Bacteria Test Guidelines

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All tests for bacteria use a nutritional broth or agar and incubation at a specific temperature to grow the target organism. Sterile equipment and careful handling techniques are necessary to prevent contamination of the sample.

Methods for bacteria testing

The following descriptions give a general overview of the different methods for bacteria tests.

- **Presence/absence (P/A)**—the sample is added to a bottle containing broth and incubated. A color change indicates the presence of the target bacteria.
- **Most Probable Number (MPN)**—the sample is diluted and added to a series of tubes containing broth. The tubes are incubated and then examined for the presence of gas.
- **Membrane Filtration (MF)**—the sample is filtered and the filter is placed on a pad containing growth media. After incubation, the filter is examined for the growth of the target organism.
- **Plate count agar**—the sample is mixed with an agar in a large petri dish and incubated. After incubation, the agar is examined for bacteria colonies. This test is usually used for total or heterotrophic bacteria.

Presumptive and confirmation tests

Two tests are necessary for most methods, a presumptive test and a confirmation test.

- **Presumptive test**—uses growth media that facilitates the growth of the target organism. A positive result is an indication of the target organism but can include a false positive result. The P/A, MPN and MF methods are presumptive tests.
- **Confirmation test**—uses media that is more selective for the target organism and sometimes uses a higher incubation temperature. Some media, such as the m-ColiBlue24® broth used with the MF method, is selective enough for the target organism (E. coli) that no confirmation test is required.

Techniques for microbiological tests

Good laboratory technique is essential for microbiological tests. To make sure the results are reliable, collect and preserve samples carefully. Use high-quality laboratory equipment and ready-to-use media to save time and minimize errors.

Prepare the work area

- Start the incubator while preparing other materials. Set the incubator to the temperature required by the specific procedure (usually 35 ± 0.5 °C for total coliforms and enterococci and 44.5 ± 0.2 °C for fecal coliforms).
- Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray or dilute iodine solution. Wash hands thoroughly with soap and water.
- Mark each sample container with the sample number, dilution, date and other necessary information. Avoid contaminating the inside of the sample container in any way.
- Use presterilized Whirl-Pak® bags or bottles for sample collection. If the sample has been disinfected, use bags or bottles that contain a dechlorinating agent.

Prepare sample containers

To collect samples, use any of the following: sterilized plastic bags, sterilized disposable bottles, autoclavable glass or plastic bottles.

Sterilized plastic bags or disposable bottles
Presterilized plastic bags and bottles are available with or without dechlorinating agent. The bottles are available with a 100-mL fill-to line.

Dechlorinating reagent should be used with potable or chlorinated water samples. It is not necessary for unchlorinated or non-potable water samples. However, dechlorinating reagent will not interfere with unchlorinated samples so, for simplicity, plastic bags containing dechlorinating reagent may be used for all samples.

Autoclavable glass or plastic bottles:
Glass or plastic bottles (125-mL size) may be used instead of sterilized plastic bags or disposable bottles. These containers should be prepared as follows:

1. Wash in hot water and detergent.
2. Thoroughly rinse with hot tap water, followed by a distilled water rinse to make sure that all detergent is removed.
3. If dechlorinating agent is needed (for chlorinated, potable water), add the contents of one Dechlorinating Reagent Powder Pillow for each 125-mL of container volume. (A 250-mL sample container will require two powder pillows.)
4. Steam sterilize glass and autoclavable plastic containers at 121 °C (250 °F) for 15 minutes. Glass sample containers may be sterilized by hot air at 170 °C (338° F) for one hour.
5. Store sterile containers, tightly capped, in a clean environment until needed.

Autoclave option for sterilization

Use presterilized laboratory equipment and media to save time and minimize errors. When numerous samples must be run on a routine basis, sterilization of non-disposable materials with an autoclave may be preferable.

Procedure

1. Wash sample bottles, pipets, petri dishes, filter holder with stopper and graduated cylinder (if needed) with hot water and detergent.
2. Rinse several times with tap water and then with demineralized water. Dry thoroughly.
3. Prepare all equipment for the autoclave as follows:
   - Loosely thread caps on sample bottles and cover caps and bottle necks with foil or paper.
   - Cover the openings of graduated cylinders with foil or paper.
   - Insert the filter funnel base into an autoclavable rubber stopper that will fit the filter flask.
   - Wrap the two parts of the filter funnel assembly separately in heavy wrapping paper and seal with masking tape.
   - Wrap petri dishes (borosilicate glass) in paper or place in aluminum or stainless cans.
4. Sterilize equipment in an autoclave at 121 °C (250 °F) for 15 minutes. Borosilicate glass items may be sterilized with dry heat at 170 °C (338 °F) for a minimum of 1 hour.

