeration or agitation when determining the pH (Skougstad and others, 1979). If sample is to be agitated, agitate at the same rate when standardizing the meter with the buffers.

2.3.7 Operating and measurement conditions for other ion-selective procedures are detailed in the analytical procedures. The response time of electrodes increases as the concentration for the ion of interest decreases. Allow adequate time for equilibrium to be achieved to obtain an accurate reading for the samples.

2.3.8 Check the stability of the ion-selective electrode instrument by measuring, in random order, one of the calibration standards after every tenth sample. If drift is indicated, restandardize the instrument before continuing with the analysis.

2.4 Read-out and graphical techniques

2.4.1 The pH results are read directly in pH units. If a recorder is used, follow the manufacturer's instructions.

2.4.2 The output from other ion-selective electrode procedures is read either on the "mV" or "log" scales. For the "mV" mode, plot a graph of the potential in microvolts versus concentration on semilong paper with the concentrations plotted on the logarithmic axis. Obtain the concentrations of the ions of interest from this graph.

Selected References

Reilley, C. N., and Sawyer, D. T., 1961, Experiments for instrumental methods: New York, McGraw-Hill, p. 11.

Skougstad, M. W., Fishman, M. J., Friedman, L. C., Erdmann, D. E., and Duncan, S. S., eds., 1979, Methods for determination of inorganic substances in water and fluvial sediments: U.S. Geological Survey Techniques of Water-Resources Investigations, Book 5, Chapter A1, 626 p.

U.S. Environmental Protection Agency, 1979, Handbook for analytical quality control in water and wastewater laboratories: U.S. Environmental Protection Agency EPA–600/4–79–019, Cincinnati, p. 3–1—3–15.

Reference Material

The use of reference material is an integral part of any quality assurance program. For water analyses, two types are primarily prepared: ampouled concentrates and natural waters.

In the Central Laboratories System of the U.S. Geological Survey, ampouled concentrates are obtained from the U.S. Environmental Protection Agency, the National Bureau of Standards, and commercial sources, or are prepared by a Geological Survey quality assurance project which is independent of the analytical laboratories. Ampouled concentrates can easily be used to develop precision and bias data for methods-development and methods-comparison studies.

Reference samples with working level concentrations in natural water are also prepared by the independent quality assurance project. These samples are easily introduced into the laboratory as blind samples since they need no dilution. The Standard Reference Water Sample (SRWS) program has, since 1962, distributed reference materials semiannually, and has involved an increasing number of participating laboratories. In April 1978, for example, a set of reference samples for 41 constituents was distributed to 8 U.S. Geological Survey laboratories, to 50 other laboratories in the continental United States, and to 6 laboratories in Puerto Rico, Brazil, and Saudi Arabia.

Preparation of Ampouled Concentrates

1. Application or scope

1.1 This practice details procedures to be followed in preparing ampouled reference material.

1.2 Most constituents can be prepared as ampouled concentrates and later quantitatively diluted with either distilled or natural water to provide a variety of matrices and concentrations. The concentrates can also be used to perform standard-additions analysis.

2. Practice

2.1 Materials and equipment

2.1.1 Ampoules, prescored to break at a non-painted part of the ampoules, such as Wheaton 176780 or equivalent.

2.1.2 Ampoule washer, Cozzoli or equivalent (NOTE 1).

NOTE 1. If an organic solvent wash is required, ampoule washer must be explosion proof.

2.1.3 Automatic ampouling equipment, Cozzoli or equivalent (NOTE 2).

NOTE 2. Ampoules may also be washed and filled by hand.

2.1.4 Oven, 105°C.

2.2 Procedure

2.2.1 Dissolve carefully weighed primary standard(s), or equivalent quality chemicals, in demineralized water or other solvent, and dilute to obtain a concentrated solution which, when further diluted, will yield the desired "working-level" concentration (NOTE 3). Acidify or chemically preserve the solution, if necessary.

NOTE 3. Ordinarily, the concentration of each constituent is prepared so that an aliquot of the ampouled concentrated solution can be diluted to 1 liter to yield working level concentrations. For example: dissolve x grams of iron wire which has been cleaned in diluted HCl, rinsed and dried. Dilute to 10,000 mL with demineralized water. Ampoule 15 mL of solution. Withdraw 10 mL of ampouled solution and dilute to 1,000 mL with demineralized water, for a final concentration of y µg/L.

