9020 QUALITY ASSURANCE/QUALITY CONTROL*

9020 A. Introduction

1. General Considerations

The growing emphasis on microorganisms in water quality standards and enforcement activities and their continuing role in research, process control, and compliance monitoring require the establishment and effective operation of a quality assurance (QA) program to substantiate the validity of analytical data.

A laboratory quality assurance program is the integration of intralaboratory and interlaboratory quality control (QC), standardization, and management practices into a formal, documented program with clearly defined responsibilities and duties to ensure that the data are of the type, quality, and quantity required.

The program must be practical and require only a reasonable amount of time or it will be bypassed. Generally, about 15% of overall laboratory time should be spent on different aspects of a quality assurance program. However, more time may be needed for more important analytical data, e.g., data for enforcement actions. When properly administered, a balanced, conscientiously applied QA program will optimize data quality without adversely affecting laboratory productivity.

Because microbiological analyses measure constantly changing living organisms, they are inherently variable. Some quality control tools used by chemists, such as reference standards, instrument calibration, and quality control charts, may not be available to the microbiologist.

Because QA programs vary among laboratories as a result of differences in organizational mission, responsibilities, and objectives; laboratory size, capabilities, and facilities; and staff skills and training, this section provides only general guidance. Each laboratory should determine the appropriate QA level for its purpose.

2. Guidelines for a Quality Assurance Program

Develop a QA program to meet the laboratory's specific needs and the planned use of the data. Emphasis on the use of data is particularly important where significant and costly decisions depend on analytical results. An effective QA program will confirm the quality of results and increase confidence in the data.

a. Management responsibilities: Management must recognize the need for quality assurance, commit monetary and personnel resources, assume a leadership role, and involve staff in development and operation of the QA program. Management should meet with the laboratory supervisor and staff to develop and maintain a comprehensive program and establish specific responsibility for management, supervisors, and analysts.

b. Quality assurance officer: In large laboratories, a QA officer has the authority and responsibility for application of the QA program. Ideally, this person should have a staff position reporting directly to upper management, not a line position. The QA officer should have a technical education, be acquainted with all aspects of laboratory work, and be familiar with statistical techniques for data evaluation. The QA officer is responsible for initiating the program, convincing staff of its value, and providing necessary information and training to the staff. Once the QA program is functioning, the coordinator conducts frequent (weekly to monthly) reviews with the laboratory supervisor and staff to determine the current status and accomplishments of the program and to identify and resolve problems. The QA officer also reports periodically to management to secure backing in actions necessary to correct problems that threaten data quality.

c. Staff: Laboratory and field staffs participate with management in planning the QA program, preparing standard operating procedures, and most importantly, implementing the QC program in their daily tasks of collecting samples, conducting analyses, performing quality control checks, and calculating and reporting results. Because the staffs are the first to see potential problems, they should identify them and work with the supervisor to correct and avoid them. It is critical to the success of the QA program that staff understand and actively support it.

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3. Quality Assurance Program Objectives

The objectives of a QA program include providing data of known quality, ensuring a high quality of laboratory performance, maintaining continuing assessment of laboratory operations, identifying weaknesses in laboratory operations, detecting training needs, and improving documentation and recordkeeping.

4. Elements of a Quality Assurance Program

Each laboratory should develop and implement a written QA plan describing the QA program and QC activities of the laboratory. The plan should address the following basic common aspects:

a. Statement of objectives, describing the specific goals of the laboratory.

b. Sampling procedures, including selection of representative sites and specified holding time and temperature conditions. If data may be subjected to litigation, use chain-of-custody procedures.

c. Personnel policies, describing specific qualification and training requirements for supervisors and analysts.

d. Equipment and instrument requirements, providing calibration procedures and frequency and maintenance requirements.

e. Specifications for supplies, to ensure that reagents and supplies are of high quality and are tested for acceptability.

f. Analytical methods, i.e., standardized methods established by a standards-setting organization and validated. Ideally, these laboratory methods have documented precision, bias, sensitivity, selectivity, and specificity.

g. Analytical quality control measures, including such analytical checks as duplicate analyses, positive and negative controls, sterility checks, and verification tests.

h. Standard operating procedures (SOPs), i.e., written statement and documentation of all routine laboratory operations.

i. Documentation requirements, concerning data acquisition, recordkeeping, traceability, and accountability.

j. Assessment requirements:

1) Internal audits of the laboratory operations, performed by the QA officer and supervisor.

2) On-site evaluations by outside experts to ensure that the laboratory and its personnel are following an acceptable QA program.

3) Performance evaluation studies, in which the QA officer works with the supervisor to incorporate unknown challenge samples into routine analytical runs and laboratories are encouraged to participate in state and national proficiency testing and accreditation programs. The collaborative studies confirm the abilities of a laboratory to generate acceptable data comparable to those of other laboratories and identify potential problems.

k. Corrective actions: When problems are identified by the staff, supervisor, and/or QA coordinator, use standard stepwise procedures to determine the causes and correct them. Nonconformances identified by external laboratory evaluation are corrected, recorded, and signed off by the laboratory manager and QA officer.

Detailed descriptions of quality assurance programs are available.[1–4]

The QC guidelines discussed in 9020B and 9020C are recommended as useful source material, but all elements need to be addressed in developing a QA program.

5. References

1. GASKIN, J.E. 1992. Quality Assurance in Water Quality Monitoring. Inland Water Directorate, Conservation & Protection, Ottawa, Ont., Canada.
2. RATLIFF, T.A., JR. 1990. The Laboratory Quality Assurance System. A Manual of Quality Procedures with Related Forms. Van Nostrand Reinhold, New York, N.Y.
3. GARFIELD, F.M. 1984. Quality Assurance Principles of Analytical Laboratories. Assoc. Official Analytical Chemists, Arlington, Va.
4. DUX, J.P. 1983. Quality assurance in the analytical laboratory. *Amer. Lab.* 26:54.

9020 B. Intralaboratory Quality Control Guidelines

All laboratories have some intralaboratory QC practices that have evolved from common sense and the principles of controlled experimentation. A QC program applies practices necessary to minimize systematic and random errors resulting from personnel, instrumentation, equipment, reagents, supplies, sampling and analytical methods, data handling, and data reporting. It is especially important that laboratories performing only a limited amount of microbiological testing exercise strict QC. A listing of key QC practices is given in Table 9020:I. Other sources of QC practices are available.[1–3] These practices and guidelines will assist laboratories in establishing and improving QC programs. Laboratories should address all of the QC guidelines discussed herein, but the depth and details may differ for each laboratory.

1. Personnel

Microbiological testing should be performed by a professional microbiologist or technician trained in environmental microbiology whenever possible. If not, a professional microbiologist should be available for guidance. Train and evaluate the analyst in basic laboratory procedures. The supervisor periodically should review procedures of sample collecting and handling, media and glassware preparation, sterilization, routine analytical testing, counting, data handling, and QC techniques to identify and eliminate problems. Management should assist laboratory personnel in obtaining additional training and course work to advance their skills and career.

