

# Exercise 1

## SIGNIFICANCE OF HANDWASHING

### Introduction

The external surfaces of the human body, skin and mucous membranes, are sterile during fetal development, but are colonized by multiple different types of microorganisms during and following the birth process. Many of these acquired organisms persist throughout the life of the individual, while others change over time. As humans grow and age, their bodies are exposed to many different environments, and new microbial populations are provided opportunities to colonize. Microorganisms established as more-or-less permanent residents are referred to as **normal microbiota**, while those present only temporarily, e.g., for a few hours, days or weeks are called **transient microbiota** (Note – the terms **normal flora** and **transient flora** are sometimes used in place of microbiota because for many years, bacteria and fungi were categorized as plants; **bacteria are not plants**). The number of prokaryotic cells inhabiting the human body far exceeds the number of human cells present, and the impacts of these organisms are multiple and varied.

The significance of bacteria as **etiological** (disease-causing) agents was documented by **Robert Koch** in 1876; but long before that, some physicians recognized the potential for disease transmission between individuals. **Ignaz Philipp Semmelweis**, an official with the obstetrical clinics of the Vienna General Hospital (1847), noticed that surgeons and medical students frequently traveled directly from autopsy rooms to medical wards where they assisted women during childbirth. After studying hospital records, he concluded that materials transferred from cadavers were directly related to the incidence of **puerperal** or **childbirth fever**, a common cause of patient mortality. Semmelweis then instituted a policy requiring all medical students to **wash their hands** using a solution of chlorinated lime before attending to patients. Infection rates within the obstetrical clinics were significantly reduced while this practice was required, but Semmelweis was ridiculed for his policy, and ultimately resigned his position.

Current documentation provided by the Centers for Disease Control and Prevention (CDC) states that "**handwashing is the single most important procedure for preventing nosocomial (institutionally acquired) infections**". Although fast-acting hand sanitizers are used in many clinical settings, evidence suggests these are less effective than handwashing with soap and water, particularly in the control of methicillin-resistant strains of *Staphylococcus aureus* (MRSA). Washing with soap helps to remove a layer of oil and some of the microbes associated with the epithelial surfaces of dry skin. Prior to surgery, medical doctors use a complete surgical scrub to remove transient microorganisms as well as some normal flora by scrubbing with soap (or a halogen such as chlorine or iodine) for 10 minutes.

New information gained through studies of the **human microbiome** indicates that microbial diversity on human skin is great, varies from one location to another, and plays a significant role in human health. Microbial communities inhabit all skin surfaces, live within pores and sweat glands, and along hair shafts. These microbes are not passive bystanders but are playing active roles in the dynamic balance between health and disease. Common inhabitants such as *Staphylococcus epidermidis* help to inhibit the growth of potential pathogens such as MRSA, so help prevent infection. *Staphylococcus lugdunensis*, another member of our normal microbiota, has recently been found to produce a new type of antibiotic called lugdunin that kills both MRSA and Vancomycin-resistant *Enterococcus* species. In some cases, disease symptoms may result from the disturbance of microbial communities, altered proportions of constituents or altered behaviors due to stress (all forms of **dysbiosis**). Consequently, the **frequent use of antibacterial soaps and other antimicrobial agents** that significantly alter the skin microbiome **are likely to have detrimental effects**. Commonly used antibacterial agents (Triclosan, Triclo-carban, etc.), have now been shown to adversely affect both human and environmental health.

During this exercise, students will attempt to assess the effectiveness of handwashing, by observing transient flora as well as a portion of the normal flora readily grown in an aerobic environment on nutrient agar plates. After completing the exercise, students will be expected to know how handwashing effectiveness is evaluated, if or not handwashing effectively decreases skin microbiota, and to understand the importance of this aspect of aseptic technique in the hospital environment.