2.2.2 Determine the concentration of each constituent to assure that the concentrate was correctly prepared.

2.2.3 Wash all ampoules with tap water and demineralized water using ampoule washer. If organic concentrates are being prepared, also wash with an organic solvent.

2.2.4 Dry ampoules at 105°C for 1 hour (NOTE 4).

NOTE 4. If organic solvent was used, air-dry ampoules before drying in an oven.

2.2.5 If automated equipment is used to fill the ampoules, adjust settings according to manufacturer's instructions. Make test run(s) to assure that dispensing and sealing alignments are properly set.

2.2.6 Fill and seal ampoules.

2.2.7 Label ampoules, including date of preparation.

2.2.8 Analyze sufficient samples, randomly selected, to assure that correct concentration is obtained (NOTE 5).

NOTE 5. Refer to the section, "Materials Evaluation," to determine the number of ampoules to be analyzed and use a random numbers table (available in most statistic books) to select which ampoules should be analyzed.

Preparation of Natural Water Reference Material

1. Application or scope

1.1 This practice gives general procedures to be followed in preparing reference materials with working level concentrations of stable constituents in natural water matrices.

1.2 Reference materials for major inorganic constituents and trace metals should be prepared so that they will be stable from 5 to 10 years. Reference materials for nutrient and pesticide constituents are expected to be stable for 4 to 12 months.

1.3 In the U.S. Geological Survey, reference materials in natural water are prepared at least semiannually for use in the Standard Reference Water Sample (SRWS) program (NOTE 1).

NOTE 1. In 1980, it is expected that each SRWS set will consist of a major constituent sample, a trace-metal sample, a nutrient sample, an insecticide sample, and a herbicide sample.

2. Practice

2.1 Materials and equipment

2.1.1 Autoclave.

2.1.2 Bag, paper, rated for 12 lb load.

2.1.3 Bag, polyethylene.

2.1.4 Bands, opaque white cellulose.

2.1.5 Bottles, 1-liter Teflon for major inorganic constituents and trace metal samples; 500-mL polyethylene for nutrient samples; 1-liter glass, for organic samples.

2.1.6 Filter, 0.45-μm, in-line, Acroflow II cartridge single pen-end, epoxy coated top and bottom, Model 12611, Gelman Institute Co., or equivalent.

2.1.7 Sterilizer, ultraviolet, flow-through with flow-rate capacity of 6 liters per minute.

2.1.8 Hood, equipped with ultraviolet light.

2.1.9 Oven.

2.1.10 Stirrer, polyethylene or Teflon coated for inorganic samples; stainless steel for organic samples.

2.1.11 Tank, of sufficient size for entire sample; 300-gallon (1,140-liter) polyethylene for major inorganic constituent and trace metal samples; 55-gallon (210-liter) polyethylene for nutrient sample; 55-gallon (210-liter) stainless steel for organic sample.

2.2 Procedure

2.2.1 Collect sample at specified site (NOTE 2).

NOTE 2. For SRWS sample collection, site will be specified by the SRWS project chief. Collect a minimum of 750 gallons to prepare samples to be analyzed for major inorganic constitutents, trace metals, and nutrients.

2.2.2 Allow samples to come to room temperature.

2.2.3 Filter 300 gallons (each) for major inorganic and trace metal samples or 55 gallons (each) for nutrient and organic samples through 0.45 micrometer filter into appropriate mixing tank (NOTE 3). For organic samples, use a stainless steel filter and refrigerate water after filtration.

NOTE 3. Minimum movement of water during temperature equilibration will aid filtration.

2.2.4 Add 1.5 g thymol to solution for major inorganic constituents. Add 1.5 g thymol and 2,100 mL of concentrated nitric acid to solution for trace metals. Add 10 g $HgCl_2$ + 93 g NaCl to solution for nutrients.

2.2.5 Prepare any desired spiking solutions individually in deionized water or acetone for inorganic or organic samples, respectively. Then slowly add, with stirring, to solution in tank.

2.2.6 Return solution for organic reference material to refrigerator until ready to bottle. Stir solution for nutrient samples overnight. Stir solutions for major inorganic constituents and trace-metal reference materials several times a day for 2 to 3 days.