Collect and preserve samples

Collect a sufficient volume of sample for analysis (usually a minimum of 100 mL of sample). World Health Organization guidelines prescribe 200 mL per sample while Standard Methods for the Examination of Water and Wastewater prescribes 100 mL per sample. Avoid sample contamination.

No dechlorination is necessary if the sample is added directly to the growth medium on site. Otherwise, treat the samples to destroy chlorine residual. Sodium thiosulfate that has been sterilized within the collection vessel is used to remove chlorine residual. Transport for analysis immediately after collection.

Analyze as soon as possible after collection. Allow no more than 6 hours to elapse between collection and examination for non-potable water samples and 30 hours for potable water samples. For best results, maintain the sample at or below 10 °C, but do not freeze. Failure to properly collect and transport samples will cause inaccurate results.

Collect at least 100 mL of sample in a presterilized plastic bag or bottle or in a sterile glass or plastic bottle. Do not fill sample containers completely. Maintain at least 2.5 cm (approximately one inch) of air space to allow adequate space for mixing the sample prior to analysis.

Faucets, spigots, hydrants or pumps
Collect representative samples by allowing the water to run from a faucet, hydrant or pump at a moderate rate for 2 to 3 minutes before sampling. Adjust the flow rate before sample collection to avoid splashing during collection. Do not adjust the rate of flow while collecting the sample. Avoid valves, spigots and faucets that swivel or leak or those with attachments such as aerators and screens or remove the attachments prior to sample collection.

Handle the sample containers carefully. Open the sample containers just prior to collection and close immediately following collection. Do not lay the lid or cap down. Do not touch the lip or inside surface of the container. Do not rinse the containers before use. Label the sample containers immediately and analyze promptly.

Rivers, lakes and reservoirs
When sampling a river, lake or reservoir, do not sample near the edge or bank. Remove the cap, grasp the sample container near the bottom and plunge the container, mouth down, into the water in order to exclude any surface scum. Fill the container entirely under water by positioning the mouth into the current or, in non-flowing water, by tilting the container slightly and allowing it to fill slowly. Do not rinse the container before use. Label the sample containers immediately and analyze promptly.

Dilute non-potable samples

Non-potable water samples must be diluted to a level at which the bacteria can be measured.

Procedure

1. Wash hands.
2. Open a bottle of sterile Buffered Dilution Water.
3. Invert the sample container in a Belt to Ear motion, approximately 25 times for 30 seconds.
4. Use a sterile pipet to add 11 mL of sample into the dilution water bottle.
5. Put the cap on the dilution water bottle and invert the sample container in a Belt to Ear motion 25 times for 30 seconds. This is a 10-fold or 10x dilution (sample is diluted by a factor of 10).
6. Add 11 mL of the 10x dilution to another dilution bottle and mix well (100x dilution).
7. Add 11 mL of the 100x dilution to a third bottle and mix well (1000x dilution).
8. Continue to make dilutions until the necessary dilution level has been reached.

Dispose of bacteria cultures

To safely dispose of bacterial cultures left in the broth tubes, use one of the following methods:

Bleach
Sterilize used test containers with household bleach. Add 1–2 mL of the bleach to each test tube. Allow 10 to 15 minutes contact time with the bleach. Pour the liquid down a drain.

Autoclave
Place used test tubes in a contaminated-items bag or a biohazard bag to prevent leakage into the autoclave. Autoclave the used test tubes in the unsealed bag at 121 °C for 30 minutes at 15 pounds pressure. When cool, seal the bag, place it in another garbage bag and tie tightly.

The use of indicator organisms in water tests

Many serious diseases, such as typhoid fever and dysentery, can be traced directly to pathogenic microorganisms in polluted water. These disease-producing organisms are discharged in fecal wastes and are difficult to detect in water supplies. People may come in contact with these pathogens in drinking water or in recreational waters such as swimming pools, rivers, streams and bathing beaches.

Testing directly for bacterial pathogens is impractical for many reasons, not the least of which is the need for lengthy and involved test procedures. It has become customary to use indicator organisms such as coliform bacteria instead. Indicator organisms are usually not pathogenic and are present when pathogens are present and absent when pathogens are absent. Indicator organisms are usually of fecal origin as well.

No one organism or group of organisms satisfies all of the criteria for an indicator. For example, in temperate climates total coliform bacteria are commonly used as indicator organisms in potable water supplies. In many tropical climates, however, indigenous Escherichia coli (E. coli) bacteria are present in pristine water sources where no fecal contamination exists, yet they will produce positive results in total coliform tests.

In such cases, other bacteria, known to be associated with fecal contamination, can be used as indicator organisms in place of the coliforms. Hydrogen sulfide-producing bacteria have been shown to be associated with the presence of fecal contamination and total coliform bacteria and they may be used as an indicator organism in place of coliforms.

Total coliform tests are used for potable water supplies. Fecal coliform tests are used on untreated (non-potable) water, wastewater, bathing water and swimming water.

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