Sterilize by autoclaving for 15 minutes at 121 °C. Prepared peptone water contained in tightly-closed screw-capped bottles may be stored for up to 3 months at <30 °C. Handle aseptically, and discard if turbidity develops.

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9060 SAMPLES

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9060 A. COLLECTION

1. Containers

Collect samples for microbiological examination in clean, sterile, wide-mouth, nonreactive borosilicate glass or plastic bottles, or in presterilized plastic bags appropriate for microbiological use. The bottles must have nonleaking ground glass stoppers or caps with nontoxic liners that withstand repeated sterilization. For sludge analysis, disposable wide-mouth sterile cups may be convenient. If legal action may be involved, consider using tamper-evident closures.

2. Dechlorination

Add a reducing agent to containers intended for the collection of water containing residual chlorine or other halogens, unless they contain broth for direct incubation of sample. Sodium thiosulfate ($Na_2S_2O_3$) is a satisfactory dechlorination agent that neutralizes any residual halogen and prevents bactericidal action from continuing during transit.

When sampling chlorinated wastewater effluents, add enough $Na_2S_2O_3$ to a clean sample bottle so the final concentration in the sample is 100 mg/L. For example in a 120-mL bottle, 0.1 mL of a 10% solution of $Na_2S_2O_3$ neutralizes a sample containing up to 15 mg/L residual chlorine. The dechlorination agent may be less concentrated in drinking water samples: 0.1 mL of a 3% solution of $Na_2S_2O_3$ in a 120-mL bottle neutralizes up to 5 mg/L residual chlorine. See Table 9060:1 for preparation of $Na_2S_2O_3$ solutions. When possible, determine typical residual chlorine before sampling at a new site (e.g., pool water may contain a higher chlorine level than tap water) so the laboratory can prepare an adequate amount of dechlorination agent per sample bottle. Discard 10% $Na_2S_2O_3$ stock solutions that are turbid due to bacterial growth.

Loosely cap the bottle and sterilize by either dry or moist heat, as directed (Section 9040) and perform sterility checks as noted in Section 9020 B.5*d*. Presterilized plastic bags or bottles containing $Na_2S_2O_3$ are available commercially that neutralize up to 15 mg/L residual chlorine. Check and record efficacy of dechlorination agent, one per batch or lot (see Section 9020 B.5*d*). The dechlorinating agent's efficacy can be checked by the use of a sodium hypochlorite (NaOCl) control at 5 or 15 mg/L (or equivalent), for example.

3. Sampling Procedures

Proper sample-collection technique is important to maintain the sample's integrity. Improper sample handling can invalidate the results of any laboratory analysis.

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 number of samples collected depend on ultimate data usage.

Use bottles for sample collection that are large enough to collect the desired sample volume and still maintain adequate headspace (2.5 cm) to ensure proper sample mixing by shaking before analyses. If a sample bottle arrives at the laboratory without adequate headspace for proper mixing, either reject it and request resampling or maintain sample integrity by pouring the entire sample volume into a sterile container large enough to ensure adequate mixing. Then aseptically remove 100 mL or the required sample volume into another suitably sized sterile container.

Keep the sample bottle closed until just before collecting the sample. Remove the cap or stopper as a unit and do not set on any surface. Avoid external contamination during sample collection and do not contaminate the inner surface of stopper or cap and bottle neck. Fill the container without rinsing, replace the stopper or cap immediately, and, if using a hood, secure it

around the neck of the bottle. Take precautions to avoid contaminating the sample (e.g., wear clean disposable gloves when collecting the sample and avoid touching bottle mouth with either hands or the faucet tap).