2.2.7 Clean bottles.

2.2.7a For major inorganic constituent and trace metal samples, clean at least 800 1-liter Teflon bottles. Clean the outside thoroughly. Add 15 mL of concentrated HNO_3 (sp gr 1.41) to each bottle, fill with demineralized water and allow to stand for 24 hours. Soak caps in dilute HNO_3. Rinse bottles and caps three times with demineralized water. Package two bottles per brown bag and fold top of bag and staple. Put 20 or more caps in an autoclavable plastic bag. Sterilize bottles with dry heat at 160°C for 3 hours and sterilize caps in autoclave (NOTE 4).

NOTE 4. Dry sterilization will shrink caps.

2.2.7b For organic samples, clean at least 200 1-liter glass bottles. Clean the bottles thoroughly and rinse bottles and caps three times with demineralized water. Heat bottles at 350°C for 12 hours and tightly cap.

2.2.8 Bottle the sample as soon as all bottles for a particular reference material are sterilized (NOTE 5).

NOTE 5. Teflon bottles will remain sterile in the bags for 4 to 5 days.

2.2.9 For major constituent and trace metal samples, pass the water through an in-line, 0.45 micrometer filter and ultraviolet sterilizer at a flow rate of less than 6 liters per minute. Package in Teflon bottles in a hood equipped with ultraviolet light, putting on caps and tightening them while in the hood. Do not refilter organic samples and do not use ultraviolet light for either nutrient or organic samples.

2.2.10 Place a cellulose band around Teflon caps.

2.2.11 Label each bottle "Standard Reference Water Sample No._____."

2.2.12 Store all nutrient or organic samples at 4°C.

Selected References

Schroder, L. J., Fishman, M. J., Friedman, L. C., and Darlington, G. W., 1980, The use of standard reference samples by the U.S. Geological Survey: U.S. Geological Survey Open-File Report 80–738, 11 p.

Skougstad, M. W., and Fishman, M. J., 1975, Standard reference water samples: Proceedings of the AWWA Water Quality Technology Conference, December 1974, p. XIX–1–XIX–6.

Development of Statistical Data for Standard Reference Water Samples

1. Application or scope

1.1 This practice describes how the interlaboratory statistics for the Standard Reference Water Sample (SRWS) program are developed. Most of the computations are handled by a computer program.

1.2 The mean, average deviation, standard deviation, range, 95 percent confidence interval of the mean, and the percent deviation of each value from the mean are calculated for each constituent. The mean and standard deviation for each method are also computed for each constituent.

1.3 Inasmuch as the validity of the most probable concentration of each constituent is dependent upon the competence of the laboratories (as well as the number of laboratories analyzing the sample), the most probable means and standard deviations are refined by eliminating laboratories with inferior overall ratings.

2. Practice

2.1 Reported statistics

2.1.1 Values are rounded to conform with Geological Survey reporting policy.

2.1.2 The mean, average deviation, standard deviation, and total range are calculated for each determination. "Less than" values are not included. Outlying values are rejected using the *T* test described in the practice "Single operator precision" in section "Analytical Methods Development Procedures" (*T* values are listed in table A1).

2.1.3 The 95 percent confidence interval about the mean is calculated:

$$CI = \bar{x} \pm \frac{ts}{\sqrt{n}} \qquad (20)$$

where

CI = the confidence interval

$\bar{x}$ = mean of all unrejected values,

s = standard deviation,

n = number of values, and

t = value from Student t table at the 95 percent level for the (n-1) degrees of freedom.

2.1.4 The percent deviation of each value from the mean is calculated:

$$\frac{\bar{x} - x_i}{\bar{x}} \times 100 \text{ percent} \qquad (21)$$

where

$\bar{x}$ = the mean of all unrejected values from all laboratories, and

x_i = value from one laboratory.

2.1.5 Histograms are plotted showing each laboratory's result (figure 4). Because the computer program is designed to show differences in the significant figures appropriate to the determination and to depict only 25 columns, values which are very different from the mean may not be shown in the computerized plot.

2.1.6 The mean and standard deviation for each method in which there are three or more unrejected values are also calculated (NOTE 1).

NOTE 1. After a sufficient number of reference materials have been distributed for a constituent, these data are also used to develop precision statements for the analytical methods. Usually this precision is reported as a regression line and (or) as the relative percent standard deviation (coefficient of variation). See practice "Interlaboratory precision" in section "Analytical Methods Development Procedures." Also see individual precision statements in Skougstad and others, 1979.

