

9060 SAMPLES*

9060 A. Collection

1. Containers

Collect samples for microbiological examination in clean, sterile, nonreactive borosilicate glass or plastic bottles or pre-sterilized plastic bags appropriate for microbiological use. Where legal action may be involved, consider the use of tamper-evident closures.

2. Dechlorination

Add a reducing agent to containers intended for the collection of water having residual chlorine or other halogen unless they contain broth for direct incubation of sample. Sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) is a satisfactory dechlorination agent that neutralizes any residual halogen and prevents continuation of bactericidal action during sample transit. The examination then will indicate more accurately the true microbial content of the water at the time of sampling.

For sampling chlorinated wastewater effluents add sufficient $\text{Na}_2\text{S}_2\text{O}_3$ to a clean sample bottle to give a concentration of 100 mg/L in the sample. In a 120-mL bottle 0.1 mL of a 10% solution of $\text{Na}_2\text{S}_2\text{O}_3$ will neutralize a sample containing up to 15 mg/L residual chlorine. For drinking water samples, the concentration of dechlorination agent may be reduced: 0.1 mL of a 3% solution of $\text{Na}_2\text{S}_2\text{O}_3$ in a 120-mL bottle will neutralize up to 5 mg/L residual chlorine. See Table 9060:I for preparation of sodium thiosulfate solutions. Where possible, determine normal residual chlorine before sampling at a new site (e.g., pool water may contain a higher chlorine level than normal) to enable laboratory to prepare an adequate amount of dechlorination agent per sample bottle. Discard turbid (bacterial growth) 10% sodium thiosulfate stock solutions.

Loosely cap bottle and sterilize by either dry or moist heat, as directed (Section 9040) and perform sterility checks as noted in Section 9020B.5d. Presterilized plastic bags or bottles containing $\text{Na}_2\text{S}_2\text{O}_3$ are available commercially.

3. Sampling Procedures

Maintain consistent sampling procedures. When the sample is collected, leave ample air space in the bottle (at least 2.5 cm) to facilitate mixing by shaking, before examination. Reject sample bottles that are overfilled and request resampling or, alternatively, add overfilled samples to a larger sterile sample bottle in the laboratory to assure adequate mixing.

Keep sampling bottle closed until it is to be filled. Remove cap or stopper, if used, as a unit. Do not place cap down on any surface. Avoid external contamination during sample collection

TABLE 9060:I. SODIUM THIOSULFATE EQUIVALENTS

Solution Strength and $\text{Na}_2\text{S}_2\text{O}_3$ Form	Weight of Compound Required
3%, anhydrous	3 g/100 mL
3%, pentahydrate	4.6 g/100 mL
10%, anhydrous	10 g/100 mL
10%, pentahydrate	15.21 g/100 mL

and do not contaminate inner surface of stopper or cap and bottle neck. Fill container without rinsing, replace stopper or cap immediately, and secure hood, if used, around neck of bottle.

Systematically plan to collect samples that are representative of the water being tested. When planning sample collection activities, consider temporal, spatial (horizontal and vertical), and hydrodynamic conditions (e.g., wet versus dry weather and seasonal lake turnover effects). Sampling frequency and the number of samples to be collected will depend on ultimate data usage needs.

a. Potable water: If the water sample is to be taken from a distribution-system tap without attachments, select a tap that is supplying water from a service pipe directly connected with the main, and is not, for example, served from a cistern or storage tank. Remove from the tap any attachments, such as filters, aerators, flow directors, or screens. Open cold water tap fully and let water run to waste for 2 or 3 min, or for a time sufficient to permit clearing the service line. Reduce water flow to permit filling bottle without splashing. If tap cleanliness is questionable, choose another tap. If a questionable tap is required for special sampling purposes, disinfect the faucet (inside and outside) by applying a solution of sodium hypochlorite (100 mg NaOCl/L) to faucet or by flaming before sampling; let water run for additional 2 to 3 min after treatment. Do not sample from leaking taps that allow water to flow over the outside of the tap. If sampling from a mixing faucet cannot be avoided, run hot water for 2 min, then cold water for 2 to 3 min, and collect sample as indicated above.

