

Normal Flora Lab

Objectives

1. Evaluate the advantages of normal flora.
2. Describe the factors involved in effective hand washing.
5. Chart and interpret the data from the handwashing experiment.
6. Analyze factors affecting hand washing.
7. Integrate information from the hand washing experiment and the importance of normal flora with patient care.

Introduction

The transmission of organisms as a result of hand contact is well documented. Regulations mandate signs directing handwashing in all public restrooms.

Beginning with Semmelweis, physicians slowly became more aware of the presence of bacteria normally present on their skin. Many microorganisms belong to the normal flora of our skin and represent no threat to a healthy individual; these are called **resident flora**. Resident flora can cause serious infections in immunocompromised patients, such as patients. Physicians may not be aware, but the lack of proper handwashing techniques may inadvertently harm the patient. Inadvertent inoculation of skin organisms into susceptible areas (such as those introduced into the bladder with a urinary catheter or the blood with an I.V. catheter) may represent life-threatening conditions. Organisms that are present for a short period of time, as a result of contact, are termed **transient flora**. These organisms, if pathogenic in nature, represent a real threat if introduced into a patient's treatment.

Removal of these organisms requires dedicated handwashing techniques. Transient flora tends to be more susceptible to antiseptic washes and is easily removed with thorough scrubbing. Resident flora is more difficult to detach. A layer of oil, hair, and dead skin obstruct removal of these organisms. They are usually more resistant to soaps and antiseptics, but generally less pathogenic.

Hospital procedures require personnel to wash their hands before and after patient contact and with each glove change. Follow the protocol below and determine the effectiveness of handwashing with regards to different handwashing techniques through the ages.

Materials:

3 Nutrient Agar Plates per group

gloves

Soap (liquid)

Antiseptic

Sterilized Scrub brushes wrapped in foil (one per person)

Q-tips

Read the lab. Then write a hypothesis, a prediction, about the outcome of this experiment.

Hypothesis – WRITE YOUR HYPOTHESIS DOWN ON A RECIPE CARD. Store them in your binder.

Methods:

Each lab partner:

1. Each person in the group will inoculate one plate. The plates are divided into 4 sections and are called quad plates. Notice that the quadrants are numbered I, II, III, IV. You may want to number them with the sharpie so the sections are more easily visible. Assign each person a quadrant.

2. Label a plate "**No Soap**" and will swab their hands and arms with a q-tip (before washing.) Gently touch your q-tip to the agar on the petri dish. Each person should stay only on their quadrant.

3. Label a plate "Chlor-lime". Each student in your group should wash with chlorinated lime water (on back table) for 15 seconds. Dry off your hands with a hand towel. With a new Q-tip, swab your hands and arms, then gently inoculate your petri plate in your quadrant.

For the remaining plates, each individual student can choose one method of handwashing to test:

Hand sanitizer	Surgical hand washing	Other?
Take two pumps of handsanitizer into your hands, rub vigorously and allow to air dry. Then innoulate a petri plate labelled "sani"	Follow the directions posted by the surgical sink. Then innoculate a petri plate labelled "Surgical"	Please ask the teacher if you have another idea you would like to test. (Different soaps, different scrub times, ?)

Aseptic technique-

*Lids should remain on the petri dish until you are ready to inoculate with your swabs. Keeping them closed prevents bacteria from the air accidentally inoculating your petri plates.

* Be careful to avoid touching the agar. Agar is the nutrient media present on your petri dish that provides the nutrients that normal skin bacteria need to grow. We will be incubating these petri plates for a few weeks for colonies to form.



Example of proper swab technique on quad plates.

Lab Session #2

Read the plates and count the colonies. Record the data on the table.

The unwashed hand serves as a control between the four individuals.

Record the relative growth using:

(0) = No growth

(+) = Very little growth (5 colonies or less)

(2+) = moderate growth (6-20 colonies)

(3+) for heavy growth (20 - 50 colonies)

(4+) for confluent growth; uncountable, possible growing over the quadrant lines

On graph paper, represent this data using a bar graph.

Answer the following questions in a formal lab write up.

1. How and when do humans get normal flora?
2. Identify the control, dependant, and independant variables in this experiment.
- 2b. What was your hypothesis concerning the hand washing experiment? Was your hypothesis supported? Why or Why not?
3. Compare the effectiveness of initial scrub with the vigorous scrub.
4. Why have we switched from Semmelweis' original suggestion for handwashing, to our current surgical methods?
5. What was the effect of the hand sanitizer?
6. What was the effect of any other treatments you did?
7. What might explain differences amongst class results as far as the number of colonies that grew?
8. Discuss the effect of handwashing on transient versus residential bacteria.
9. Why did we do the methods of handwashing in the order that we did? What would have happened if we had done it in reverse?
10. How might we proceed to identify the types of bacteria we incubated?
11. Surgeons always wear surgical gloves when touching a patient, even if they wash their hands.... why?
12. Prescribed antibiotics can be used to help the body eliminate patholicial bacteria. As a unattended side effect, the antibiotics sometimes kill off all natural normal flora. What might happen if you kill all the good bacteria in your body with antibiotics?
(You may need to research this on the internet!)

