

## INTRODUCTION

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In microbiology, epidemiology is the study of the spread and survival of infectious agents through a group of susceptible hosts. Awareness of how a microbe is transmitted between individuals is imperative to developing methods of its control.

Hand washing is one of the most important methods of controlling microbes in any setting including laboratory and clinical. Transferring of microbes from person-to-person is termed **direct contact**. Other sources of microbial infection are termed **fomites**. Fomites are inanimate objects that are contaminated with an infectious agent and when mishandled can be passed on and cause infection.

Very often, personal care products or especially our everyday portable phones can act as fomites or reservoirs for infectious microbes. Most personal care products have bacteriostatic agents added to inhibit the growth of bacteria. However, everyday objects such as doorknobs, handles, phones, and especially our hands need to be sanitized properly in order to avoid formation of fomites and transmission of microbes.

In order to avoid the accidental transmission of pathogens that could induce diarrhea or cholera or even viral influenza, good handwashing etiquette is paramount. When it comes to handwashing, using soaps is traditionally a good measure to ensure removal of bacteria, especially coliform bacteria. Soaps are **surfactants**, or detergent compounds that lower the surface tension between liquid and solid compounds. Soaps contain detergents that are *amphipathic*, those that are both polar and nonpolar. Detergents help solvate the lipid cell membranes of bacteria, and thus detaching them from surfaces and washing them away. The nonpolar side of the detergents in soap react with the oils and dead cells on our hands and pulls them off along with the bacteria, while the polar side of the detergents help wash it away.

Some soap may contain antibacterial compounds, most commonly Triclosan (0.3%), in order to aid in removal and killing of bacteria. Antibacterial soaps, however, should be used in moderation if at all since the antibiotics work on a limited range of microbes and widespread use may contribute to increase in

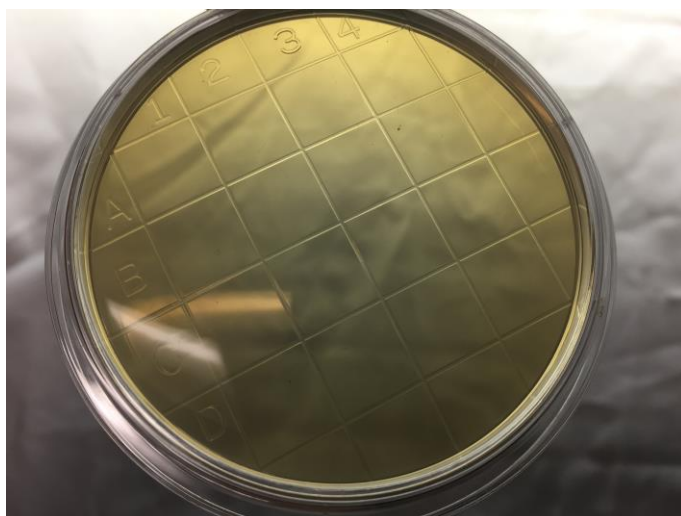
antimicrobial resistance. Another popular method of hand sanitization is hand antiseptics/sanitizer. Hand sanitizers are typically isopropyl or ethanol alcohol-based gels used to kill bacteria. These evaporating gels are very effective in killing bacteria depending on the time of contact.

The ultimate determinant of properly cleaned and sanitized hands is the **time of contact** used along with a proper surfactant. Time of contact refers to the amount of time spent actively scrubbing, rubbing, working in, and keeping the detergents onto the hands. The longer the time of contact, the better the removal of bacteria.

A very easy and effective method of enumerating bacteria on surfaces involves the use of RODAC plates. **RODAC** (replicate Organism Detection and Counting) plates are designed specifically with a domed or convex agar surface that is pressed onto any surface that is being tested for traces of bacteria. RODAC plates are often used in the monitoring of environmental surfaces, laboratory, medical, and even home surfaces. The gridlines present on the back of the plate allows easy enumeration of bacteria or CFUs per 10mm square of the surface tested (Figure 1). Table 1 below is often used to establish whether a surface exposed to RODAC plates have been properly cleaned and sanitized.

Table 1. RODAC plate CFU Classifications

RODAC Colony Count Results	
Excellent Sanitation	0 CFU
Adequate Sanitation	1-10 CFU
Marginal Sanitation	11-20 CFU
Unsatisfactory Sanitation	>20 CFU



**Figure 1.** Rodac Nutruent Agar plate with labeled 10mm gridlines showing before inoculation and after inoculation of a tested surface.

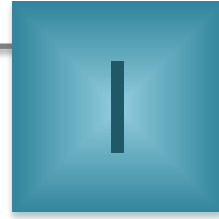
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***Materials:***

1. (10) Ten Nutrient Agar RODAC plates
2. (2) Two EMB Agar RODAC plates
3. Liquid disinfectant/antibacterial soap
4. Hand sanitizer gel (Antibacterial)
5. Broth culture of E. Coli
6. Gloves
7. One large test tube
8. Common everyday item (phone, notebook, bottle, doorknob)
9. Ruler