If the sample is to be taken from a well fitted with a hand pump, pump water to waste for about 5 to 10 min or until water temperature has stabilized before collecting sample. If an outdoor sampling location must be used, avoid collecting samples from frost-proof hydrants. If there is no pumping machinery, collect a sample directly from the well by means of a sterilized bottle fitted with a weight at the base; take care to avoid contaminating samples by any surface scum. Other sterile sampling devices, such as a trip bailer, also may be used.

In drinking water evaluation studies, collect samples of finished water from distribution sites selected to ensure systematic coverage during each month. Carefully choose distribution system sample locations to include dead-end sections to demonstrate bacteriological quality throughout the network and to

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Joint Task Group: Margo E. Hunt (chair), Ellen B. Braun-Howland, Terry C. Covert, Gil Dichter, Nancy H. Hall, Robin K. Oshiro.

ensure that localized contamination does not occur through cross-connections, breaks in the distribution lines, or reduction in positive pressure. Sample locations may be public sites (police and fire stations, government office buildings, schools, bus and train stations, airports, community parks), commercial establishments (restaurants, gas stations, office buildings, industrial plants), private residences (single residences, apartment buildings, and townhouse complexes), and special sampling stations built into the distribution network. Preferably avoid outdoor taps, fire hydrants, water treatment units, and backflow prevention devices. Establish sampling program in consultation with state and local health authorities.

b. Raw water supply: In collecting samples directly from a river, stream, lake, reservoir, spring, or well, obtain samples representative of the water that is the source of supply to consumers. It is undesirable to take samples too near the bank or too far from the point of drawoff, or at a depth above or below the point of drawoff.

c. Surface waters: Stream studies may be short-term, high-intensity efforts. Select bacteriological sampling locations to include a baseline location upstream from the study area, industrial and municipal waste outfalls into the main stream study area, tributaries except those with a flow less than 10% of the main stream, intake points for municipal or industrial water facilities, downstream samples based on stream flow time, and downstream recreational areas. Dispersion of wastewaters into the receiving stream may necessitate preliminary cross-section studies to determine completeness of mixing. Where a tributary stream is involved, select the sampling point near the confluence with the main stream. Samples may be collected from a boat or from bridges near critical study points. Choose sampling frequency to be reflective of changing stream or water body conditions.

To monitor stream and lake water quality, establish sampling locations at critical sites. Sampling frequency may be seasonal for recreational waters, daily for water supply intakes, hourly where waste treatment control is erratic and effluents are discharged into shellfish harvesting areas, or even continuous.

d. Bathing beaches: Sampling locations for recreational areas should reflect water quality within the entire recreational zone. Include sites from upstream peripheral areas and locations adjacent to drains or natural contours that would discharge stormwater collections or septic wastes. Collect samples in the swimming area from a uniform depth representative of physical contact through swimming and splashing, approximately 30 cm or 1 ft below the surface. Consider sediment sampling of the water–beach (soil) interface because of exposure of young children at the water's edge.

To obtain baseline data on marine and estuarine bathing water quality, include sampling at low, high, and ebb tides.

Relate sampling frequency directly to the peak bathing period, which generally occurs in the afternoon. Preferably, collect daily samples during the recognized bathing season; at a minimum include Friday, Saturday, Sunday, and holidays.¹ When limiting sampling to days of peak recreational use, preferably collect a sample in the morning and the afternoon. Correlate bacteriological data with turbidity levels and rainfall over the watershed to make rapid assessment of water quality changes. Heterotroph levels can also vary in response to sunlight conditions.²

e. Sediments and biosolids: The bacteriology of bottom sediments is important in water supply reservoirs, in lakes, rivers, and coastal waters used for recreational purposes, and in shellfish-growing waters. Sediments may provide a stable index of the general quality of the overlying water, particularly where there is great variability in its bacteriological quality.