2.2 Development of most probable value for reference material.

2.2.1 All laboratories are confidentially rated on a scale ranging from 0–4 that is based upon the number of standard deviations from the most probable mean as follows:

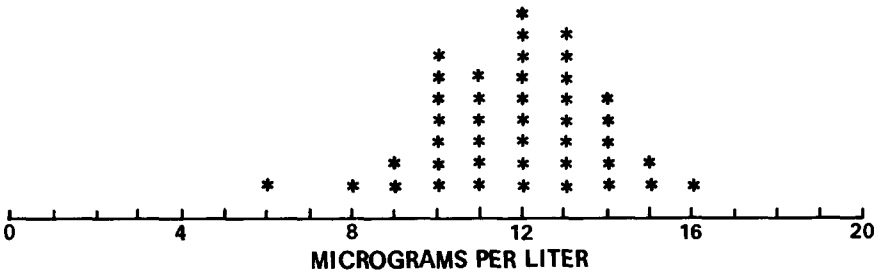

Figure 4.—Standard Reference Water Sample 65 histogram for cadmium. Each * equals a value from one laboratory.

Number of deviations from mean	*Rating number*
0–0.50	4
0.51–1.00	3
1.01–1.50	2
1.51–2.00	1
>2.00	0

2.2.2 All laboratories with an overall rating of less than 2.5 are eliminated, and the means and standard deviations are recomputed to determine the most probable concentration for each constituent.

Selected References

Schroder, L. J., Fishman, M. J., Friedman, L. C., and Darlington, G. W., 1980, The use of standard reference water samples by the U.S. Geological Survey: U.S. Geological Survey Open-File Report 80–738, 11 p.

Skougstad, M. W., and Fishman, M. J., 1975, Standard reference water samples: Proceedings, AWWA Water Quality Technology Conference, December, 1974, American Water Works Association, p. XIX–1–XIX–6.

Skougstad, M. W., Fishman, M. J., Friedman, L. C., Erdmann, D. E., and Duncan, S. S., eds., 1979, Methods for determination of inorganic substances in water and fluvial sediment: U.S. Geological Survey Techniques of Water-Resources Investigations, Book 5, Chapter A1, 626 p.

Laboratory Quality Control

Fundamental to the quality assurance of analytical data are quality control procedures in the laboratory. The practices which follow are interrelated with practices in other sections in this manual, particularly with the sections on "Standard Quantitative Techniques" and on "Instrumental Techniques." Each analyst must be familiar with and participate in the laboratory's quality control program.

Biological Quality Control

Analysis of Aquatic Organisms (benthic invertebrates, phytoplankton, and periphyton)

1. Application or scope

1.1 This practice specifies some general factors which are necessary for qualitative and quantitative measurements of aquatic biological samples. Specifically, this practice applies to taxonomic identification and determination of biomass of benthic invertebrates, phytoplankton, and periphyton.

1.2 Refer to applicable methods in Book 5, Chapter A4, of Techniques of Water-Resources Investigations of the U.S. Geological Survey (Greeson and others, 1977) and in the supplement to chapter A4 (Greeson, 1979). Also refer to other practices in this manual such as the practice, "Required documentation and review of data" and the practice, "Gravimetry."

2. Practice

2.1 Collection and preservation

2.1.1 In general, collect samples from downstream to upstream. When using a sieving device to collect benthic invertebrates, for example, stand in the downstream side and take samples in an upstream or a lateral direction (Tracor Jitco, Inc., 1978).

2.1.2 Collect samples at a depth appropriate to the object of the study; in order to lessen the chance of collecting terrestrial insects, collect samples from below the surface. To collect periphyton, be sure substrates are submerged (NOTE 1).

NOTE 1. Because of the amount of time needed to collect a periphyton sample and because of possible fluctuations in water levels, possibility of vandalism, and so forth, it is recommended that four replicate substrates be taken and duplicate samplers used (Tracor Jitco, Inc., 1978).

2.1.3 Prepare and use a formaldehyde-cupric sulfate solution or Lugol's solution to preserve phytoplankton and periphyton samples which are collected for taxonomic identification. Use ethyl alcohol or isopropyl alcohol to preserve benthic invertebrate samples collected for taxonomic identification; do not use formaldehyde (Greeson and others, 1977). If possible, freeze samples collected for the determination of biomass instead of using a chemical preservative (NOTE 2).

