



40 minutes total

Overview of Activities

1. Introduction
2. Microbiological Testing Methods
3. Evaluation of Microbiological Testing Methods
4. Review

Learning Outcomes

At the end of this session participants will be able to:

1. Identify microbiological testing methods
2. Evaluate different microbiological testing methods
3. Determine when to use Presence Absence (P-A), Most Probable Number (MPN) and Membrane Filtration (MF) tests

Materials & Preparation

- Activity: Microbiological testing method demonstration
- Prepare: Sample of P-A test (e.g., Hach Pathoscreen or Enpho)
- Prepare: Sample of MPN test (e.g., Hach Pathoscreen MPN field kit or Aquagenx Compartment Bag test)
- Prepare: Sample of Membrane Filtration test: (e.g., Palintest or Delagua kit)
- PowerPoint (optional): Microbiological Testing Methods (use if samples of each testing method are not available)
- Activity: Microbiological testing method evaluation debate
- Flip chart

Recommended Reading

- Technical Brief: Microbiological Aspects in Drinking Water

5 minutes

Introduction



1. Present the following scenarios:
 - You are a project implementer and have a new borehole being drilled in a remote village. You want to see if the borehole has been properly disinfected after commissioning but you can't get out to the site easily and you have no trained lab staff available.
 - You have a trained water quality technician and laboratory and need to verify the effectiveness of a water treatment technology (e.g., biosand filter).
 - You do not have laboratory equipment but you need to know approximate numbers of E. coli contaminating a water source.
2. Ask if the same test could be used in each scenario. Ask participants to suggest reasons why they might not be able to/or want to use the same tests.
 - There are different levels of investigation or examination depending on several factors (including):
 - i. Objectives
 - ii. Resources
 - iii. Time
 - iv. Degree of accuracy, etc.
3. Explain that during this session, we are going to identify and evaluate particular microbiological testing methods and decide when to use each method.
4. Present the learning outcomes or an overview of the lesson.

15 minutes

Microbiological Testing Methods



1. Remind participants that there are different ways to test and we will be looking at the three main methods of microbiological testing.
2. Introduce the three main methods of microbiological testing:
 - Presence-Absence (P-A): a simple test that indicates contamination based on a colour change
 - Most Probable Number (MPN): uses the same principle as P-A but estimates the number of indicator bacteria likely to be present in the water
 - Membrane Filtration (MF): the most accurate method to determine the number of bacteria in a water sample and is the internationally recognized standard
3. Demonstrate examples of each method by passing around sample tests or kits and explaining how to use each test. *(Optional) Use PowerPoint: Microbiological Testing Methods for this presentation if samples of each testing method are not available.*

Presence Absence (P-A)

- Simplest method
- Add water sample to a bottle containing culture media and let it sit for 24-48 hours
- Colour will change if indicator organism is present
- Does not show numbers of bacteria
- If the sample is positive, the water should be re-tested using membrane filtration to determine the number of bacteria

Most Probable Number (MPN)

- Tells the number of bacteria that are most likely to be in the water sample
- Add water sample to 5-10 test tubes (or trays of 10-96 wells or compartment bag) containing culture media
- Incubate for 24-48 hours
- It is a statistical method based on the number of positive tubes (or wells)
- Using a table provided, the number of positive tubes is reported as the number of colonies per 100 mL of sample (MPN/100 mL) where MPN = most probable number

Membrane Filtration — MF

- Most accurate method to count bacteria (internationally recognized method)
- Filter 100 mL of a water sample using filtration equipment
- Add culture media to a Petri dish to provide nutrients for indicator organisms to grow
- Transfer the filter paper to the Petri dish
- Incubate for 24-48 hours depending on the culture media
- If the indicator organism is present, colonies will appear on the filter paper and can be counted
- Results are reported as the number of colonies per 100 mL of water sample (CFU/100 mL) where CFU = colony forming units

4. Explain that they will be practising the membrane filtration technique on their water samples later in the workshop.

15 minutes

Evaluation of Microbiological Testing Methods

1. Explain that participants will be split into groups and will be discussing the advantages and disadvantages of the three testing methods.
2. Explain that each group should discuss their topic and prepare to present their findings before voting on the testing method they think is the most appropriate.

3. Divide participants into six groups by having them count off one to six and organize them into their number groups (all ones together, twos together etc.).
4. Assign each group a topic to discuss.
 - Group 1 discuss P-A advantages
 - Group 2 discuss P-A limitations
 - Group 3 discuss MPN advantages
 - Group 4 discuss MPN limitations
 - Group 5 discuss MF advantages
 - Group 6 discuss MF limitations
5. Allow five minutes for groups to deliberate and record their ideas.
6. Invite corresponding groups to present their ideas comparing advantages and limitations of each testing method.
7. Record ideas on a flip chart.
8. Add any missed points (see Key Points below for reference).
9. Invite participants to record important points in their workbooks.

Key Points

- P-A Advantages: 1. Simple to understand and use; 2. Rapid results (within 24 hours); 3. Doesn't require extensive equipment; 4. Portable and durable in field; 5. Relatively inexpensive
- P-A Disadvantages: 1. Does not show how many bacteria are in the sample; 2. Not recommended by WHO for analysis of surface water and untreated community water supplies; 3. Not recommended for testing the efficiency of household water treatment technologies (e.g., biosand filter)
- MPN Advantages: 1. Semi-quantitative results; 2. Simple to understand and use; 3. Relatively inexpensive; 4. Can be used with turbid water
- MPN Disadvantages: 1. More labour intensive than P-A; 2. Requires some training; 3. Requires incubator and other equipment; 4. Disposable trays generate a lot of waste
- MF Advantages: 1. Able to count the number of bacteria; 2. Most accurate testing method; 3. Internationally recognized method; 4. Ability to test many samples at once
- MF Disadvantages: 1. More labour intensive than MPN, P-A; 2. Requires more training; 3. Requires additional equipment; 4. Cost of consumables can be high in many countries; 5. More difficult to use with turbid water (dilution required)

5 minutes

Review Quiz



1. Explain that we are going to return to the scenarios from the introduction activity and identify the appropriate test for each.
2. Ask: "What testing method would you use if presented with the following scenarios?"
3. Read each scenario aloud and allow a few minutes for participants to decide and share.

- **Scenario 1:** “You are a project implementer and have a new borehole being drilled in a remote village. You want to see if the borehole has been properly disinfected after commissioning but you can’t get out to the site easily and you have no trained lab staff available. What microbiological testing method could you have the driller perform while in the field?”
 - i. Presence-absence
- **Scenario 2:** “You have a trained water quality technician and laboratory and need to verify the effectiveness of a water treatment technology (e.g., biosand filter). What microbiological testing method would you use?”
 - i. Membrane filtration
- **Scenario 3:** “You do not have laboratory equipment but you need to know approximate numbers of E. coli contaminating a water source. What microbiological testing method would you use?”
 - i. Most probable number

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