TABLE 9020:I. KEY QUALITY CONTROL PRACTICES

Item	Action	Frequency	Further Information in Section 9020B, ¶
Reagent water	Monitor quality	See Table 9020:II	
Bench surface	Monitor for contamination	Weekly	2*e*
Air in workplace	Monitor bacterial density	Monthly	2*e*
Thermometers	Check accuracy	Semiannually	3*a*
Balances and weights	Check accuracy	Monthly	3*b*
Balances	Service and recalibrate	Annually	3*b*
pH meter	Standardize	Each use	3*c*
	Check against another meter	Monthly	3*c*
Media-dispensing apparatus	Check volume accuracy	Each use	3*f*
Hot-air oven	Check performance	Monthly	3*g*
Autoclave	Check performance	Monthly	3*h*, 4*i*2)
Refrigerator	Check temperature	Daily	3*i*
Freezer	Check temperature	Daily	3*j*
	Defrost	Semiannually	3*j*
Membrane filtration equipment	Check for leaks and surface scratches	Each use	3*k*
UV lamps	Test with UV meter	Quarterly	3*l*
Biohazard hood	Monitor air and UV lamps	Monthly	3*m*
	Inspect for airflow	Quarterly	3*m*
Incubator	Check temperature	Twice daily	3*n* and *o*
Microscope	Clean optics and stage	Each use	3*p*
Glassware	Inspect for cleanliness, chips, and etching	Each use	4*a*
	Check pH	Each batch	4*a*1)
	Conduct inhibitory residue test	Annually	4*a*2)
Dilution water bottles	Check pH and volume	Each use	4*c*
Media	Check pH and appearance	Each use	4*i*1)
Plate counts	Perform duplicate analyses	Weekly	8*a*4)
	Repeat counts	Monthly	8*a*2)

2. Facilities

a. Ventilation: Plan well-ventilated laboratories that can be maintained free of dust, drafts, and extreme temperature changes. Whenever possible, laboratories should have air conditioning to reduce contamination, permit more stable operation of incubators, and decrease moisture problems with media and instrumentation.

b. Space utilization: Design and operate the laboratory to minimize through traffic and visitors, with a separate area for preparing and sterilizing media, glassware, and equipment. Use a vented laminar-flow hood for dispensing and preparing sterile media, transferring microbial cultures, or working with pathogenic materials. In smaller laboratories it may be necessary, although undesirable, to carry out these activities in the same room.

c. Laboratory bench areas: Provide at least 2 m of linear bench space per analyst and additional areas for preparation and support activities. For stand-up work, typical bench dimensions are 90 to 97 cm high and 70 to 76 cm deep. For sit-down activities such as microscopy and plate counting, benches are 75 to 80 cm high. Specify bench tops of stainless steel, epoxy plastic, or other smooth, impervious surface that is inert and corrosion-resistant, has a minimum number of seams, and has adequate sealing of any crevices. Install even, glare-free lighting with about 1000 lux (100 ft-candles) intensity at the working surface.

d. Walls and floors: Assure that walls are covered with a smooth finish that is easily cleaned and disinfected. Specify floors of smooth concrete, vinyl, asphalt tile, or other impervious, sealed washable surfaces.

e. Work-area monitoring: Maintain high standards of cleanliness in work areas. Monitor air, at least monthly, with air density plates. The number of colonies on the air density plate test should not exceed $160/m^2/15$ min exposure (15 colonies/plate/15 min).

Plate or the swab method[1] can be used weekly or more frequently to monitor bench surface contamination. Although uniform limits for bacterial density have not been set, each laboratory can use these tests to establish a base line and take action on a significant increase.

f. Laboratory cleanliness: Regularly clean laboratory rooms and wash benches, shelves, floors, and windows. Wet-mop floors and treat with a disinfectant solution; do not sweep or dry-mop. Wipe bench tops and treat with a disinfectant before and after use. Do not permit laboratory to become cluttered.

3. Laboratory Equipment and Instrumentation

Verify that each item of equipment meets the user's needs for precision and minimization of bias. Perform equipment maintenance on a regular basis as recommended by the manufacturer or obtain preventive maintenance contracts on autoclave, balances, microscopes, and other equipment. Directly record all quality control checks in a permanent log book.

Use the following quality control procedures:

a. Thermometer/temperature-recording instruments: Check accuracy of thermometers or temperature-recording instruments

semiannually against a certified National Institute of Standards and Technology (NIST) thermometer or one traceable to NIST and conforming to NIST specifications. For general purposes use thermometers graduated in increments of 0.5°C or less. Maintain in water or glycerol for air incubators and refrigerators and glycerol for freezers and seal in a flask. For a 44.5°C water bath, use a submersible thermometer graduated to 0.2°C or less. Record temperature check data in a quality control log. Mark the necessary NIST calibration corrections on each thermometer and incubator, refrigerator, or freezer. When possible, equip incubators and water baths with temperature-recording instruments that provide a continuous record of operating temperature.

b. Balances: Follow manufacturer's instructions in operation and routine maintenance of analytical and top-loading balances. Balances should be serviced and recalibrated by a manufacturer technician annually or more often as conditions change or problems occur. In weighing 2 g or less, use an analytical balance with a sensitivity less than 1 mg at a 10-g load. For larger quantities use a pan balance with sensitivity of 0.1 g at a 150-g load.

Wipe balance before use with a soft brush. Clean balance pans after use and wipe spills up immediately with a laboratory tissue. Inspect weights with each use and replace if corroded. Use only a plastic-tip forceps to handle weights. Check balance and working weights monthly against a set of reference weights (ANSI/ASTM Class 1 or NIST Class S) for accuracy, precision, and linearity.[4] Record results.

c. pH meter: Use a meter graduated in 0.1 pH units or less, that includes temperature compensation. Preferably use digital meters and commercial buffer solutions. With each use, standardize meter with two buffers that bracket the pH of interest and record. Date buffer solutions when opened and check monthly against another pH meter. Discard solution after each use and replace buffer supply before expiration date. For full details of pH meter use and maintenance, see Section 4500-H^+.

d. Water purification system: Commercial systems are available that include some combination of prefiltration, activated carbon, mixed-bed resins, and reverse-osmosis with final filtration to produce a reagent-grade water. The life of such systems can be extended greatly if the source water is pretreated by distillation or by reverse osmosis to remove dissolved solids. Such systems tend to produce the same quality water until resins or activated carbon are near exhaustion and quality abruptly becomes unacceptable. Some deionization components are available now that automatically regenerate the ion exchange resins. Do not store reagent water unless a commercial UV irradiation device is installed and is confirmed to maintain sterility.

Monitor reagent water continuously or daily with a calibrated conductivity meter and analyze at least annually for trace metals. Replace cartridges at intervals recommended by the manufacturer based on the estimated usage and source water quality. Do not wait for column failure. If bacteria-free water is desired, include aseptic final filtration with a 0.22-μm-pore membrane filter and collect in a sterile container. Monitor treated water for contamination and replace the filter as necessary.

e. Water still: Stills produce water of a good grade that characteristically deteriorates slowly over time as corrosion, leaching, and fouling occur. These conditions can be controlled with proper maintenance and cleaning. Stills efficiently remove dissolved substances but not dissolved gases or volatile organic chemicals. Freshly distilled water may contain chlorine and ammonia (NH_3). On storage, additional NH_3 and CO_2 are absorbed from the air. Use softened water as the source water to reduce frequency of cleaning the still. Drain and clean still and reservoir according to manufacturer's instructions and usage.

f. Media dispensing apparatus: Check accuracy of volumes dispensed with a graduated cylinder at start of each volume change and periodically throughout extended runs. If the unit is used more than once per day, pump a large volume of hot reagent water through the unit to rinse between runs. Correct leaks, loose connections, or malfunctions immediately. At the end of the work day, break apparatus down into parts, wash, rinse with reagent water, and dry. Lubricate parts according to manufacturer's instructions or at least once per month.