NOTE 2. All samples collected for a particular determination as part of a specific study should be preserved in a similar fashion and the method of preservation should be clearly documented.

2.1.4 In preserving benthic invertebrates, fill containers almost to the top (half of volume in container should be preservative) to avoid damage to specimens during transport. If unsorted samples are to be stored for more than a few weeks, drain preservative and replace with fresh preservative after a week (Greeson and others, 1977).

2.1.5 Label container with pertinent information including date, time, location, volume or area of sample, name of collector, preservative, and mesh or sieve size. If a sample is

sorted into categories (either in the field or in the laboratory), include the total number of containers per sample and the name of the sorter on the label; keep the sample together as a unit.

2.2 Calibration and measurement

2.2.1 Calibrate new microscopes or a microscope which has not been used for several months using an optical reticle and stage micrometer.

2.2.2 After every use, clean optics and stage of microscope with lens paper.

2.2.3 Check temperature of oven prior to each use to make sure it is correct (NOTE 3).

NOTE 3. The setting on the outside may be incorrect and not reflect the actual temperature in the oven. The thermometer, rather than the setting, must be read.

2.2.4 Carefully maintain records of dilution or concentration, if either is necessary, and apply the appropriate dilution or concentration factor in reporting analyses. When concentrating a phytoplankton sample, be careful that it has settled sufficiently before siphoning the supernatant liquid since different shapes and sizes of particles will have different sedimentation rates (Tracor Jitco, Inc., 1978); in general, allow the sample to sit undisturbed for 4 hours per centimenter of depth before siphoning the supernatant liquid (Greeson and others, 1977).

2.2.5 For sorting benthic invertebrates, consider use of the optional procedures (density separation, differential staining, and (or) subsampling) specified in the analytical procedure (see Greeson and others, 1977).

2.2.6 For phytoplankton or periphyton, use the magnification specified in the analytical procedure. Count, in randomly chosen fields, the minimum number of organisms or minimum number of fields specified in the procedure. In counting, enumerate all forms wholly within the grid boundaries and all forms which intersect two adjacent grid borders, but not those intersecting the opposite two borders (see Greeson and others, 1977, and Greeson, 1979).

2.2.7 Be sure taxonomic references are adequate and are readily available to each analyst. Consult references if taxonomy identification is uncertain.

2.2.8 Develop and maintain an "in-house" reference specimen collection.

2.2.9 Consult with outside taxonomic experts on unusual specimens to confirm or provide identification.

2.3 Calibration checks

2.3.1 Using an optical reticle and stage micrometer, recalibrate each microscope at least semiannually. Record the date of calibration in a notebook kept near the microscope.

2.3.2 Check calibration of oven thermometer with a U.S. National Bureau of Standards certified themometer at least every 3 months. Record date checked in a notebook kept near the oven.

2.3.3 Check calibration of analytical balance at least every 3 months using Class S weights. Record date of calibration check in a notebook kept near the balance. If recalibration is necessary, consult the manufacturer's directions.

2.3.4 For taxonomic identification, have every 20th slide checked by another analyst. Preferably, the analyst should be from another laboratory.

Selected References

American Public Health Association and others, 1976, Standard methods for the examination of water and wastewater (14th ed): Washington, D.C., American Public Health Association, 1193 p.

Greeson, P. E., ed., 1979, A supplement to—Methods for collection and analysis of aquatic biological and microbiological samples: (U.S. Geological Survey Techniques of Water-Resources Investigations, Book 5, Chapter A4): U.S. Geological Survey Open-File Report 79–1279, 92 p.

Greeson, P. E., Ehlke, T. A., Irwin, G. A., Lium, B. W., and Slack, K. V., eds., 1977, Methods for collection and analysis of aquatic biological and microbiological samples: U.S. Geological Survey Techniques of Water-Resources Investigations, Book 5, Chapter A4, 332 p.

Tracor Jitco, Inc., 1978, Quality assurance guidelines for biological testing: U.S. Environmental Protection Agency EPA–600/4–78–043, Las Vegas, 474 p.

Bacteriological Analysis

1. Application or scope

1.1 This practice applies to the measurement of bacteria in water (for example, the measurement of total or fecal coliform bacteria).

