# Assessment of Over-the-Counter

### Introduction

Use the following bacterial culture test to observe the effectiveness of antibacterial household products in preventing microbial growth.

#### Concepts

- Antibacterial agents
- Bacterial inoculation

#### Materials

Bleach solution, 10% (for disposal)\*
Household products (i.e., mouthwash, cleaners, antibacterial soaps, etc.)
Isopropyl alcohol, (CH<sub>3</sub>)<sub>2</sub>CHOH (for sterilization)
Autoclave (recommended)
Bacterial stock cultures (recommended)
Forceps
Incubator (optional)
\*Dilute household bleach by a factor of 10.

• Zones of inhibition

Marking pens
Sterile paper disks, 4 per dish, or hole-punch to make paper disks, ≈ 7 mm
Petri dishes filled with nutrient agar
Cotton swabs
Ruler
Tape, transparent

BIO FAX!

#### Safety Precautions

After use, agar plates may contain viable pathogenic microbes. Do not open the plates unnecessarily. Use sterile techniques at all times while handling bacterial cultures. Before disposal, plates should be soaked in a 10% bleach solution for at least 6 hours or overnight or autoclaved. The plates should then be disposed of directly into the garbage. Set a good example of sterile technique for students when handling culture plates. Be sure to sterilize work areas before and after bandling microbes and have students wash their bands with soap before leaving the laboratory area. Bleach solution is a corrosive liquid, and may cause skin burns. Avoid contact with heat, acids, and organic materials. Isopropyl alcohol is a flammable liquid and causes serious eye irritation, mild skin irritation and may cause drowsiness or dizziness. Avoid breathing mist, vapors or spray. Please consult current Safety Data Sheets for additional safety, bandling, and disposal information.

#### Procedure

- 1. Draw quadrants on the bottom of the nutrient agar-filled Petri dish using a marking pen or wax pencil.
- 2. Using a sterile moistened cotton swab, touch the bacterial stock culture.
- 3. Streak the entire agar plate and wait a few minutes for the plate to dry.
- 4. Sterilize forceps using isopropyl alcohol.
- 5. Soak different paper disks in four different household products and use forceps to transfer the disks to the Petri dish.
- 6. Gently blot the disks on a paper towel to remove excess product before placing the disks on the agar plate.
- 7. Using forceps, place one paper disk in a quadrant. Press down lightly with forceps to set the disk in place.
- 8. Sterilize the forceps using isopropyl alcohol between samples.
- 9. Label the bottom of the plate along the plate edge. Do not write under the disk—this area needs to remain clear in order to measure the zone of inhibition.
- 10. Repeat steps 5–8 using the three remaining disks.
- 11. Have students write their initials and the date on the bottom of the plate, and tape the lid shut.
- 12. Stack the Petri dishes in a cabinet, on a countertop, or in an incubator set at 37 °C for a few days until bacteria growth is sufficient that zones of inhibition can be easily seen around the paper disks. (This may take only a day if an incubator is used.)

13. Measure the zone of inhibition around each disk from the bottom of the plate. Which products had the largest zone of inhibition? The smallest?

#### Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures governing the disposal of laboratory waste. The agar plates may be disposed of according to Flinn Suggested Biological Disposal Method Type I.

#### NGSS Alignment

This laboratory activity relates to the following Next Generation Science Standards (2013):

#### **Disciplinary Core Ideas: Middle School**

MS-LS1 From Molecules to Organisms: Structures and Processes LS1.A: Structure and Function

LS1.B: Growth and Development of Organisms

Science and Engineering Practices Planning and carrying out investigations Analyzing and interpreting data **Crosscutting Concepts** Patterns Cause and effect Structure and function

#### Tips

- Isopropyl alcohol and bleach solution are used as disinfectants. Try them as test chemicals to observe their effectiveness!
- Have students bring in their own household products. Try some products that may not claim to be antibacterial but would be interesting to test (i.e., acidic beverages or fruit juices, regular soaps, spicy sauces, bug repellent, salt water, oils, etc.).
- Once the agar plates have been taped shut, do not reopen except for sterilization before disposal.
- Purchase stock cultures from Flinn Scientific to test how different bacterial strains react to antibacterial products.
- The rate of diffusion for the household products into the agar also affects the zone of inhibition.

#### Discussion

Triclosan, a chlorophenol, is commonly found in over-the-counter products that claim antibacterial properties. This chemical has the ability to kill both gram-positive and gram-negative bacteria and to inhibit bacterial growth. Zones of inhibition are areas where bacteria cannot grow due to the presence of an inhibiting substance such as triclosan. Many people are not aware that bacteria need to be exposed to an antibacterial agent for at least two minutes in order for the chemical to effectively kill bacteria. Recently scientists have been concerned with bacterial resistance leading to the development of resistant strains. Antibacterial products kill not only pathogenetic bacteria (capable of causing disease), but also bacteria that make up our natural flora as well. Bacteria normally found on our bodies help inhibit growth of parasitic strains by occupying space and resources that pathogens require in order to flourish. The FDA has prohibited the sale of "consumer antiseptic washes" that contain triclosan, effective September 2017.

#### Reference

2

Scheppler, Judith A.; Sethakorn, Nan; Styer, Susan. The Science Teacher. November 2003, 56-61.

## Materials for Assessment of Over-the-Counter Antibacterial Products are available from Flinn Scientific, Inc.

Catalog No.	Description
FB0526	Nutrient Agar, 10 plates
LM1006	Escherichia coli
AP7696	Swab Applicators, Sterile
FB1148	Blank Sterile Disks

Consult your Flinn Scientific Catalog/Reference Manual for current prices.