BACTERIAL SAMPLING*

Sampling Containers

Collect samples for microbiological examination in bottles that have been cleansed and rinsed carefully, given a final rinse with distilled water, and sterilized. (Please refer to Standard Methods for the Examination of Water and Wastewater, 18th edition) for wash and sterilization protocols. Alternatively, purchase pre-sterilized containers and integrity check each sealed vessel prior to use.

Sample 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 plating of sample. Sodium thiosulfate ($Na_2S_2O_3$) is a satisfactory dechlorinating agent that neutralizes any residual halogen and prevents continuation of bactericidal action during sample transit. Alternately, purchase pre-sterilized containers appropriate for the volume of sample desired which contain Sodium thiosulfate in pellet form. The examination then will indicate more accurately the true microbial content of the water at the time of sampling.

For drinking water samples, the concentration of dechlorinating agent: 0.1 mL of a 3% solution of Sodium thiosulfate in a 120-mL bottle will give a final concentration of 18 mg/L in the sample and will neutralize up to 5 mg/L residual chlorine. In emergency disinfection with higher concentrations of chlorine add sufficient dechlorination agent to give a concentration of 100 mg/L in the sample.

Sample Collection - General

1. When the sample is collected, leave ample air space in the bottle (at least 2.5 cm) to facilitate mixing by shaking, before examination.
2. Collect samples that are representative of the water being tested, flush or disinfect sample ports, and use aseptic techniques to avoid sample contamination.
3. Keep sampling bottle closed until it is to be filled.
4. Remove stopper and cap as a unit. DO NOT contaminate inner surface of stopper or cap and neck of bottle.
5. Fill container without rinsing.
6. Replace stopper or cap immediately, and if used, secure hood around neck of bottle. Alternately, secure “tamper proof” devices applicable to sample container.

Sample Collection - 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. Open tap fully and let water run to waste for 2-3 minutes, 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, apply solution of sodium hypochlorite (100 mg NaOCl/L) to faucet before sampling; let water run for additional 2 to 3 minutes after treatment. Do not sample from leaking taps that allow water to flow over the outside of the tap. In sampling from a mixing faucet remove faucet attachments such as screen or splash guard, run hot water for 2 minutes, then cold water for 2 to 3 minutes, 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 minutes before collecting sample. If the well is equipped with a mechanical pump, collect sample from a tap on the discharge. 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.

In drinking water evaluation, collect samples of finished water and from distribution sites selected to assure 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 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), and buildings, and townhouse complexes, and special sampling stations built into the distribution network. Establish sampling program in consultation with state and local health authorities.

Sample Collection - Raw Water Supply

In collecting samples directly from a river, stream, lake, reservoir, spring, or shallow 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.

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Sample Collection - 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 stream or water body conditions. For example, to evaluate waste discharges, sample every 4 to 6 hours and advance the time over a 7 to 10 day period.

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.

Sampling Collection - 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 of approximately 1 meter. 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.

Sample Collection - Sediments and Sludges

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 related more to seasonal changes in water temperatures and storm water 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.

Bacteriological examination of sludges from water and wastewater treatment processes is desirable to determine the impact of their disposal into receiving waters, ocean dumping, or burial in landfill operations. Sludge monitoring also may indicate the effectiveness of wastewater treatment processes.

Sample Collection - Manual Sampling

Take samples from a river, stream, lake, or reservoir by holding the bottle near its base in the hand 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, created a current artificially by pushing bottle forward horizontally in the 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.

Size of Sample

The volume of sample should be sufficient to carry out all tests required, preferably not less than 100 mL.

Sample Identification

Accompany samples by complete and accurate identifying and descriptive data. Do not accept for examination inadequately identified samples.

Sample Preservation and Storage and Shipment

1. Samples must be stored and shipped cold via same-day or overnight delivery.
2. HOLDING TIME: All bacterial samples must be placed on-test within 24 hours of sampling

*Adapted from Standard Methods for the Examination of Water and Wastewater, 18th Edition.

Additional Information

For more information concerning Cryptosporidium spiking studies, sampling, detection, immunofluorescent assay and current regulation, please call BioVir Laboratories at 1-800-GIARDIA (442-7342).

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