1.2 Refer to applicable methods in Book 5, Chapter A4 of Techniques of Water-Resources

Investigations of the U.S. Geological Survey (Greeson and others, 1977) and in the supplement to chapter A4 (Greeson, 1979).

2. Practice

2.1 Preparation of reagents

2.1.1 Use demineralized water free from traces of dissolved metals, nutrients, residual chlorine, and other bactericidal compounds. Test demineralized water semiannually to determine if it contains substances which are toxic to bacteria or which will stimulate the growth of bacteria (NOTE 1). Record dates and results of the tests.

NOTE 1. The test procedure and specifications are described in Standard Methods (American Public Health Association and others, 1976) and in the U.S. Environmental Protection Agency EPA–600/8–78–077 (Bordner and Winter, 1978). As noted in the latter publication, the test is ". . . a complex method that requires skill and experience, (and) is very sensitive to toxicants . . ."

2.1.2 Follow directions specified in the analytical method for the preparation of all reagents. Record date of preparation in notebook and on container.

2.1.3 Follow storage requirements, including refrigerating or storing in dark, specified in the method. Do not exceed maximum allowable storage times.

2.1.4 Keep a record of each bottle of media including its lot number, date of receipt, date of opening bottle, and date of expiration (NOTE 2).

NOTE 2. The U.S. Environmental Protection Agency recommends that, as a general guideline, "storage of unopened bottles of cultural media (be limited) to 2 years" (Bordner and Winter, 1978).

2.1.5 When preparing a new batch of media, indicate in notebook the number of tubes or plates prepared, date of preparation, bottle lot number, and name of preparer. Be extremely careful not to overheat media. Test 5 percent of noninhibitory media by overnight incubation at 35°C. Discard the entire batch if two out of five plates show contamination (McClelland and others, 1978).

2.1.6 Check each set of washed glassware for acid or alkaline residue by adding a few drops of a 0.04 percent solution of bromothymol blue indicator to a few pieces, randomly chosen from the set (Bordner and Winter, 1978). The indicator will show a yellow color at pH less than 6.2 and blue at pH greater than 7.6 (Dean, 1973).

2.2 Calibration and measurement

2.2.1 Temperature is critical to bacteriological tests. At least quarterly, check calibration of thermometer(s) against a U.S. National Bureau of Standards certified thermometer. Record the date checked.

2.2.2 Follow sterilization procedures specified in the method. Once a week or with every batch, whichever is less frequent, include in autoclave load a sterilization indicator (such as a Diack control ampoule or Sterilometer tape). Place indicator in center of load. Record date and result of indicator test. If sterilization is shown to be incomplete, locate problem and correct.

2.2.3 When in use, daily check the temperature of all water baths and incubators upon first opening. Temperature must be within limits specified in the method. Record daily temperature.

2.2.4 As noted in Greeson and others (1977), when determining total coliform bacteria by the most probable number method (B–0035–77), check broth in inverted tubes for air bubbles before use. Discard any tubes which contain a bubble.

2.2.5 Prior to use, check each batch of medium by inoculating two tubes or plates with pure cultures of organisms which will produce positive or negative reactions (Bordner and Winter, 1978). See table 7 for organisms which can be used.

2.2.6 Weekly or the day before use, whichever is less frequent, check phosphate buffer dilution water for sterility. Follow method B–0030–77, Total Coliform Bacteria (Membrane Filter Method) from Greeson and others (1977), after selecting three bottles of buffer at random and filtering. Record all information, including date, in notebook. If any plate shows a count greater than 2 colonies/mL, resterilize all buffer water prepared on same date (McClellend and others, 1978).

2.3 Calibration checks

2.3.1 Monthly, test a pure culture known to give positive results. Record data in notebook.

Table 7.—Cultures for use in testing media[a]

