Hand washing and Surface Bacteria Sampling

Background:

Hand washing with soap and water has long been considered a standard of personal hygiene and its efficacy dates back to the 19th century. In 1846, Ignaz Semmelweis observed that women whose babies were delivered by medical students and physicians at the Vienna General Hospital had a much higher mortality rate compared to women whose babies were delivered by midwives. He observed that physicians were going directly from the autopsy room to the obstetrics ward to deliver babies without washing their hands, and he made the connection. Beginning in mid 1847, he became a proponent of students and physicians washing their hands with a chlorine solution between patients; and the mortality rate significantly decreased. This represents the first evidence indicating that hand washing between patients will contribute to the prevention of the transmission of disease between health care workers (HCW) and patients. In 1843, Oliver Wendell Holmes associated the spread of puerperal fever by the hands of HCWs. He was an advocate of hand washing between patients, but initially ignored. However, over time due to the observations and advocacy of Semmelweis and Holmes, hand washing has now become recognized as an important measure to be practiced by HCWs in preventing the transmission of pathogens.

While hand washing is recognized as a basic measure for preventing infections, compliance with hand washing in in most environments is limited. For example, studies by the American Society for Microbiology showed that 97% of females and 92% of males say they wash their hands regularly. Of these, only 75% females and 58% males actually washed their hands under observational studies. Furthermore, correct hand washing technique, particularly in respect of duration, is often not practiced.
Most pathogens that become associated with the skin originate on another person’s skin, or on surfaces in the surrounding environment. Fomites are inanimate objects that harbor viable pathogenic organisms. Equipment and objects used by many individuals, such as eating utensils, hospital surfaces, and shared surfaces such as phones, ATM machines, and money, are common objects that act as vehicles of dissemination for bacteria fungi and viruses. Public health microbiologists routinely examine relevant surfaces and are able to assess the prevailing sanitary conditions based on the number of organisms detected on the surface. Various procedures are employed for quantification and each involves transferring the organisms from the surface in question to solid media, followed by incubation and colony counting.

In this laboratory exercise, you will perform two experiments that connect the concepts of hand hygiene and treatment of surface contamination. You will perform the “glove juice” method to determine the efficacy of three hand washing techniques, and then you will compare the ability of five common surface disinfection methods to reduce the bacteria load on a shared surface.

A. Glove juice method to determine the efficacy of hand washing

Materials (per group):

Three, large, powder-free latex gloves
6 x 50 ml bacteria extraction solution

Dissolve 0.4 g of monobasic anhydrous potassium phosphate (KH$_2$PO$_4$), 10.1 g of tribasic, dodecahydrate sodium phosphate (Na$_2$PO$_4$) and 1.0 ml of Tween 80 or Triton X-100, in 1 L of distilled water. Adjust the solution to pH 7.8 and autoclave. Dispense 50 ml into three, sterile, 50 ml Falcon tubes.
Plastic rack for the tubes of bacteria extraction solution
24 x 9 ml sterile bacteria extraction solution, in 15 ml Falcon tubes
Pipette and 200 µl tips (sterile)
Eighteen Petri dishes containing tryptic salt agar (TSA)
Eighteen Petri dishes containing mannitol salt agar (MSA)
Glass cell spreader with Bunsen burner or three disposable, sterile cell spreaders
Marker
Kim wipes
Three rubber bands
Antibacterial soap
Alcohol-based hand sanitizer

Procedure:

1. “Inoculate” the hands of three group members by vigorously shaking hands with the other members of the group. This will provide a bacteria load that can be measured.

2. Each subject will don a glove on their LEFT hand, and a group member will carefully add 50 mL of bacteria extraction solution inside the glove. Rubber bands can be used to secure the gloves at the wrists.

3. Another group member (not the ones wearing the gloves) will massage the extraction solution throughout the glove for one minute. Then, carefully remove the gloves and empty the solution into the original Falcon tube (labeled with the student’s name and “control”).

4. Generate a dilution series to 10⁴ in 15 ml Falcon tubes for each of the three control glove juice samples.

5. Each of the three group members will perform a different hand washing technique; (i) 10-second rinse (rub hands together according to Figure 1, under flowing, warm water), (ii) 30-second wash with antibacterial soft soap (per procedures in Figure 1), and (iii) treatment with alcohol-based hand sanitizer (apply a dime sized amount of waterless hand sanitizer to the palm of one hand, and rub hands together covering all surfaces of hands and fingers until sanitizer is absorbed.)
6. Following hand washing procedures, each subject will don a glove on their RIGHT hand, and a group member will carefully add 50 mL of bacteria extraction solution inside the glove. Rubber bands can be used to secure the gloves at the wrists.