13. Your doctor often prescribes a course of antibiotics to be taken for a specific amount of time (usually around 1 week). Often antibiotic courses cause patients to start feeling better before their prescription has been fully taken. What might happen if you stop taking your antibiotics before your doctor recommends? Draw a diagram or picture showing what happens if you stop taking your antibiotics. (You may need to research this on the internet!)

Answer key:

Hypothesis writing: hypothesis' should take the form of a statement, "I think that the surgical hand washing procedure will reduce the amount of bacteria to 0 in the cultures. Hypothesis should not be written in the form of a question!

1. How and when do humans get normal flora?

Humans get normal flora from their daily lives. Who they interact with commonly (family), their home, pets, environment, and their lifestyles all contribute to their normal flora.

2. Identify the control, dependant, and independant variables in this experiment.

Control- no hand washing agar plate

Dependant variable- handwashing techniques (hand sanitizer, chlorinated lime water, surgical hand washing etc)

2b. What was your hypothesis concerning the hand washing experiment? Was your hypothesis supported? Why or Why not?

Individual answers may vary.

3. Compare the effectiveness of initial scrub with the vigorous scrub.

Surgical scrubs are much more vigourous, and time consuming then Semmelweis' version. The time involved, amount of cleaning products, and the attention to nails, and the spaces between fingers are much more detailed.

4. Why have we switched from Semmelweis' original suggestion for handwashing, to our current surgical methods?

According to the data, surgical methods of hand washing have the best effect on eliminating bacteria on our hands and arms. Semmelweis' recommendation still shows some bacteria growth, which could cause potential ill effects in patients.

5. What was the effect of the hand sanitizer?

Answers may vary.

6. What was the effect of any other treatments you did?

Answers may vary.

7. What might explain differences amongst class results as far as the number of colonies that grew?

Each individual has a different normal flora. This is the resident flora for them, according to their lifestyle, homelife, family, etc. This is non-pathogenic to them, but may be pathogenic to others who are not used to these bacterias.

8. Discuss the effect of handwashing on transient versus residential bacteria.

Residential bacteria requires much more vigourous scrubbing methods to remove. As

mentioned, these bacteria are often present on the top layer of skin, clinging to hair cells, in oil glands, etc. Surgical scrubbing is necessary to fully clean the skin before patient interaction.

9. Why did we do the methods of handwashing in the order that we did? What would have happened if we had done it in reverse?

During our no scrubbing, we were able to get an accurate incubation of our normal floras. We then proceeded to do Semmelweis' technique, with chlorinated lime water which will likely remove some of our normal flora, but not all. Our next step was to use hand sanitizer or surgical hand washing techniques, which will again eliminate more bacteria. The handwashing techniques were done in a progressive manner.

Had we done the procedure in the opposite way, we would have inaccurate data. Our control would have shown similar results to our surgical handwashing techniques, which would obviously be inaccurate.

10. How might we proceed to identify the types of bacteria we incubated?

Colonies can be identified by different techniques such as differential agar, morphology, microscopy, and analyzing them against normal flora keys.

11. Surgeons always wear surgical gloves when touching a patient, even if they wash their hands.... why?

Despite the vigourousness of surgical hand washing, some bacteria may remain on the surgeons hands. The surgical gloves provide an additional layer of protection for the surgeon and helps them obtain accurate readings when doing cultures.

The gloves also are key to preventing surgeons from being infected with the patients normal flora. Patients often have compromised immune systems, which can lead them to carrying many bacteria and viruses that the surgeon needs protection from.

12. Prescribed antibiotics can be used to help the body eliminate pathogenic bacteria. As a unattended side effect, the antibiotics sometimes kill off all natural normal flora. What might happen if you kill all the good bacteria in your body with antibiotics?

(You may need to research this on the internet!)

Patients often feel better as the bacteria begin to be killed by antibiotic treatments. This does not mean that all the bacteria have been killed, just that the concentration of bacteria is too low to cause symptoms. By stopping the prescription early, there may be residual bacteria that are still present in the body. Without an antibiotic, these bacteria will begin to reinfect the patient, and cause a return of the symptoms and disease.