g. Hot-air oven: Test performance monthly with commercially available *Bacillus subtilis* spore strips or spore suspensions. Monitor temperature with a thermometer accurate in the 160 to 180°C range and record results. Use heat-indicating tape to identify supplies and materials that have been exposed to sterilization temperatures.

h. Autoclave: Record items sterilized, temperature, pressure, and time for each run. Optimally use a recording thermometer. Check and record operating temperature weekly with a minimum/maximum thermometer. Test performance with *Bacillus stearothermophilus* spore strips, suspensions, or capsules monthly. Use heat-indicating tape to identify supplies and materials that have been sterilized.

i. Refrigerator: Maintain temperature at 1 to 4°C. Check and record temperature daily and clean monthly. Identify and date materials stored. Defrost as required and discard outdated materials quarterly.

j. Freezer: Maintain temperature at −20°C to −30°C. Check and record temperature daily. A recording thermometer and alarm system are highly desirable. Identify and date materials stored. Defrost and clean semiannually; discard outdated materials.

k. Membrane filtration equipment: Before use, assemble filtration units and check for leaks. Discard units if inside surfaces are scratched. Wash and rinse filtration assemblies thoroughly after use, wrap in nontoxic paper or foil, and sterilize.

l. Ultraviolet lamps: Disconnect lamps monthly and clean bulbs with a soft cloth moistened with ethanol. Test lamps quarterly with an appropriate (short- or long-wave) UV light meter* and replace bulbs if output is less than 70% of the original. For short-wave lamps used in disinfecting work areas, expose plate count agar spread plates containing 200 to 300 organisms of interest, for 2 min. Incubate plates at 35°C for 48 h and count colonies. Replace bulb if count is not reduced 99%.

CAUTION: *Although short-wave (254-nm) UV light is known to be more dangerous than long-wave UV (365-nm), both types of UV light can damage eyes and skin and potentially are carcinogenic.[5] Protect eyes and skin from exposure to UV light. (See Section 1090B.)*

m. Biohazard hood: Once per month expose plate count agar plates to air flow for 1 h. Incubate plates at 35°C for 48 h and examine for contamination. A properly operating biohazard hood

* Fisher Scientific, short wave meter (Cat. No. 11-924-54) and long wave meter (Cat. No. 11-984-53), Pittsburgh, PA 15219-4785, or equivalent.

should produce no growth on the plates. Disconnect UV lamps and clean monthly by wiping with a soft cloth moistened with ethanol. Check lamps' efficiency as specified above. Inspect cabinet for leaks and rate of air flow quarterly. Use a pressure monitoring device to measure efficiency of hood performance. Have laminar-flow safety cabinets containing HEPA filters serviced by the manufacturer. Maintain hoods as directed by the manufacturer.

n. Water bath incubator: Verify that incubators maintain test temperature, such as 35 ± 0.5°C or 44.5 ± 0.2°C. Keep an appropriate thermometer (¶ 3*a*, above) immersed in the water bath; monitor and record temperature twice daily (morning and afternoon). For optimum operation, equip water bath with a gable cover. Use only stainless steel, plastic-coated, or other corrosion-proof racks. Clean bath as needed.

o. Incubator (air, water jacketed, or aluminum block): Verify that incubators maintain appropriate test temperatures. Also, verify that cold samples are incubated at the test temperature for the required time. Check and record temperature twice daily (morning and afternoon) on the shelves in use. If a glass thermometer is used, submerge bulb and stem in water or glycerine to the stem mark. For best results use a recording thermometer and alarm system. Place incubator in an area where room temperature is maintained between 16 and 27°C (60 to 80°F).

p. Microscopes: Use lens paper to clean optics and stage after each use. Cover microscope when not in use.

Permit only trained technicians to use fluorescence microscope and light source. Monitor fluorescence lamp with a light meter and replace when a significant loss in fluorescence is observed. Log lamp operation time, efficiency, and alignment. Periodically check lamp alignment, particularly when the bulb has been changed; realign if necessary. Use known positive 4 + fluorescence slides as controls.

4. Laboratory Supplies

a. Glassware: Before each use, examine glassware and discard items with chipped edges or etched inner surfaces. Particularly examine screw-capped dilution bottles and flasks for chipped edges that could leak and contaminate the analyst and the area. Inspect glassware after washing for excessive water beading and rewash if necessary. Make the following tests for clean glassware as necessary:

1) pH check—Because some cleaning solutions are difficult to remove completely, spot check batches of clean glassware for pH reaction, especially if soaked in alkali or acid. To test clean glassware for an alkaline or acid residue add a few drops of 0.04% bromthymol blue (BTB) or other pH indicator and observe the color reaction. BTB should be blue-green (in the neutral range).

To prepare 0.04% bromthymol blue indicator solution, add 16 mL 0.01*N* NaOH to 0.1 g BTB and dilute to 250 mL with reagent water.

2) Test for inhibitory residues on glassware and plasticware—Certain wetting agents or detergents used in washing glassware may contain bacteriostatic or inhibiting substances that require 6 to 12 rinsings to remove all traces and insure freedom from residual bacteriostatic action. Perform this test annually and before using a new supply of detergent. If prewashed, presterilized plasticware is used, test it for inhibitory residues. Although the following procedure describes testing of petri dishes for inhibitory residue, it is applicable to other glass or plasticware.

a) Procedure—Wash and rinse six petri dishes according to usual laboratory practice and designate as Group A.

Wash six petri dishes as above, rinse 12 times with successive portions of reagent water, and designate as Group B.

Rinse six petri dishes with detergent wash water (in use concentration), and air-dry without further rinsing, and designate as Group C.

Sterilize dishes in Groups A, B, and C by the usual procedure.

For presterilized plasticware, set up six plastic petri dishes and designate them as Group D.

Prepare and sterilize 200 mL plate count agar and hold in a 44 to 46°C water bath.

Prepare a culture of *E. aerogenes* known to contain 50 to 150 colony-forming units/mL. Preliminary testing may be necessary to achieve this count range. Inoculate three dishes from each test group with 0.1 mL and the other three dishes from each group with 1 mL culture.

Analyze the four sets of six plates each, following heterotrophic plate count method (Section 9215B), and incubate at 35°C for 48 h. Count plates with 30 to 300 colonies and record results as CFU/mL.

b) Interpretation of results—Difference in averaged counts on plates in Groups A through D should be less than 15% if there are no toxic or inhibitory effects.

Differences in averaged counts of less than 15% between Groups A and B and greater than 15% between Groups A and C indicate that the cleaning detergent has inhibitory properties that are eliminated during routine washing. Differences between B and D greater than 15% indicate an inhibitory residue.

b. Utensils and containers for media preparation: Use utensils and containers of borosilicate glass, stainless steel, aluminum, or other corrosion-resistant material (see Section 9030). Do not use copper utensils.

c. Dilution water bottles: Use scribed bottles made of nonreactive borosilicate glass or plastic with screwcaps containing inert liners. Clean before use. Sterile disposable plastic bottles prefilled with dilution water are available commercially and are acceptable. Before use of each lot, check pH and volume and examine sterile bottles of dilution water for a precipitate; discard if present. Reclean bottles with acid if necessary, and remake the dilution water. If precipitate repeats, procure a different source of bottles.

d. Reagent-grade water quality: The quality of water obtainable from a water purification system differs with the system used and its maintenance. See 3*d* and *e* above. Recommended limits for reagent water quality are given in Table 9020:II. If these limits are not met, investigate and correct or change water source. Although pH measurement of reagent water is characterized by drift, extreme readings are indicative of chemical contamination.

e. Use test for evaluation of reagent water, media, and membranes: When a new lot of culture medium, membrane filters, or a new source of reagent-grade water is to be used make comparison tests, at least quarterly, of the current lot in use (reference lot) against the new lot (test lot).