**Procedure – Work in groups of two or three:**

1. Obtain a plate of nutrient agar and draw two straight lines on the bottom surface dividing the agar into four equal parts. Label one quadrant of the plate "before", one quadrant "transient", one quadrant "sanitize", and the last quadrant "scrub & air dry". Be sure to include student names and the date on the plate bottom also.
2. Gently press one fingertip from each participant onto the agar surface of the "before" quadrant of the plate. Outline the position of each finger indicating where the plate was inoculated and to identify each participant (label each outline with initials).
3. Obtain a sample of "transient" flora by touching the "patient" provided. Make certain the fingertips used in the first inoculation come in contact with the surface provided.
4. Gently inoculate the "transient" quadrant of the plate as described in #2 above. Label the finger positions and participants as indicated above.
5. Sanitize your hands (or individual fingers) using one or more of the hand-sanitizing solutions provided. Allow your fingertips to air dry, and then gently inoculate the "sanitize" section of the plate as described earlier. Label the finger positions and participants as before, and record the type of hand sanitizer used for each one.
6. Wash your hands thoroughly in either hot or cold water, using one of the soap solutions provided and a soft brush. If you choose to use an alternative cleaning agent (e.g., laboratory disinfectant), or to wash for an extended period of time, be sure to document these variations.
7. After rinsing thoroughly and shaking off excess water (**but without using a paper towel for drying**), gently inoculate the "scrub & air dry" quadrant of the plate as described above. Record finger positions and participants as indicated.

**Note – The paper towels provided in this laboratory are not sterile, and the bacteria they carry along their outer edges would invalidate the handwashing procedure if used for hand drying.**

8. Incubate the plate at room temperature until the next laboratory period. Examine the agar surface and note the number, size and morphological features of any colonies present to evaluate the effectiveness of sanitizing and handwashing. Record qualitative and quantitative variation as indicated in the data sheet provided.
9. Discuss your findings with other members of the class to determine the effectiveness of hand cleaning methods as demonstrated by this exercise.

**Questions:**

1. Why are microbiology students advised to never eat or drink in the Microbiology laboratory?  
\_\_\_\_\_
2. Assuming you have a liquid microbial culture (broth culture) that you must dispose of; what are you expected to do with it? \_\_\_\_\_  
\_\_\_\_\_
3. While working in this laboratory, you will encounter a number of potentially harmful microorganisms. Aside from these, what are some of the hazards associated with working in the microbiology lab? \_\_\_\_\_
4. According to the CDC, handwashing is \_\_\_\_\_.
5. Did the application of hand sanitizer effectively eliminate or prevent the growth of the "transient" flora provided? How do you know? \_\_\_\_\_
6. Did thoroughly scrubbing the hands reduce the number and variety of cells present? How do you know? \_\_\_\_\_
7. Did the data you obtained meet your expectations, or did the results differ from what you expected?  
\_\_\_\_\_
8. Were variations in growth consistent throughout the laboratory, or did they vary with different individuals, solutions used and/or times used for washing? \_\_\_\_\_  
\_\_\_\_\_

Name \_\_\_\_\_

Lab Section \_\_\_\_\_

**WORKSHEET**  
**Introduction & Exercise 1**  
**Significance**  
**of Handwashing**

**Goals:** \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

**Materials & Methods:**

Sanitize step - Hand sanitizer used: \_\_\_\_\_  
Scrub & Air Dry Step – Soap used: \_\_\_\_\_  
Water temperature – Indicate hot, warm or cold: \_\_\_\_\_  
Date: \_\_\_\_\_ Medium used: \_\_\_\_\_  
Incubation temperature: \_\_\_\_\_ Duration of Incubation: \_\_\_\_\_

**Results:**

<b>Quadrant</b>	<b>Number of Colonies</b>	<b>Description of Colony Types (color, texture, etc.)</b>	<b>Did any colony type disappear?</b>	<b>Did any colony type appear?</b>
Before				
Transient				
Sanitize				
Scrub & Air Dry				

**Conclusions:**

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_