Sampling frequency in reservoirs and lakes may be determined by seasonal changes in water temperatures and stormwater runoff. Bottom sediment changes in river and estuarine waters may be more erratic, being influenced by stormwater runoff, increased flow velocities, and sudden changes in the quality of effluent discharges.

Microbiological examination of biosolids from water and wastewater treatment processes is desirable to determine the impact of their disposal into receiving waters, ocean dumping, land application, or burial in landfill operations.

Collect and handle biosolids with less than 7% total solids using the procedures discussed for other water samples. Biosolids with more than 7% solids and exhibiting a “plastic” consistency or “semisolid” state typical of thickened sludges require a finite shear stress to cause them to flow. This resistance to flow results in heterogeneous distribution of biosolids in tanks and lagoons. Use cross-section sampling of accumulated biosolids to determine distribution of organisms within these impoundments. Establish a length–width grid across the top of the impoundment, and sample at intercepts. A thief sampler (e.g., a Van Dorn or Kemmerer sampler) that samples only the solids layer may be useful. Alternatively, use weighted bottle samplers that can be opened up at a desired depth to collect samples at specific locations. Use gloves when sampling. Rinse exterior of sample bottles onsite and place them in plastic bags.

Processed biosolids having no free liquids are best sampled when they are being transferred. Collect grab samples across the entire width of the conveyor and combine into a composite sample. If solids are stored in piles, classification occurs. Exteriors of uncovered piles are subject to various environmental stresses such as precipitation, wind, fugitive dusts, and fecal contamination from scavengers. Consequently, surface samples may not reflect the microbiological quality of the pile. Therefore, use cross-section sampling of these piles to determine the degree of heterogeneity within the pile. Establish a length–width grid across the top of the pile, and sample intercepts. Sample augers and corers may prove to be ineffective for sampling piles of variable composition. In such cases, use hand shovels to remove overburden.

f. Nonpotable samples (manual sampling): Take samples from a river, stream, lake, reservoir, or pool by holding the bottle near its base in the hand (use gloves) and plunging it, neck downward, below the surface. Turn bottle until neck points slightly upward and mouth is directed toward the current. If there is no current, as in the case of a reservoir, create a current artificially by pushing bottle forward horizontally in a direction away from the hand. When sampling from a boat, obtain samples from upstream side of boat. If it is not possible to collect samples from these situations in this way, attach a weight to base of bottle and lower it into the water. In any case, take care to avoid contact with bank or stream bed; otherwise, water fouling may occur.

g. Sampling apparatus: A special apparatus that permits mechanical removal of bottle stopper below water surface is required to collect water samples from depths of a lake, reservoir,

or deep well without a pump. Various types of deep sampling devices are available. The most common is the ZoBell J-Z sampler.³ Commercial adaptations of this sampler and others are available.

Bottom sediment sampling may require a special apparatus. Petit Ponar^{®†} samplers are effective for a variety of bottom materials for remote (deep water) or hand (shallow water) sampling. Following manufacturer's instructions, drop the closed sterile sampler through the water column and open it when it reaches the sediment bed. Close after the sample is taken, bring sampler to the surface, and drain excess water. Use a sterile spatula or similar device to transfer sample into a sterile container. Clean and decontaminate sampler between sampling sites; a suggested procedure is to brush with dilute soap, rinse with tap water, soak in 0.005% bleach solution for 10 to 20 min, and then, if chlorination is of concern, soak in 0.005% sodium thiosulfate solution for 5 min.

For sampling wastewaters or effluents the techniques described above generally are adequate; in addition see Section 1060.

4. Sample Volume

The volume of sample should be sufficient to carry out all tests required. For potable water samples collect a minimum of 100 ± 2.5 mL. Larger volumes may be needed for bacterial pathogen, protozoan, and viral analyses.