1) Procedure—Use a single batch of control water (redistilled or distilled water polished by deionization), glassware, membrane filters, or other needed materials to control all variables

TABLE 9020:II. QUALITY OF REAGENT WATER USED IN MICROBIOLOGY TESTING

Test	Monitoring Frequency	Maximum Acceptable Limit
Chemical tests:		
Conductivity	Continuously or with each use	>0.5 megohms resistance or <2 μmhos/cm at 25°C
pH	With each use	5.5–7.5
Total organic carbon	Monthly	<1.0 mg/L
Heavy metals, single (Cd, Cr, Cu, Ni, Pb, and Zn)	Annually*	<0.05 mg/L
Heavy metals, total	Annually*	<0.10 mg/L
Ammonia/organic nitrogen	Monthly	<0.10 mg/L
Total chlorine residual	Monthly or with each use	<0.01 mg/L
Bacteriological tests:		
Heterotrophic plate count (See Section 9215)	Monthly	< 1000 CFU/mL
Use test (see 4*e*)	Quarterly and for a new source	Student's $t \leq 2.78$

*Or more frequently if there is a problem.

except the one factor under study. Make parallel pour or spread plate or membrane filter plate tests on reference lot and test lot, according to procedures in Sections 9215 and 9222. As a minimum, make single analyses on five different water samples positive for the target organism. Replicate analyses and additional samples can be tested to increase the sensitivity of detecting differences between reference and test lots.

When conducting the use test on reagent water, perform the quantitative bacterial tests in parallel using a known high-quality water as a control water. Prepare dilution/rinse water and media with new source of reagent and control water. Test water for all uses (dilution, rinse, media preparation, etc.).

2) Counting and calculations—After incubation, compare bacterial colonies from the two lots for size and appearance. If colonies on the test lot plates are atypical or noticeably smaller than colonies on the reference lot plates, record the evidence of inhibition or other problem, regardless of count differences. Count plates and calculate the individual count per 1 mL or per 100 mL. Transform the count to logarithms and enter the log-transformed results for the two lots in parallel columns. Calculate the difference, d, between the two transformed results for each sample, including the + or − sign, the mean, $\bar{d}$ and the standard deviation s_d of these differences (see Section 1010B).

Calculate Student's t statistic, using the number of samples as n:

$$t = \frac{\bar{d}}{s_d / \sqrt{n}}$$

These calculations may be made with various statistical software packages available for personal computers.

3) Interpretation—Use the critical t value from a Student's t table for comparison against the calculated value. At the 0.05 significance level this value is 2.78 for five samples (four degrees of freedom). If the calculated t value does not exceed 2.78, the lots do not produce significantly different results and the test lot is acceptable. If the calculated t value exceeds 2.78, the lots produce significantly different results and the test lot is unacceptable.

If the colonies are atypical or noticeably smaller on the test lot or the Student's t exceeds 2.78, review test conditions, repeat the test, and/or reject the test lot and obtain another one.

f. Reagents: Because reagents are an integral part of microbiological analyses, their quality must be assured. Use only chemicals of ACS or equivalent grade because impurities can inhibit bacterial growth, provide nutrients, or fail to produce the desired reaction. Date chemicals and reagents when received and when first opened for use. Make reagents to volume in volumetric flasks and transfer for storage to good-quality inert plastic or borosilicate glass bottles with borosilicate, polyethylene, or other plastic stoppers or caps. Label prepared reagents with name and concentration, date prepared, and initials of preparer. Include positive and negative control cultures with each series of cultural or biochemical tests.

g. Dyes and stains: In microbiological analyses, organic chemicals are used as selective agents (e.g., brilliant green), as indicators (e.g., phenol red), and as microbiological stains (e.g., Gram stain). Dyes from commercial suppliers vary from lot to lot in percent dye, dye complex, insolubles, and inert materials. Because dyes for microbiology must be of proper strength and stability to produce correct reactions, use only dyes certified by the Biological Stain Commission. Check bacteriological stains before use with at least one positive and one negative control culture and record results.

h. Membrane filters and pads: The quality and performance of membrane filters vary with the manufacturer, type, brand, and lot. These variations result from differences in manufacturing methods, materials, quality control, storage conditions, and application.

1) Membrane filters and pads for water analyses should meet the following specifications:

a) Filter diam 47 mm, mean pore diam 0.45 μm. Alternate filter and pore sizes may be used if the manufacturer provides data verifying performance equal to or better than that of 47-mm-diam, 0.45-μm-pore size filter. At least 70% of filter area must be pores.

b) When filters are floated on reagent water, the water diffuses uniformly through the filters in 15 s with no dry spots on the filters.

c) Flow rates are at least 55 mL/min/cm^2 at 25°C and a differential pressure of 93 kPa.

d) Filters are nontoxic, free of bacterial-growth-inhibiting or stimulating substances, and free of materials that directly or indirectly interfere with bacterial indicator systems in the me-

dium; ink grid is nontoxic. The arithmetic mean of five counts on filters must be at least 90% of the arithmetic mean of the counts on five agar spread plates using the same sample volumes and agar media.

e) Filters retain the organisms from a 100-mL suspension of *Serratia marcescens* containing 1×10^3 cells.

f) Water-extractables in filter do not exceed 2.5% after the membrane is boiled in 100 mL reagent water for 20 min, dried, cooled, and brought to constant weight.

g) Absorbent pad has diam 47 mm, thickness 0.8 mm, and is capable of absorbing 2.0 ± 0.2 mL Endo broth.

h) Pads release less than 1 mg total acidity calculated as $CaCO_3$ when titrated to the phenolphthalein end point with 0.02*N* NaOH.

i) If filter and absorbent pad are not sterile, they should not be degraded by sterilization at 121°C for 10 min. Confirm sterility by absence of growth when a membrane filter is placed on a pad saturated with tryptone glucose extract broth or tryptone glucose extract agar and incubated at 35 ± 0.5°C for 24 h.

j) Some lots of membrane filters yield low recoveries, poor differentiation, or malformation of colonies due to toxicity, chemical composition, or structural defects.[6] Perform the use test (¶ 4*e*) on new lots of filters.

2) Standardized tests:

Standardized tests are available for evaluating retention, recovery, extractables, and flow rate characteristics of membrane filters.[7]

Some manufacturers provide information beyond that required by specifications and certify that their membranes are satisfactory for water analysis. They report retention, pore size, flow rate, sterility, pH, percent recovery, and limits for specific inorganic and organic chemical extractables. Although the standard membrane filter evaluation tests were developed for the manufacturers, a laboratory can conduct its own tests.

To maintain quality control inspect each lot of membranes before use and during testing to insure they are round and pliable, with undistorted gridlines after autoclaving. After incubation, colonies should be well-developed with well-defined color and shape as defined by the test procedure. The gridline ink should not channel growth along the ink line nor restrict colony development. Colonies should be distributed evenly across the membrane surface.

i. Culture media: Because cultural methods depend on properly prepared media, use the best available materials and techniques in media preparation, storage, and application. For control of quality, use commercially prepared media whenever available but note that such media may vary in quality among manufacturers and even from lot to lot from the same manufacturer.