Medium	Control cultures	Expected results
M-Endo MF broth or agar	*Escherichia coli*	Golden green metallic sheen.
	Enterobacter aerogenes	Do.
	Achromobacter species	Red colonies.
	Pseudomonas species	Do.
	Salmonella species	Red colonies if medium is overheated.
M-FC broth or agar	*Escherichia coli*	Blue colonies.
	Klebsiella pneumoniae	Do.
	Enterobacter aerogenes	No growth.
Brilliant green bile lactose broth	*Escherichia coli*	Growth with gas.
	Enterobacter aerogenes	Do.
	Citrobacter freundii	Do.
	Staphylococcus aureus	No growth.
Lauryl tryptose broth	*Escherichia coli*	Growth with gas.
	Enterobacter aerogenes	Do.
	Salmonella typhimurium	Marked to complete inhibition.
	Staphylococcus aureus	Do.
Levine's eosin methylene blue agar	*Escherichia coli*	Nucleated black colonies with golden green metallic sheen.
	Enterobacter aerogenes	Pink colonies with dark centers.
	Citrobacter freundii	Colorless colonies.
	Salomnella species	Do.
	Klebsiella species	Large brown mucoid colonies.
Xylose lysine Desoxycholate agar (XLD)	*Salmonella species*	Red colonies, to red with black centers.
	Klebsiella species	Yellow colonies.
	Escherichia coli	Do.
	Enterobacter aerogenes	Do.
Bismuth sulfite agar	*Salmonella typhosa*	Black colony with black or brownish-black zone, with or without sheen.
	Other Salmonella species	Raised green colonies.
	Coliforms	Green colonies.
Brilliant green agar	*Salmonella species*	Pink-white opaque colonies surrounded by brilliant red zone.
	Escherichia coli	Inhibition or yellow green colonies.
	Proteus vulgaris	Marked to complete inhibition or red colonies.
KF streptococcus agar	*Streptococcus faecalis*	Pink to red colonies.
	Streptococcus pyogenes	No growth.
	Straphylococcus aureus	Do.
	Escherichia coli	Do.
PSE agar	*Streptococcus faecalis*	Black colonies.
	Escherichia coli	No growth.
	Straphylococcus aureus	Do.

[a] Table is modified from Bordner, Robert, and Winter, John, 1978, Microbiological methods for monitoring the environment, water and wastes: U.S. Environmental Protection Agency EPA-600/8-78-017, Cincinnati, p. 220-221.

2.3.2 Carry along a blank with each set of analyses. Blank should not show contamination.

2.3.3 Run every 20th sample in duplicate.

2.3.4 Confirm every 10th sample by having another analyst count colonies.

2.3.5 Record data from blank, duplicates, and recounts in a notebook, along with a date of analysis and identification of samples analyzed.

2.3.6 Prepare and use quality control chart (see practice "Quality control charts").

2.3.7 Within 24 hours of membrane filtering, confirm coliform colonies by using method B–0045–77, and confirm fecal streptococcal bacteria by using method B–0060–77 (Greeson and others, 1977).

Selected References

American Public Health Association and others, 1976, Standard methods for the examination of water and wastewater (14th ed): Washington, D.C, American Public Health Association, 1193 p.

Bordner, Robert, and Winter, John, eds., 1978, Microbiological methods for monitoring the environment, water and wastes: U.S. Environmental Protection Agency EPA–600/8–78–017, Cincinnati, 337 p.

Dean, J. A., ed., 1973, Lange's handbook of chemistry (11th ed.): New York, McGraw Hill, p. 5–80.

Greeson, P. E., ed., 1979, A supplement to—Methods for collection and analyses of aquatic biological and microbiological samples (U.S. Geological Survey Techniques of Water-Resources Investigations, Book 5, Chapter A4): U.S. Geological Survey Open-File Report 79–1279, 92 p.

Greeson, P. E., Ehlke, T. A., Irwin, G. A., Lium, B. W., and Slack, K. V., eds., 1977, Methods for collection and analysis of aquatic biological and microbiological samples: U.S. Geological Survey Techniques of Water-Resources Investigations, Book 5, Chapter A4, 332 p.

McClelland, N. J., Delfino, J. J., Greenberg, A. E., McDonald, D. B., and Morris, R. L., 1978, *in* Inhorn, S. L., ed., 1978, Quality assurance practices for health laboratories: Washington, D. C., American Public Health Association, p. 1175–1182.

Prier, J. E., Bartula, J. T., Friedman, Herman, 1975, Quality control in microbiology: Baltimore, University Park Press, 188 p.

Tracor Jitco, Inc., 1978, Quality assurance guidelines for biological testing: U.S. Environmental Protection Agency EPA–600/4–78–043, Las Vegas, 474 p.

U.S. Environmental Protection Agency, 1977, Manual for the interim certification of laboratories involved in analyzing public drinking water supplies: U.S. Environmental Protection Agency EPA–600/8–78–008, Washington, D.C., 92 p.