



Overview of Activities

1. Introduction
2. Presence-Absence (P-A) and Most Probable Number (MPN) Demonstration
3. Membrane Filtration (MF) Demonstration and Practice
4. Review

Learning Outcomes

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

1. Describe P-A and MPN testing procedures
2. Demonstrate MF procedures

Materials & Preparation

- Activity: Introduction (group sample)
- Basin (clean and sanitized)
- Bottled water (1-2 litre size)
- Whirl-Pak® bag
- Handout: Data Recording Form, see Appendix 3, (one page, print one per participant)
- Activity: P-A, MPN, and MF Demonstration and Practice
- Prepare: Print MF, P-A and MPN Test Procedures, see Appendix 5
- Prepare: Set up a P-A and MPN testing station including: Hach Pathoscreen MPN field kit and Aquagenx Compartment Bag Test
- Prepare: Set up MF testing stations, see end of lesson for sample layout
- Palintest (Wagtech) and/or Delagua MF apparatus (2-4)
- Nalgene disposable MF apparatus (1-2)
- Hand sanitizer (one per table)
- Disinfectant e.g., Dettol or alcohol (500 mL)
- Lighters (one per table)

Materials & Preparation (cont.)

- Methanol (at least 500 mL)
- Small wide-neck bottle or dropper bottle for methanol
- Ethanol or isopropyl alcohol (at least 500 mL)
- Alcohol swabs or cotton balls to soak in alcohol (one box or enough for two per participant)
- Sterile syringes 3 mL capacity, without needles (two per bottle of media)
- Petri dishes (aluminum or disposable plastic), one or two per participant
- Prepare: mark each petri dish with a unique number to prevent duplicates
- Pads for Petri dishes, one or two per participant
- Pad dispensers (one per table)
- Culture media (Coliscan, Hach m-ColiBlue24, or MLSB) 2 mL per dish
- (Optional), if using MLSB prepare media as per manufacturer's instructions
- Filter paper 47 mm, 0.45µm pore size (one box)
- Wash bottles (one per table)
- Sterile (boiled) water for rinsing, enough to fill wash bottles, approx. 2 litres
- (Optional), Bottled water for blank samples
- Forceps (one set per station)
- Paper towels (one roll per table)
- Wet and dry waste disposal buckets with lids (one set per table)
- Participant water samples from sampling exercise
- Incubator set to appropriate temperature for media (MLSB = 44°C, Coliscan and mColiBlue24 = 35°C)
- Ball for Learning Ball Game

Recommended Reading

- Technical Brief: Microbiological Aspects in Drinking Water

10 minutes

Introduction



1. Explain: “We are going to take a water sample for testing.”
2. Ask: “How soon after collecting a water sample should it be tested and then discarded?”
 - Tested: within six hours; discarded after 24 hours
3. Pour clean water (use bottled water) over each participant’s hands into a clean basin.
4. Pour the rinse water into a sterile sample container or Whirl-Pak® bag.
5. Ask: “How can we test this sample for microbiological contamination?”
 - P-A, MPN or MF
6. Present the learning outcomes or an overview of the lesson.

15 minutes

Presence-Absence and Most Probable Number Demonstration



1. Explain: “We will test the hand rinse sample using P-A and/or MPN test methods.”
2. Indicate the testing stations set up for the practical session and invite the participants to gather around the station with equipment for conducting P-A and MPN tests.
3. Ask for two volunteers to come forward to help demonstrate P-A and MPN testing.
4. Explain the general test procedures before the volunteers conduct the tests.
 - Sanitize hands before beginning
 - Label the test container (tube, or bag) with a reference number before adding the water sample
 - Record the number and sample information on a data recording form
 - Shake the sample for 10 seconds before testing to disperse the bacteria
 - Read the instructions fully before beginning the procedure
5. Ask the volunteers to follow the printed procedures posted at the station to conduct the tests in front of the group. Use the hand rinse water sample for testing.
6. (Optional) Have another volunteer read the instructions aloud.
7. Encourage comments and questions.

Key Points

- Shake a water sample before testing because bacteria tend to clump together, stick to the sides of the container and settle to the bottom. Shaking provides better distribution.
- Results will be ready in 24-48 hours for P-A and MPN tests.
- Always read procedures as different manufacturers have unique instructions specific to their product.
- Positive result colour change will vary by manufacturer and indicator organism being tested.
- Pathoscreen tests for H₂S producing bacteria which are not as specific to fecal contamination as coliforms, false positives can occur in waters high in sulfur or from other bacteria of non-fecal sources.

Membrane Filtration (MF) Demonstration and Practice



1. Explain: "This lesson is focused on giving you hands-on experience with some microbiological testing methods."
2. Invite participants to gather around a MF station for a demonstration of the technique they will be using on their water samples.
3. Demonstrate the procedure for membrane filtration using the Palintest (Wagtech) or Delagua apparatus.
4. Plate a blank sample using sterile (boiled) water. If boiled water is not available use unopened bottled water.
5. Divide participants into small groups of two or three people depending on the number of stations and participants.
6. Hand out participant-collected water samples.
7. Hand out one data recording form per participant.
8. Instruct: "Record your sample data on your data recording forms. Please keep the forms because all test data will be recorded, including microbiological, chemical and acceptability aspects. You will need these forms in future sessions."
9. Remind participants to shake the sample for 10 seconds before filtering.
10. Have participants test their water samples using the equipment at their station following the procedures posted at the station.
11. Ask one person to plate the hand washing sample if time allows.
12. (Optional) Demonstrate the Nalgene apparatus to the small group at that station.
13. Allow small groups to practice membrane filtration so that each participant does at least one test.
14. Rotate between stations as time permits.
15. Collect plated samples and allow to resuscitate for 1-4 hours at room temperature before placing them in the incubator.

Trainer Tips

Supervise small groups at the testing stations.

MF involves many details that can be difficult to remember the first time.

Trainer Tips

The newer Palintest kit incubator has a function that allows for a resuscitation period.

Key Points

- Blank samples are part of quality control to verify that culture media is sterile.
- If bacterial colonies grow on the blank plate then secondary contamination has occurred.
- Test one blank for every 10-20 test samples.
- Test from the least to the most contaminated sample.
- The resuscitation period allows bacteria to recover from the stress of the filtration process and ensure that they survive and grow.

Review

1. Invite participants to stand in a circle.
2. Throw a ball to a participant.
3. Ask: “What is one step in the presence-absence testing procedure?”
4. Have the participant describe one step and throw the ball to another person to describe the next step.
5. Continue until all the steps have been described.
6. Remind people to keep their data recording forms for the testing sessions the following day.

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Microbiological Testing Stations

Handout

Example Testing Station Layout