Order media in quantities to last no longer than 1 year. Use media on a first-in, first-out basis. When practical, order media in quarter pound (114 g) multiples rather than one pound (454 g) bottles, to keep the supply sealed as long as possible. Record kind, amount, and appearance of media received, lot number, expiration date, and dates received and opened. Check inventory quarterly for reordering.

Store dehydrated media at an even temperature in a cool dry place, away from direct sunlight. Discard media that cake, discolor, or show other signs of deterioration. If expiration date is given by manufacturer, discard unused media after that date. A conservative time limit for unopened bottles is 2 years at room temperature. Compare recovery of newly purchased lots of media against proven lots, using recent pure-culture isolates and natural samples.

Use opened bottles of media within 6 months. Dehydrated media are hygroscopic. Protect opened bottles from moisture. Close bottles as tightly as possible, immediately after use. If caking or discoloration of media occurs, discard media. Store opened bottles in a dessicator.

1) Preparation of media—Prepare media in containers that are at least twice the volume of the medium being prepared. Stir media, particularly agars, while heating. Avoid scorching or boil-over by using a boiling water bath for small batches of media and by continually attending to larger volumes heated on a hot plate or gas burner. Preferably use hot plate-magnetic stirrer combinations. Label and date prepared media. Prepare media in reagent water. Measure water volumes and media with graduates or pipets conforming to NIST and APHA standards, respectively. Do not use blow-out pipets. After preparation and storage, remelt agar media in boiling water or flowing steam.

Check and record pH of a portion of each medium after sterilization and cooling. Check pH of solid medium with a surface probe. Record results. Make minor adjustments in pH ($<$0.5 pH units) with 1*N* NaOH or HCl solution to the pH specified in formulation. If the pH difference is larger than 0.5 units, discard the batch and check preparation instructions and pH of reagent water to resolve the problem. Incorrect pH values may be due to reagent water quality, medium deterioration, or improper preparation. Review instructions for preparation and check water pH. If water pH is unsatisfactory, prepare a new batch of medium using water from a new source (see 9020B.3*d* and *e*). If water is satisfactory, remake medium and check; if pH is again incorrect, prepare medium from another bottle.

Record pH problems in the media record book and inform the manufacturer if the medium is indicated as the source of error. Examine prepared media for unusual color, darkening, or precipitation and record observations. Consider variations of sterilization time and temperature as possible causes for problems. If any of the above occur, discard the medium.

2) Sterilization—Sterilize media at 121 to 124°C for the minimum time specified. A double-walled autoclave permits maintenance of full pressure and temperature in the jacket between loads and reduces chance for heat damage. Follow manufacturer's directions for sterilization of specific media. The required exposure time varies with form and type of material, type of medium, presence of carbohydrates, and volume. Table 9020:III gives guidelines for typical items. Do not expose media containing carbohydrates to the elevated temperatures for more than 45 min. Exposure time is defined as the period from initial exposure to removal from the autoclave.

Some currently available autoclave models are automatic and include features such as vertical sliding, self-sealing and opening doors, programmable sterilization cycles, and continuous multipoint monitoring of chamber temperature and pressure. These units also may incorporate solution cooling and vapor removal features. When sterilizer design includes heat exchangers and solution cooling features as part of a factory-programmed liquid cycle, strict adherence to the 45-min total elapsed time in the autoclave is not necessary provided that printout records verify

TABLE 9020:III. TIME AND TEMPERATURE FOR AUTOCLAVE STERILIZATION

Material	Time at 121°C*
Membrane filters and pads	10 min
Carbohydrate-containing media (lauryl tryptose, BGB broth, etc.)	12–15 min
Contaminated materials and discarded cultures	30 min
Membrane filter assemblies (wrapped), sample collection bottles (empty)	15 min
Buffered dilution water, 99 mL in screw-cap bottle	15 min
Rinse water, volume > 100 mL	Adjust for volume

*Except for media, times are guidelines; check for sterility.

TABLE 9020:IV. HOLDING TIMES FOR PREPARED MEDIA

Medium	Holding Time
Membrane filter (MF) broth in screw-cap flasks at 4°C	96 h
MF agar in plates with tight-fitting covers at 4°C	2 weeks
Agar or broth in loose-cap tubes at 4°C	2 weeks
Agar or broth in tightly closed screw-cap tubes or other sealed containers	3 months
Poured agar plates with loose-fitting covers in sealed plastic bags at 4°C	2 weeks
Large volume of agar in tightly closed screw-cap flask or bottle at 4°C	3 months

normal cycle operation and chamber cooling during exhaust and vapor removal.

Remove sterilized media from autoclave as soon as chamber pressure reaches zero, or, if a fully automatic model is used, as soon as the door opens. Do not reautoclave media.

Check effectiveness of sterilization monthly by placing *Bacillus stearothermophilus* spore suspensions or strips (commercially available) inside glassware. Sterilize at 121°C for 15 min. Place in trypticase soy broth tubes and incubate at 55°C for 48 h. If growth of the autoclaved spores occurs after incubation at 55°C, sterilization was inadequate. A small, relatively inexpensive 55°C incubator is available commercially.†

Sterilize heat-sensitive solutions or media by filtration through a 0.22-μm-pore-diam filter in a sterile filtration and receiving apparatus. Filter and dispense medium in a safety cabinet or biohazard hood if available. Sterilize glassware (pipets, petri dishes, sample bottles) in an autoclave or an oven at 170°C for 2 h. Sterilize equipment, supplies, and other solid or dry materials that are heat-sensitive, by exposing to ethylene oxide in a gas sterilizer. Use commercially available spore strips or suspensions to check dry heat and ethylene oxide sterilization.

3) Use of agars and broths—Temper melted agars in a water bath at 44 to 46°C until used but do not hold longer than 3 h. To monitor agar temperature, expose a bottle of water or medium to the same heating and cooling conditions as the agar. Insert a thermometer in the monitoring bottle to determine when the temperature is 45 to 46°C and suitable for use in pour plates. If possible, prepare media on the day of use. After pouring agar plates for streaking, dry agar surfaces by keeping dish slightly open for at least 15 min in a bacteriological hood to avoid contamination. Discard unused liquid agar; do not let harden or remelt for later use.

Handle tubes of sterile fermentation media carefully to avoid entrapping air in inner tubes, thereby producing false positive reactions. Examine freshly prepared tubes to determine that gas bubbles are absent.

4) Storage of media—Prepare media in amounts that will be used within holding time limits given in Table 9020:IV. Protect media containing dyes from light; if color changes occur, discard the media. Refrigerate poured agar plates not used on the day of preparation. Seal agar plates with loose-fitting lids in plastic bags if held more than 2 d. Prepare broth media that will be stored for more than 2 weeks in screw-cap tubes, other tightly sealed tubes, or in loose-capped tubes placed in a sealed plastic bag or other tightly sealed container to prevent evaporation.

Mark liquid level in several tubes and monitor for loss of liquid. If loss is 10% or more, discard the batch. If media are refrigerated, incubate overnight at test temperature before use and reject the batch if false positive responses occur. Prepared sterile broths and agars available from commercial sources may offer advantages when analyses are done intermittently, when staff is not available for preparation work, or when cost can be balanced against other factors of laboratory operation. Check performance of these media as described in ¶ 5 below.