5. Identifying Data

Accompany samples by complete and accurate identifying and descriptive data, such as name of system or site; sample type; collection location; sampling depth, date, and time; sampler's name; analyses to be performed; chlorine residual; and reducing

[†] Registered trademark of Morris & Lee, Inc., d/b/a Wildlife Supply Co., Buffalo, NY.

agents if used (e.g., sodium thiosulfate and EDTA). Record abnormalities or departures from specified sample collection, handling, or receipt procedures. Where possible or required for compliance, sample receipt information should indicate chain-of-custody and shipment handling at temperatures <8°C, but not frozen. Do not accept for examination inadequately identified samples.

6. References

1. LEECASTER, M.K. & S. B. WEISBERG. 2001. Effect of sampling frequency on shoreline microbiology assessments. *Mar. Pollut. Bull.* 42:1150.
2. WHITMAN, R.L., M.B. NEVERS, G.C. KORINEK & N.B. MURULEEDHARA. 2004. Solar and temporal effects on *Escherichia coli* at a Lake Michigan swimming beach. *Appl. Environ. Microbiol.* 70:4276.
3. ZOBELL, C.E. 1941. Apparatus for collecting water samples from different depths for bacteriological analysis. *J. Mar. Res.* 4:173.

7. Bibliography

- PUBLIC HEALTH LABORATORY SERVICE WATER SUB-COMMITTEE. 1953. The effect of sodium thiosulphate on the coliform and *Bacterium coli* counts of non-chlorinated water samples. *J. Hyg.* 51:572.
- SHIPE, E.L. & A. FIELDS. 1956. Chelation as a method for maintaining the coliform index in water samples. *Pub. Health Rep.* 71:974.
- HOATHER, R.C. 1961. The bacteriological examination of water. *J. Inst. Water Eng.* 61:426.
- COLES, H.G. 1964. Ethylenediamine tetra-acetic acid and sodium thiosulphate as protective agents for coliform organisms in water samples stored for one day at atmospheric temperature. *Proc. Soc. Water Treat. Exam.* 13:350.
- DAHLING, D.R. & B.A. WRIGHT. 1984. Processing and transport of environmental virus samples. *Appl. Environ. Microbiol.* 47:1272.
- U.S. ENVIRONMENTAL PROTECTION AGENCY. 1992. Environmental Regulations and Technology Control of Pathogens and Vector Attraction in Sewage Sludge. EPA-625/R-92-013. Washington, D.C.
- NATIONAL RESEARCH COUNCIL. 2004. Health effects assessment. Chapter 2 in *Indicators for Waterborne Pathogens*. National Academies Press, Washington, D.C.

9060 B. Preservation and Storage

1. Holding Time and Temperature

a. General: Start microbiological analysis of water samples as soon as possible after collection to avoid unpredictable changes in the microbial population. Do not analyze samples submitted to the laboratory with chlorine residual or with leakage. In such cases, request resampling.

For most accurate results, ice samples during transport to the laboratory if they cannot be processed within 1 h after collection. Maintain samples in the dark and keep cool with ice or blue ice at <8°C but not frozen. Samples arriving quickly at the laboratory may not have reached this temperature. Verify and record sample temperature upon receipt either through the use of a control water sample bottle or infrared thermometer.

The regulatory holding time varies for different types of samples; observe these limits. For samples collected for non-

regulatory purposes, do not hold for more than 24 h. Follow the guidelines and requirements given below for specific water types. If the results may be used in legal action, use special means (rapid transport, express mail, courier service, etc.) to deliver the samples to the laboratory within the specified time limits and maintain chain of custody. For samples that cannot be analyzed within the required holding time, consider setting up a mobile field laboratory or pre-incubation of sample.

b. Drinking water for compliance purposes: For coliform *E. coli* analyses, the holding time from collection to analysis is 30 h. While there is no regulatory preservation temperature, attempt to keep samples at <8°C during transport to the laboratory. Maintain samples for heterotrophic plate count analysis at <8°C and do not exceed 8 h holding time. Do not freeze. Record sample receipt time and temperature in sample receipt file. Analyze samples on day of receipt whenever possible and re-

frigerate overnight if arrival is too late for processing on same day, as long as holding time conditions can still be met.