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**Procedures:**



**Part I. Handwashing**

1. Take EIGHT Nutrient agar RODAC plates for your group and **label** your pair with “before” and “after”. (Everyone gets two plates).
2. Each lab member of the bench will be responsible for a different cleaning method:
  - a. Solid Soap (20 second wash)
  - b. Antibacterial Soap (20 second wash)
  - c. Antibacterial Soap (2 minutes wash)
  - d. Antibacterial gel.
    - i. If you are missing a student, place a glove on one hand during the first process, and remove glove for next process.
3. First, each lab member will use their first RODAC plate labeled “before” and press it gently onto their bare palms on their dominant hand for few seconds. Place the lid back onto the plate.
4. Next, each lab member of the group will perform a different handwashing procedure: Solid Soap (20 second wash), Antibacterial Soap (20 second wash), Antibacterial Soap (2 minutes wash), and Antibacterial gel.
5. One member will use the solid bar soap and wash their hands, rinse and dry completely with paper towels.
6. The next member will wash their hands using the antimicrobial soap provided for 20 seconds or less, rinse and dry completely with paper towels.
7. Next student will wash their hands using the antimicrobial soap provided for 2 minutes, rinse and dry completely with paper towels.
8. The last student will place a workable amount of antibacterial gel onto their hands and work it into their hands for 30 seconds and allow to air dry completely.

9. After all the hand cleansing procedures, each student will grab their respective “after” plates and gently press it onto their cleansed hand for a few seconds. Place lid back onto plate.
10. Incubate plates at 30°C for 48 hours. Next lab you will count the number of total bacteria, the number of bacteria types (refer to Procedures Figure 3), the percent increase/decrease and record in your worksheet. Formula for percent increase/decrease is found below.
11. After observing and counting the colonies on the plates, use parafilm to seal your plate for the next lab.

$$\left( \frac{\#Bacteria\ Before - \#Bacteria\ After}{\#Bacteria\ Before} \right) \times 100 = Percent\ Change$$

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## Part II Enumeration of Microbes on Fomite

1. Every pair of lab members should acquire one RODAC plate.
2. Choose an object of everyday use such as a notebook, cell phone, hairbrush, or eyeglasses.
3. Carefully remove the plate lid, and gently touch the surface of the Rodac plate onto the chosen surface for 5 seconds. Do NOT rub or move the plate around onto the surface.
4. Remove the Rodac plate after touching and place the lid back. Gently press the top of the lid to form a seal. Incubate at 37°C for 48 hours.

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## Part III. Direct Transmission of Microbes

1. Obtain two EMB RODAC agar plates. Label one “Fomite” and with your group #. Label the second plate “Direct” also with your group number.
2. Two students per group needs to place one glove on. One student will handle the fomite, while the other will perform a handshake.
3. The TA will be the “source of infection”.
4. One test tube contaminated with a known bacterium will be placed into the gloved hand of the source to be passed on to the next student in the first row and then onto the next student in the NEXT group until the last group is reached.
5. The “source of infection” will then shake hands with the first student in the first group; the handshake should be between students in another bench or group.
6. After everyone has been “exposed”, students will gently press their gloved hand onto their assigned and labeled EMB agar RODAC plate.

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***Procedures Week 2:***

1. Obtain your Nutrient Agar RODAC plates and choose one colony from either of your plates or your lab mate's.
2. Aseptically transfer the chosen colony into a sterile Nutrient Agar Slant (see Appendix Figure 8).
3. Thee bacteria will be incubated at 30°C for 48 hours.
4. The bacteria from the slant will be used in the next experiment #6.

**Part I & II Handwashing and Enumeration of Microbes on Fomite (3 points)**

**Directions:** Include you and your group’s observations from the Nutrient agar RODAC plates and your object of choice. Calculate the percent difference between the RODAC plates “before” and “after” cleansing.

		Number of Bacteria Colonies	Number of Bacteria types	Percent % Difference Colonies and Types	
<b>Bar Soap (20 sec)</b>	Before				
	After				
<b>Antibacterial Soap (20 sec)</b>	Before				
	After				
<b>Antibacterial Soap (2 min)</b>	Before				
	After				
<b>Hand Sanitizer</b>	Before				
	After				
<b>Fomite</b>				-	-

**Part III. Direct Transmission of Microbes (2 points)**

**Directions:** Record the number of colonies present on the plates. Include your classmates’ results.

From Fomite	Student #	From Handshake
	1	
	2	
	3	
	4	
	5	
	6	
	7	



#### **Part IV Questions (5 points)**

**Directions:** Answer the following questions by using your experimental results.

1. Was there an increase or decrease of CFUs after your Handwashing procedure? **(0.5 points)**
  
2. Which hand cleaning procedure was the most effective in removing bacteria among your group members? Which was the least? Use the *percent* increase/decrease. **(0.5 points)**
  
3. Do you expect more or less growth on the handwashing plates after the hands are “cleaned” or before? Why? Were the results what you expected? **(0.5 points)**
  
4. Based on the results of the handwashing plates with antibacterial soap, did time of contact have any effect on sanitation? Explain.
  
5. According to the number of colonies and types (different colony morphology) of bacteria from your RODAC plates (selected object), do you believe it to be sanitary? Refer to Table 1. **(0.5 points)**

6. If the area of the palm of your hand is about  $175 \times 75 \text{ mm}^2$ , what is the total bacterial titer on your hand before cleansing? On your object? **(use a ruler and show your work).**
  
7. Observe the growth of the bacteria on the transmission RODAC plates. Which mechanism, fomite or direct contact, allowed more microbes to be transmitted?
  
8. Did the number of bacteria decrease after being transmitted between people? Explain.
  
9. Taking all the experimental results into consideration from handwashing, fomite, and direct transmission, how will your results affect your everyday habits? **(0.5 points)**