a. Potable water: Carefully choose sample locations that are conveniently located and readily accessible to collectors so routine sample collection may occur. If testing distribution-system water, for example, avoid taps connected to private water-treatment equipment, such as water softeners or filters. Also, avoid taps that are subject to exterior contamination because they are too close to a sink bottom or the ground. If taking samples from a distribution-system tap without attachments, select a tap that is supplying water from a service pipe directly connected with the main (e.g., not one served from a cistern or storage tank). Remove any attachments (e.g., filters, aerators, flow directors, or screens) from the tap because these may harbor bacteria that do not reflect the source's water quality. Open the cold-water tap fully and let the water run to waste just long enough to clear the service line (~2 or 3 min). Reduce the water flow so the bottle can be filled 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 sodium hypochlorite solution (100 mg/L NaOCl) to the faucet and let the water run at a steady consistent flow for another 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 the hot water for 2 min, then cold water for 2 to 3 min, and then collect the sample as indicated above. Collect a grab sample by collecting all of the sample at once and conducting no further manipulations, such as pouring off or adding to sample, because such activities could contaminate sample. Collect a sufficient volume to perform analyses but do not try to manipulate the collected sample further to adjust the sample volume. It is the laboratory's responsibility, not the sample collector's, to measure sample volume for analyses.

If collecting a sample from a well fitted with a hand pump, pump the water to waste until water temperature has stabilized (~5 to 10 min) before collecting a sample. If an outdoor sampling location must be used, avoid frost-proof hydrants. Also, outdoor taps may need additional disinfection (bleach or equivalent) due to the tap's condition. If there is no pumping machinery, collect a sample directly from the well via a sterilized bottle fitted with a weight at the base. Avoid contaminating samples with 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 ensure that localized contamination does not occur via cross connections, breaks in distribution lines, or a reduction in positive pressure. Sample locations may be

- public sites (e.g., police and fire stations, government office buildings, schools, bus and train stations, airports, and community parks);
- commercial establishments (e.g., restaurants, gas stations, office buildings, and industrial plants);
- private residences (e.g., 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 a sampling program in consultation with state and local health authorities.

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

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, or
- downstream recreational areas.

Wastewater dispersion into the receiving stream may necessitate preliminary cross-section studies to determine the completeness of mixing. For tributaries, select a sampling point near the confluence with the main stream. Samples may be collected from a boat or from bridges near critical study points. Adjust the sampling frequency to reflect changing stream or waterbody 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 ideally reflect water quality within the entire recreational zone. Include sites from upstream peripheral areas and locations next to drains or natural contours that may discharge accrued stormwater 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). For specific sampling procedures, follow the guidance listed in Section 9213 B.2*b*. Consider sediment sampling of the water-beach interface to account for young children's exposure at the water's edge.

To obtain baseline data on marine and estuarine bathing-water quality, include sampling at low, high, and ebb tides and at different times of the day (AM and PM) for all waters.

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

Table 9060:1. Sodium Thiosulfate Equivalents

Solution Strength and $Na_2S_2O_3$ 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

sample in both morning and 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 overlying water's general quality, particularly if its bacteriological quality varies greatly.

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 because they are influenced by storm-water runoff, increased flow velocities, and sudden changes in effluent-discharge quality.

Microbiological examination of biosolids from water and wastewater treatment processes is desirable to determine their effects on receiving waters, land application, or landfilling.

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 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 the distribution of organisms in these impoundments. Establish a length-width grid across the top of the impoundment, and sample at intercepts. A thief sampler (e.g., a VanDorn 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 the exterior of sample bottles onsite and place them in plastic bags.

Processed biosolids without 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. Exteriors of uncovered piles are subject to various environmental stresses (e.g., precipitation, wind, fugitive dusts, and fecal contamination from scavengers), so surface samples may not reflect the microbiological quality of the entire pile. Therefore, use cross-section sampling of these piles to determine the degree of heterogeneity in the pile. Establish a length-width grid across the top of the pile, and sample intercepts. If sample augers and corers are ineffective in sampling piles of variable composition, 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 (with gloves) near its base and plunging it, neck downward, below the surface. Turn the bottle until the neck points slightly upward and the mouth is directed toward the current. If there is no current (e.g., in a reservoir), create one artificially by pushing the bottle forward horizontally away from the hand. When sampling from a boat, obtain samples from the upstream side of boat. If it is impossible to collect samples by hand, attach a weight to the base of the bottle and lower it into the water. In any case, avoid contact with the bank or stream bed, which might cause water fouling to occur.

g. Sampling apparatus: When collecting water samples from the depths of a lake, reservoir, or deep well without a pump, use a special apparatus that enables users to mechanically remove the bottle stopper below the water surface. 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 the 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 the sampler to the surface, and drain the excess water. Use a sterile spatula or similar device to transfer the sample into a sterile container. Clean and decontaminate the sampler between sampling sites. A suggested cleaning 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 or rinse with tap water (and check for chlorine residual).

