

## WATER PURIFICATION

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### Objectives/Rationale

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Although waterborne diseases are rare in the United States, water is still a universal source of infectious disease. The student will recognize water as a potential source for the transmission of disease, identify diseases caused by contaminated water supplies, and analyze water for possible contaminants.

TEKS 121.14 5C, 3B

TAKS ELA 1, 4  
Mathematics 8  
Science 1

National Science Education Standards A9-12;C9-12; F9-12

National Health Care Skills Standards .01, .09

National Curriculum Standards for School Mathematics S1; S3

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### Key Points

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- I. Water suitable for human consumption, or “potable” water, is determined by the presence of acceptable levels of specific “indicator” microorganisms normally found in human and animal gastrointestinal tracts.
- II. The coliforms are the indicator organisms used for determining the presence of fecal contamination.
  - a. Gram negative
  - b. Rod-shaped bacteria that are nonspore forming
  - c. Facultative aerobes that ferment lactose with gas formation within 48 hrs. at 35C.
- III. Contaminated water may cause serious infections.
  - a. Cholera
  - b. Giardiasis
  - c. Cryptosporidiosis
  - d. Salmonellosis
  - e. Shigellosis
  - f. Leptospirosis
- IV. Water community preparation stages:
  - a. Collection
  - b. Treatment
    1. Sedimentation
    2. Filtration
    3. Chlorination
  - c. Distribution
- VI. Lab Procedure
  - Preparation
    1. Wash hands and put on gloves and goggles.

2. Assemble equipment and materials.
  3. Prepare work area.
- Presumptive test
    - A. Inoculate 5 double-strength lactose broth tubes with 10 ml. of water sample
    - B. Inoculate 5 single-strength lactose broth tubes with 1 ml. of water sample
    - C. Inoculate 5 single-strength lactose broth tubes with 0.1 ml. of water sample
    - D. Incubate at 35 C for 24 - 48 hrs.
  - Confirmed test
    - A. Streak one EMB plate for each gas-positive tube.
    - B. Incubate at 35 C for 24 - 48 hrs.
    - C. Examine plates for lactose-fermenting colonies.
  - Completed test
    - A. Inoculate one lactose broth tube and one nutrient agar slant for each plate with one lactose-fermenting colony
    - B. Incubate at 35 C for 24 - 48 hrs.
    - C. Examine for the presence of fermentation, i.e. Gas-positive
    - D. Gram-stain growth in nutrient agar slant, report findings.
    - E. Clean work area with surface disinfectant. Remove goggles and gloves and wash hands.

*\*Teacher note: Invite environmental engineer as an industry partner to lead lab inventory.*

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### Activities

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- I. Complete the **Water Analysis Laboratory Investigation**.
- II. Visit the local water treatment plant and observe the different processes involved in making water safe for consumption (potable). Explain the steps taken to purify water.

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### Material/Resources

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100 ml. Water samples  
5 tubes of double-strength lactose broth  
10 tubes of single-strength lactose broth  
Nutrient agar slant  
Incubator  
10 EMB plates  
Inoculating loop  
Pipette and bulb  
Bunsen burner  
Gloves  
Lab coat  
Goggles  
MPN Statistical Chart (can be obtained in Microbiology textbook, local water utilities plant, American Public Health Association)

<http://www.alpha.org/contact/>

<http://www.thesciencelab.com>  
<http://www.lamotte.com>  
<http://members.aol.com/savemodoe2/emergency.htm>  
<http://www.usa-ro.com>  
Pamphlets obtained from local water treatment plant.

‘Brock Biology of Microorganisms’, Madigan, et al., Prentice Hall, 2000; chapter 11.

“Microbiology--Principles and Health Science Application”, Bergquist & Pogosian, W.B. Saunders Co., 2000, unit 7, chapter 23.

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**Assessment**

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**Laboratory Investigation Rubric**  
**Writing Rubric**

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**Accommodations**

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For reinforcement, the student will create a chart showing examples of waterborne diseases and the causative agent.

For enrichment, the student will research and report on a recent epidemic caused by a contaminated water supply. Include in the report the steps taken by the local health department to identify the source of contamination and method of containment.

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**Reflections**

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## **Environmental Microbiology- Water Analysis**

### **Purpose:**

In this laboratory exercise, the student will employ one of two commonly used methods for determining the presence of organisms, known as coliforms, in unknown water samples. The multiple tube fermentation technique is a three-part test which includes the presumptive, confirmed, and completed tests.

### **Background Information:**

### **Materials**

100 ml. Water samples  
5 tubes of double-strength lactose broth  
10 tubes of single-strength lactose broth  
Nutrient agar slant  
Incubator  
10 EMB plates  
Inoculating loop  
Pipette and bulb  
Bunsen burner  
Gloves  
Lab coat  
Goggles  
MPN Statistical Chart

### **Procedure**

- I. Preparation
  1. Wash hands and put on gloves and goggles.
  2. Assemble equipment and materials.
  3. Prepare work area.
- II. Presumptive Test
  - A. Flame and cool the loop. Inoculate 5 double-strength lactose broth tubes with 10 ml. of water sample.
  - B. Flame and cool the loop. Inoculate 5 single-strength lactose broth tubes with 1 ml. of water sample.
  - C. Flame and cool the loop. Inoculate 5 single-strength lactose broth tubes with 0.1 ml. of water sample. Flame and cool the loop.
  - D. Incubate at 35 C for 24 - 48 hrs.
- III. Confirmed Test
  - A. Streak one EMB plate for each gas-positive tube.

- B. Incubate at 35 C for 24 - 48 hrs.
  - C. Examine plates for lactose-fermenting colonies.
- IV. Completed Test
- A. Inoculate one lactose broth tube and one nutrient agar slant for each plate with one lactose-fermenting colony
  - B. Incubate at 35 C for 24 - 48 hrs.
  - C. Examine for the presence of fermentation, i.e. Gas-positive (determined by the presence of a clear bubble of gas in the test tube.
  - D. Gram-stain growth in nutrient agar slant, report findings.
  - E. Clean work area with surface disinfectant. Remove goggles and gloves and wash hands.

**Data:**

Using the statistical chart, most probable numbers (mpn) of coliforms per 100ml. Of water, determine the mpn for the presumptive, confirmed, and completed testing phases of this experiment.

**Conclusion:**

1. In the presumptive test, what do bubbles in the tubes indicate?
  
  
  
  
  
  
  
  
  
  
2. In the confirmed test, what does the presence of purple or metallic green colonies indicate?
  
  
  
  
  
  
  
  
  
  
3. In the completed test, what does the presence of gas in the lactose- broth tube indicate?
  
  
  
  
  
  
  
  
  
  
4. Predict what stage or phase the water's potability is determined?

5. What are coliforms and what bacteria does this term apply to? What are some other common coliforms that may serve as indicator organisms?
  
6. How does the EPA require that the potability of water be reported?
  
7. What are some sources of contamination of a city's water supply?
  
8. Does water analysis include testing for pathogenic viruses?
  
9. Is drinking water tested for the presence of specific pathogens? Why or why not?
  
10. What are the three processes of water purification used by municipal water suppliers to ensure the safety of drinking water?