For analysis of protozoa (*Cryptosporidium* sp. and *Giardia* sp.) the holding time of 96 h is calculated from the time of sample collection to elution for those samples shipped to the laboratory in bulk, and is calculated from sample filtration to elution for samples filtered in the field. Samples should arrive at <20°C.

c. Nonpotable water for compliance purposes: Hold source water, stream pollution, recreational water, and wastewater samples at <8°C during a maximum transport time of 6 h. Do not freeze. Record sample receipt time and temperature in sample receipt files. Refrigerate these samples upon receipt in the laboratory and process within 2 h. When transport conditions necessitate delays in delivery of samples longer than 6 h, consider using either field laboratory facilities located at the site of collection or delayed incubation procedures.

For bacterial samples in wastewater sludge (fecal coliforms and *Salmonella* sp.) the regulatory holding time is 24 h.

For analyses of protozoa see ¶ *b* above.

d. Other water types for noncompliance purposes: Hold samples at <8°C during transport and until time of analysis for no more than 24 h. Do not freeze. Record sample receipt time and temperature in sample receipt files.

2. Bibliography

CALDWELL, E.L. & L.W. PARR. 1933. Present status of handling water samples—Comparison of bacteriological analyses under varying temperatures and holding conditions, with special reference to the direct method. *Amer. J. Pub. Health* 23:467.

COX, K.E. & F.B. CLAIBORNE. 1949. Effect of age and storage temperature on bacteriological water samples. *J. Amer. Water Works Assoc.* 41:948.

PUBLIC HEALTH LABORATORY SERVICE WATER SUB-COMMITTEE. 1952. The effect of storage on the coliform and *Bacterium coli* counts of water samples. Overnight storage at room and refrigerator temperatures. *J. Hyg.* 50:107.

PUBLIC HEALTH LABORATORY SERVICE WATER SUB-COMMITTEE. 1953. The effect of storage on the coliform and *Bacterium coli* counts of water samples. Storage for six hours at room and refrigerator temperatures. *J. Hyg.* 51:559.

MCCARTHY, J.A. 1957. Storage of water sample for bacteriological examinations. *Amer. J. Pub. Health* 47:971.

LONSANE, B.K., N.M. PARHAD & N.U. RAO. 1967. Effect of storage temperature and time on the coliform in water samples. *Water Res.* 1:309.

LUCKING, H.E. 1967. Death rate of coliform bacteria in stored Montana water samples. *J. Environ. Health* 29:576.

STANDRIDGE, J.H. & D.J. LESAR. 1977. Comparison of four-hour and twenty-four hour refrigerated storage of nonpotable water for fecal coliform analysis. *Appl. Environ. Microbiol.* 39:398.

MCDANIELS, A.E. & R.H. BORDNER. 1983. Effect of holding time and temperature on coliform numbers in drinking water. *J. Amer. Water Works Assoc.* 75:458.

MCDANIELS, A.E., R.H. BORDNER, P.S. GARTSIDE, J.R. HAINES, K.P. BRENNER & C.C. RANKIN. 1985. Holding effects on coliform enumeration in drinking water samples. *Appl. Environ. Microbiol.* 50:755.

POPE, M.L., M. BUSSEN, M.A. FEIGE, L. SHADIX, S. GONDER, C. RODGERS, Y. CHAMBERS, J. PULZ, K. MILLER, K. CONNELL & J. STANDRIDGE. 2003. Assessment of the effects of holding time and temperature on *Escherichia coli* in surface water samples. *Appl. Environ. Microbiol.* 69:6201.