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

4. Sample Volume

Collect sample volumes sufficient to carry out all tests required. For potable, surface, recreational, and waste water samples, collect a minimum of 100 mL. Larger volumes are needed for some bacterial pathogen, protozoan, and viral analyses.

Note: For tolerances of glassware and vessels, refer to Section 9020 B.5*a* for Class A tolerance of graduated cylinders (<2.5%) and Section 9020 B.5*d* for verification of 100 mL mark.

5. Identifying Data

Samples must be accompanied by complete, accurate sample-information forms that include the following information, as applicable:

- 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 agent (e.g., sodium thiosulfate) and EDTA (a chelating agent if sample contains metals), if used.

Record abnormalities or departures from specified sample-collection, -handling, or -receipt procedures. If sample results are likely to be used in legal proceedings, follow chain-of-custody procedures including a chain-of-custody form with appropriate signatures, dates, and times. If samples are submitted to the laboratory either refrigerated or stored in a foam chest with gel packs, document the handling and storage temperatures [cold but unfrozen (<10 °C)]. Refer to 9060 B for preservation and storage. Also, make sure that sample containers are labeled with enough information to adequately identify samples. Do not accept inadequately identified samples.

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(9060) B. Preservation and Storage

1. Holding Time and Temperature

a. General: Start the microbiological analysis of water samples as soon as possible after collection to avoid unpredictable changes in the microbial population. Do not analyze compromised samples (e.g., leakage, chlorine residual) submitted to the laboratory; instead, request resampling.

For most accurate results, ice the samples during transport to the laboratory if they cannot be processed within 1 h after collection. Maintain samples in the dark and keep cool but unfrozen (<10 °C) with ice or blue ice. Do not place samples in direct contact with frozen ice packs. Insulate the sample with bubble wrap, crumpled paper, or equivalent. Samples arriving quickly at the laboratory (within 1 h of collection) may not have reached this temperature. Upon receipt, verify and record the sample temperature via a control water-sample bottle, infrared thermometer, or other equivalent devices (e.g., i-buttons). Observe regulatory holding-time limits, which vary for different types of samples and in different countries. If the results may be used in legal action, use special means (rapid transport, express mail, courier service) to deliver the samples to the laboratory within the specified time limits and maintain the chain of custody. If the samples cannot be analyzed at a laboratory within the required holding time, consider setting up a mobile field laboratory or pre-incubating the sample. In paragraph *b* below are examples of US EPA guidelines and requirements.

b. Drinking water for compliance purposes: For total coliform and *E. coli* analyses, the holding time from collection to analysis is 30 h. Although regulations do not specify a preservation temperature, try to keep samples cold but unfrozen (<10 °C) during transport to the laboratory. Similarly, keep samples for heterotrophic plate count analysis cold but unfrozen (<10 °C) and do not exceed an 8-h holding time (collection to analyses). Do not accept any water sample for microbiological analyses that shows evidence of freezing. Record the sample-receipt time (and temperature if submitted on ice) in a sample-receipt file. Analyze the samples on the day of receipt whenever possible. If samples arrive too late for processing on the same day, refrigerate them overnight if holding time limits can still be met.

For the analysis of *Cryptosporidium* sp. and *Giardia* sp., a 96-h holding time applies to the period between sample collection and elution for samples shipped to the laboratory in bulk and to the period between sample filtration and elution for samples filtered in the field. Ensure that samples are <20 °C during transport.

c. Nonpotable water for compliance purposes: Keep source water, stream pollution, recreational water, and wastewater samples cold but unfrozen (<10 °C) during transport (<8 h between collection and lab analysis). When samples arrive at the laboratory, refrigerate them, record receipt time and temperature in sample-receipt files, and process samples within 2 h. Whenever transport conditions prevent samples from being delivered within 6 h, consider using either field laboratory facilities at the collection site or delayed incubation procedures.

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

For analyses of protozoa, see paragraph *b* above.

d. Other water types for noncompliance purposes: Keep samples cold but unfrozen (<10 °C) between collection and analysis (≤24 h). Record sample-receipt time and temperature in sample-receipt files.

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