5) Quality control of prepared media—Maintain in a bound book a complete record of each prepared batch of medium with name of preparer and date, name and lot number of medium, amount of medium weighed, volume of medium prepared, sterilization time and temperature, pH measurements and adjustments, and preparations of labile components. Compare quantitative recoveries of new lots with previously acceptable ones. Include sterility and positive and negative control culture checks on all media as described below.

5. Standard Operating Procedures (SOPs)

SOPs are the operational backbone of an analytical laboratory. SOPs describe in detail all laboratory operations such as preparation of reagents, reagent water, standards, culture media, proper use of balances, sterilization practices, and dishwashing procedures, as well as methods of sampling, analysis, and quality control. The SOPs are unique to the laboratory. They describe the tasks as performed on a day-to-day basis, tailored to the laboratory's own equipment, instrumentation, and sample types. The SOPs guide routine operations by each analyst, help to assure uniform operations, and provide a solid training tool.

6. Sampling

a. Planning: Microbiologists should participate in the planning of monitoring programs that will include microbial analyses. They can provide valuable expertise on the selection of sampling sites, number of samples and analyses needed, workload, and equipment and supply needs. For natural waters, knowledge of the probable microbial densities, and the impact of

† 3M Health Care, St. Paul, MN 55144, or equivalent.

season, weather, tide and wind patterns, known sources of pollution, and other variables, are needed to formulate the most effective sampling plan.

b. Methods: Sampling plans must be specific for each sampling site. Prior sampling guidance can be only general in nature, addressing the factors that must be considered for each site. Sampling SOPs describe sampling equipment, techniques, frequency, holding times and conditions, safety rules, etc., that will be used under different conditions for different sites. From the information in these SOPs sampling plans will be drawn up.

7. Analytical Methods

a. Method selection: Because minor variations in technique can cause significant changes in results, microbiological methods must be standardized so that uniform data result from multiple laboratories. Select analytical methods appropriate for the sample type from *Standard Methods* or other source of standardized methods and ensure that methods have been validated in a multilaboratory study with the sample types of interest.

b. Data objectives: Review available methods and determine which produce data to meet the program's needs for precision, bias, specificity, selectivity, and detection limit. Ensure that the methods have been demonstrated to perform within the above specifications for the samples of interest.

c. Internal QC: The written analytical methods should contain required QC checks of positive and negative control cultures, sterile blank, replicate analyses (precision), and a known quantitative culture, if available.

d. Method SOPs: As part of the series of SOPs, provide each analyst with a copy of the analytical methods written in stepwise fashion exactly as they are to be performed and specific to the sample type, equipment, and instrumentation used in the laboratory.

8. Analytical Quality Control Procedures

a. General quality control procedures:

1) New methods—Conduct parallel tests with the standard procedure and a new method to determine applicability and comparability. Perform at least 100 parallel tests across seasons of the year before replacement with the new method for routine use.

2) Comparison of plate counts—For routine performance evaluation, repeat counts on one or more positive samples at least monthly and compare the counts with those of other analysts testing the same samples. Replicate counts for the same analyst should agree within 5% and those between analysts should agree within 10%. See 9020B.11*b* for a statistical calculation of data precision.

3) Control cultures—For each lot of medium check analytical procedures by testing with known positive and negative control cultures for the organism(s) under test. See Table 9020:V for examples of test cultures.

4) Duplicate analyses—Perform duplicate analyses on 10% of samples and on at least one sample per test run. A test run is defined as an uninterrupted series of analyses. If the laboratory conducts less than 10 tests/week, make duplicate analyses on at least one sample each week.

TABLE 9020:V. CONTROL CULTURES FOR MICROBIOLOGICAL TESTS

Group	Control Culture	
	Positive	Negative
Total coliforms	*Escherichia coli* *Enterobacter aerogenes*	*Staphylococcus aureus* *Pseudomonas* sp.
Fecal coliforms	*E. coli*	*E. aerogenes* *Streptococcus faecalis*
Escherichia coli	*E. coli*	*E. aerogenes*
Fecal streptococci	*Enterococcus faecalis*	*Staphylococcus aureus* *E. coli*
Enterococci	*S. faecalis*	*S. mitis/salivarius*

5) Sterility checks—For membrane filter tests, check sterility of media, membrane filters, buffered dilution and rinse water, pipets, flasks and dishes, and equipment as a minimum at the end of each series of samples, using sterile reagent water as the sample. If contaminated, check for the source. For multiple-tube and presence-absence procedures, check sterility of media, dilution water, and glassware. To test sterility of media, incubate a representative portion of each batch at an appropriate temperature for 24 to 48 h and observe for growth. Check each batch of buffered dilution water for sterility by adding 20 mL water to 100 mL of a nonselective broth. Alternatively, aseptically pass 100 mL or more dilution water through a membrane filter and place filter on growth medium suitable for heterotrophic bacteria. Incubate at 35 ± 0.5°C for 24 h and observe for growth. If any contamination is indicated, determine the cause and reject analytical data from samples tested with these materials. Request immediate resampling and reanalyze.

b. Precision of quantitative methods: Calculate precision of duplicate analyses for each different type of sample examined, for example, drinking water, ambient water, wastewater, etc., according to the following procedure:

1) Perform duplicate analyses on first 15 positive samples of each type, with each set of duplicates analyzed by a single analyst. If there is more than one analyst, include all analysts regularly running the tests, with each analyst performing approximately an equal number of tests. Record duplicate analyses as D_1 and D_2.

2) Calculate the logarithm of each result. If either of a set of duplicate results is <1, add 1 to both values before calculating the logarithms.

3) Calculate the range (R) for each pair of transformed duplicates as the mean ($\bar{R}$) of these ranges.

See sample calculation in Table 9020:VI.

4) Thereafter, analyze 10% of routine samples in duplicate. Transform the duplicates as in ¶ 2) and calculate their range. If the range is greater than 3.27 $\bar{R}$, there is greater than 99% probability that the laboratory variability is excessive. Determine if increased imprecision is acceptable; if not, discard all analytical results since the last precision check (see Table 9020:VII). Identify and resolve the analytical problem before making further analyses.

5) Update the criterion used in ¶ 4) by periodically repeating the procedures of ¶s 1) through 3) using the most recent sets of 15 duplicate results.

TABLE 9020:VI. CALCULATION OF PRECISION CRITERION

Sample No.	Duplicate Analyses		Logarithms of Counts		Range of Logarithms (R_{log}) ($L_1 - L_2$)
	D_1	D_2	L_1	L_2	
1	89	71	1.9494	1.8513	0.0981
2	38	34	1.5798	1.5315	0.0483
3	58	67	1.7634	1.8261	0.0627
.	.	.	.	.	.
.	.	.	.	.	.
.	.	.	.	.	.
14	7	6	0.8451	0.7782	0.0669
15	110	121	2.0414	2.0828	0.0414

Calculations:

1) Σ of $R_{log} = 0.0981 + 0.0483 + 0.0627 + \ldots + 0.0669 + 0.0414 = 0.718\,89$

2) $\bar{R} = \frac{\Sigma R_{log}}{n} = \frac{0.718\,89}{15} = 0.0479$

3) Precision criterion $= 3.27\,\bar{R} = 3.27\,(0.0479) = 0.1566$

9. Verification

For the most part, the confirmation/verification procedures for drinking water differ from those for other waters because of specific regulatory requirements.

a. Multiple-tube fermentation (MTF) methods:

1) Total coliform procedure (9221B)

a) Drinking water—Carry samples through confirmed phase only. Verification is not required. For QC purposes, if normally there are no positive results, analyze at least one positive source water quarterly to confirm that the media produce appropriate responses. For samples with a history of heavy growth without gas in presumptive-phase tubes, carry the tubes through the confirmed phase to check for false negative responses for coliform bacteria. Verify any positives for fecal coliforms or *E. coli.*

b) Other water types—Verify by performing the completed MTF Test on 10% of samples positive through the confirmed phase.

2) Enzyme substrate coliform test (total coliform/*E. coli*) (9223B)

a) Drinking water—Verify at least 5% of total coliform positive results from enzyme substrate coliform tests by inoculating growth from a known positive sample and testing for lactose fermentation or for β-D-galactopyranosidase by the *o*-nitrophenyl-β-D-galactopyranoside (ONPG) test and indophenol by the cytochrome oxidase (CO) test. See 9225D for these tests. Coliforms are ONPG-positive and cytochrome-oxidase-negative. Verify *E. coli* using the EC MUG test (see 9221F).

b) Other water types—Verify at least 10% of total coliform positive samples as in ¶ 2*a* above.

3) Fecal streptococci procedure—Verify as in 9230C.5. Growth of catalase-negative, gram-positive cocci on bile esculin agar at 35°C and in brain-heart infusion broth at 45°C verifies the organisms as fecal streptococci. Growth at 45°C and in 6.5% NaCl broth indicates the streptococci are members of the enterococcus group.

4) Include known positive and negative pure cultures as a QC check.

b. Membrane filter methods:

1) Total coliform procedures

a) Drinking water—Pick all, up to 5 typical and 5 atypical (nonsheen) colonies from positive samples on M-Endo medium and verify as in 9222B.5*f*. Also verify any positives for fecal coliforms or *E. coli*. If there are no positive samples, test at least one known positive source water quarterly.

b) Other water types—Verify positives monthly by picking at least 10 sheen colonies from a positive water sample as in 9222B.5*f*. Adjust counts based on percent verification.

c) To determine false negatives, pick representative atypical colonies of different morphological types and verify as in 9222B.5*f*.

2) Fecal coliform procedure

a) Verify positives monthly by picking at least 10 blue colonies from one positive sample. Verify in lauryl tryptose broth and EC broth as in 9221B.1 and 9221E. Adjust counts based on percent verification.

b) To determine false negatives, pick representative atypical colonies of different morphological types and verify as in 9221B.3 and 9221E.

3) *Escherichia coli* procedure

a) Drinking water—Verify at least 5% of MUG-positive and MUG-negative results. Pick from well-isolated sheen colonies that fluoresce on nutrient agar with MUG (NA-MUG), taking care not to pick up medium, which can cause a false positive response. Also verify nonsheen colonies that fluoresce. Verify by performing the citrate test and the indole test as described in 9225D, but incubate indole test at 44.5°C. *E. coli* are indole-positive and yield no growth on citrate.

b) Other water types—Verify one positive sample monthly as in ¶ a) above. Adjust counts based on percentage of verification.

4) Fecal streptococci procedure—Pick to verify monthly at least 10 isolated esculin-positive red colonies from m-Enterococcus agar to brain heart infusion (BHI) media. Verify as described in 9230C. Adjust counts based on percentage of verification.

5) *Enterococci* procedures—Pick to verify monthly at least 10 well-isolated pink to red colonies with black or reddish-brown precipitate from EIA agar. Transfer to BHI media as described in 9230C. Adjust counts based on percentage of verification.

6) Include known positive and negative pure cultures as a quality control check.

TABLE 9020:VII. DAILY CHECKS ON PRECISION OF DUPLICATE COUNTS*

Date of Analysis	Duplicate Analyses		Logarithms of Counts		Range of Logarithms	Acceptance of Range†
	D_1	D_2	L_1	L_2		
8/29	71	65	1.8513	1.8129	0.0383	A
8/30	110	121	2.0414	2.0828	0.0414	A
8/31	73	50	1.8633	1.6990	0.1643	U

* Precision criterion = $(3.27\bar{R}) = 0.1566$.

† A = acceptable; U = unacceptable.

10. Documentation and Recordkeeping

a. QA plan: The QA program documents management's commitment to a QA policy and sets forth the requirements needed to support program objectives. The program describes overall policies, organization, objectives, and functional responsibilities for achieving the quality goals. In addition, the program should develop a project plan that specifies the QC requirements for each project. The plan specifies the QC activities required to achieve the data representativeness, completeness, comparability, and compatibility. Also, the QA plan should include a program implementation plan that ensures maximum coordination and integration of QC activities within the overall program (sampling, analyses, and data handling).

b. Sampling records: A written SOP for sample handling records sample collection, transfer, storage, analyses, and disposal. The record is most easily kept on a series of printed forms that prompt the user to provide all the necessary information. It is especially critical that this record be exact and complete if there is any chance that litigation may occur. Such record systems are called chain of custody. Because laboratories do not always know whether analytical results will be used in future litigation, some maintain chain of custody on all samples. Details on chain of custody are available in Section 1060B and elsewhere.[1]

c. Recordkeeping: An acceptable recordkeeping system provides needed information on sample collection and preservation, analytical methods, raw data, calculations through reported results, and a record of persons responsible for sampling and analyses. Choose a format agreeable to both the laboratory and the customer (the data user). Ensure that all data sheets are signed and dated by the analyst and the supervisor. The preferable record form is a bound and page-numbered notebook, with entries in ink and a single line drawn through any change with the correction entered next to it.

Keep records of microbiological analyses for at least 5 years. Actual laboratory reports may be kept, or data may be transferred to tabular summaries, provided that the following information is included: date, place, and time of sampling; name of sample collector; identification of sample; date of receipt of sample and analysis; person(s) responsible for performing analysis; analytical method used; the raw data; and the calculated results of analysis. Verify that each result was entered correctly from the bench sheet and initialed by the analyst. If an information storage and retrieval system is used, double check data on the printouts.

11. Data Handling

a. Distribution of bacterial populations: In most chemical analyses the distribution of analytical results follows the Gaussian curve, which has symmetrical distribution of values about the mean (see Section 1010B). Microbial distributions are not necessarily symmetrical. Bacterial counts often are characterized as having a skewed distribution because of many low values and a few high ones. These characteristics lead to an arithmetic mean that is considerably larger than the median. The frequency curve of this distribution has a long right tail, such as that shown in Figure 9020:1, and is said to display positive skewness.

Application of the most rigorous statistical techniques requires the assumption of symmetrical distributions such as the normal curve. Therefore it usually is necessary to convert skewed data so that a symmetrical distribution resembling the normal distribution results. An approximately normal distribution can be obtained from positively skewed data by converting numbers to their logarithms, as shown in Table 9020:VIII. Comparison of the frequency tables for the original data (Table 9020:IX) and their logarithms (Table 9020:X) shows that the logarithms approximate a symmetrical distribution.

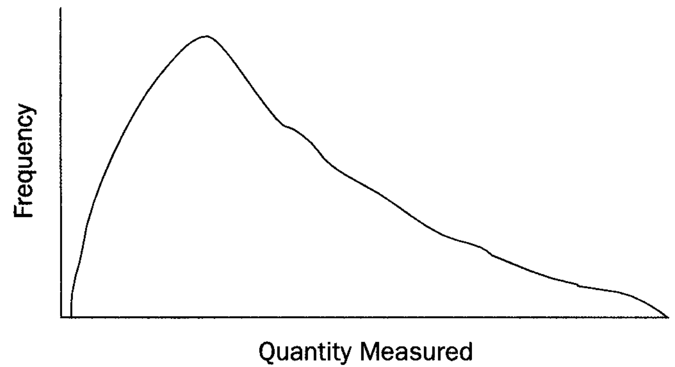

Figure 9020:1. Frequency curve (positively skewed distribution).

b. Central tendency measures of skewed distribution: If the logarithms of numbers from a positively skewed distribution are approximately normally distributed, the original data have a log-normal distribution. The best estimate of central tendency of log-normal data is the geometric mean, defined as:

$$\bar{x}_g = \sqrt[n]{(x_1)(x_2)\cdots(x_n)}$$

and

$$\log \bar{x}_g = \frac{\sum (\log x_i)}{n}$$

that is, the geometric mean is equal to the antilog of the

TABLE 9020:VIII. COLIFORM COUNTS AND THEIR LOGARITHMS

MPN Coliform Count *No./100 mL*	log MPN
11	1.041
27	1.431
36	1.556
48	1.681
80	1.903
85	1.929
120	2.079
130	2.114
136	2.134
161	2.207
317	2.501
601	2.779
760	2.881
1020	3.009
3100	3.491
$\bar{x}$ = 442	$\bar{x}_g$ = antilog 2.1825 = 152

TABLE 9020:IX. COMPARISON OF FREQUENCY OF MPN DATA

Class Interval	Frequency (MPN)
0 to 400	11
400 to 800	2
800 to 1200	1
1200 to 1600	0
1600 to 2000	0
2000 to 2400	0
2400 to 2800	0
2800 to 3200	0

arithmetic mean of the logarithms. For example, the following means calculated from the data in Table 9020:VIII are drastically different.

$$\log \bar{x}_g = \frac{\sum (\log x_i)}{n} = \frac{32.737}{15} = 2.1825$$

geometric mean

$$\bar{x}_g = \text{antilog}\ (2.1825) = 152$$

and arithmetic mean

$$\bar{x} = \frac{\sum x_i}{n} = \frac{6632}{15} = 442$$

Therefore, although regulations or tradition may require or cause microbiological data to be reported as the arithmetic mean or median, the preferred statistic for summarizing microbiological monitoring data is the geometric mean. An exception may be in the evaluation of data for risk assessment. The arithmetic mean may be a better measure for this purpose because it may generate a higher central tendency value and possibly provide a greater safety factor.[8]

TABLE 9020:X. COMPARISON OF FREQUENCY OF LOG MPN DATA

Class Interval	Frequency (log MPN)
1.000 to 1.300	1
1.300 to 1.600	2
1.600 to 1.900	1
1.900 to 2.200	5
2.200 to 2.500	1
2.500 to 2.800	2
2.800 to 3.100	2
3.100 to 3.400	0
3.400 to 3.700	1

c. "Less than" (<) values: There has always been uncertainty as to the proper way to include "less than" values in calculation and evaluation of microbiological data because such values cannot be treated statistically without modification. Proposed modifications involve changing such numbers to zero, choosing values halfway between zero and the "less than" value, or assigning the "less than" value itself, i.e., changing <1 values to 1, ½, or 0.

There are valid reasons for not including < values, whether modified or not. If the database is fairly large with just a few < values, the influence of these uncertain values will be minimal and of no benefit. If the database is small or has a relatively large number of < values, inclusion of modified < values would exert an undue influence on the final results and could result in an artificial negative or positive bias. Including < values is particularly inappropriate if the < values are <100, <1000, or higher because the unknown true values could be anywhere from 0 to 99, 0 to 999, etc. When < values are first noted, adjust or expand test volumes. The only exception to this caution would be regulatory testing with defined compliance limits, such as the <1/100 mL values reported for drinking water systems where the 100-mL volume is required.

12. References

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9020 C. Interlaboratory Quality Control

1. Background

Interlaboratory QC programs are a means of establishing an agreed-upon, common performance criteria system that will assure an acceptable level of data quality and comparability among laboratories with similar interests and/or needs.

These systems may be volunteer, such as that for the cities in the Ohio River Valley Water Sanitation Commission (ORSANCO), or regulatory, such as the Federal Drinking Water Laboratory Certification Program (see below). Often, the term "accreditation" is used interchangeably with certification. Usually, interlaboratory quality control programs have three elements: uniform criteria for laboratory operations, external review of the program, and external proficiency testing.

2. Uniform Criteria

Interlaboratory quality control programs begin as a volunteer or mandatory means of establishing uniform laboratory standards for a specific purpose. The participants may be from one organization or a group of organizations having common interests or falling under common regulations. Often one group or person may agree to draft the criteria. If under regulation, the regulating authority may set the criteria for compliance-monitoring analyses.

Uniform sampling and analytical methods and quality control criteria for personnel, facilities, equipment, instrumentation, supplies, and data handling and reporting are proposed, discussed, reviewed, modified if necessary, and approved by the group for common use. Criteria identified as necessary for acceptable data quality should be mandatory. A formal document is prepared and provided to all participants.

The QA/QC responsibilities of management, supervisors, and technical staff are described in 9020A. In large laboratories, a QA officer is assigned as a staff position but may be the supervisor or other senior person in smaller laboratories.

After incorporation into laboratory operations and confirmation that the QA program has been adapted and is in routine use, the laboratory supervisor and the QA officer conduct an internal program review of all operations and records for acceptability, to identify possible problems and assist in their resolution. If this is done properly, there should be little concern that subsequent external reviews will find major problems.

3. External Program Review

Once a laboratory has a QA program in place, management informs the organization and a qualified external QA person or team arranges an on-site visit to evaluate the QA program for acceptability and to work with the laboratory to solve any problems. An acceptable rating confirms that the laboratory's QA program is operating properly and that the laboratory has the capability of generating valid defensible data. Such on-site evaluations are repeated and may be announced or unannounced.

4. External Proficiency Testing

Whenever practical, the external organization conducts formal performance evaluation studies among all participant laboratories. Challenge samples are prepared and sent as unknowns on a set schedule for analyses and reporting of results. The reported data are coded for confidentiality and evaluated according to an agreed-upon scheme. The results are summarized for all laboratories and individual laboratory reports are sent to participants. Results of such studies indicate the quality of routine analyses of each laboratory as compared to group performance. Also, results of the group as a whole characterize the performance that can be expected for the analytical methods tested.

5. Example Program

In the Federal Drinking Water Laboratory Certification Program, public water supply laboratories must be certified according to minimal criteria and procedures and quality assurance described in the EPA manual on certification:[1] criteria are established for laboratory operations and methodology; on-site inspections are required by the certifying state agency or its surrogate to verify minimal standards; annually, laboratories are required to perform acceptably on unknown samples in formal studies, as samples are available; and the responsible authority follows up on problems identified in the on-site inspection or performance evaluation and requires corrections within a set period of time. Individual state programs may exceed the federal criteria.

On-site inspections of laboratories in the present certification program show that primary causes for discrepancies in drinking water laboratories have been inadequate equipment, improperly prepared media, incorrect analytical procedures, and insufficiently trained personnel.

6. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1997. Manual for the Certification of Laboratories Analyzing Drinking Water, 4th ed. EPA-814B-92-002, U.S. Environmental Protection Agency, Cincinnati, Ohio.