7. Another group member (not the ones wearing the gloves) will massage the extraction solution throughout the glove for one minute. Then, carefully remove the gloves and empty the solution into the original Falcon tube (labeled with the student’s name and the name of the treatment).

8. Generate a dilution series to $10^4$ in 15 ml Falcon tubes for each of the three glove juice samples.
9. Plate 100 μl of the 10^{-4}, 10^{-3} and 10^{-2} dilutions of the control (LEFT hand) dilution samples onto each of three, carefully labeled TSA plates, as well as three MSA plates.

10. Plate 100 μl of the 10^{-4}, 10^{-3} and 10^{-2} dilutions of the treatment (RIGHT hand) dilution samples onto each of three, carefully labeled TSA plates, as well as three MSA plates.

11. Incubate the plates at 35° C for 18-24 hours. Determine the bacteria load per ml of glove juice.

12. See step 13 of the surface sampling protocol for instructions to observe colonies on MSA plates.

B. Swab method for determining a surface microbial load

Materials (per group):

Tape to section off areas of your bench top
200 ml pipette and sterile, 200 μl tips
Chlorox wipes (one for each group to disinfect their bench top before the experiment)
Disinfection methods; Windex cleaner spray, warm water-soaked sponge, soapy water-soaked sponge, 70% EtOH, Chlorox wipes (one method per group)
One small sponge (for the warm water and soapy water groups only)
Clean, dry paper towels
Two sampling swabs
Sampling template
Scissors
4 x 5 ml of sterile bacteria extraction buffer, in 15 ml Falcon tubes (for extraction)
2 x 9 ml of sterile, bacteria extraction buffer, in 15 ml Falcon tubes (for dilution)
Vortex
Three Petri dishes containing tryptic salt agar (TSA)
Three Petri dishes containing mannitol salt agar (MSA)
Glass cell spreader with Bunsen burner or three disposable, sterile cell spreaders
Marker
Kim wipes
**Procedure:**

1. Each group will define a 2’ x 2’ section of their bench top by marking it with masking tape. Divide this square evenly to produce two, 2’ x 1’ sections. One of these sections will be your “control” section, while the other will be your “treatment” section.

2. Wipe both sections with a Chlorox wipe to disinfect the surface so that you are starting with a standardized bacteria load (hopefully a very low one).

3. “Contaminate” both sections with bacteria by having each group member liberally rub the palms of their hands across the surface. One section will remain contaminated, while the other section will be disinfected.

4. Each group will be assigned one disinfection method including; (i) Chlorox wipes (a single pass across the surface with a wipe), (ii) Windex surface cleaner (a single pass across the surface with a moistened paper towel), (iii) 70% EtOH (a single pass across the surface with a moistened paper towel), (iv) soap and warm water (a single pass across the surface with a soapy sponge), and (v) warm water (a single pass across the surface with a moistened sponge).

5. Disinfect the “treatment” section of your bench top. Remove any excess moisture with a clean paper towel. Be careful not to disinfect any part of the “control” section.

6. Following disinfection, remove a sampling swab from its wrapper and carefully soak it in the bacteria extraction buffer tube containing 5 ml of buffer. Press the swab against the inside of the tube to squeeze excess buffer from the swab.

7. Sample each section of the bench top by swabbing five, 100 cm² areas using the provided template. Be sure to swab the entire area of the template and to twist the swab as you sample to maximize the swab surface utilized for collecting bacteria. You will generate two sampling swabs, one from the “control” section and one from the “treatment” section. NOTE: under normal experimental conditions, we would perform this experiment in triplicate.

8. Carefully transfer the swab to a Falcon tube containing 5 ml of bacteria extraction buffer by cutting the swab from the stick with clean scissors.
The swab should drop directly into the tube.

9. Vortex the buffer/swab for 1 minute on the highest setting to liberate bacteria from the swab.

10. Generate a dilution series to $10^2$ in 15 ml Falcon tubes for each of the two buffer samples.

11. Plate 100 µl of the $10^2$, $10^1$ and $10^0$ dilutions of the control and treatment samples onto each of three, carefully labeled TSA plates, as well as three MSA plates.

12. Incubate the plates at 35°C for 18-24 hours.

13. Examine the TSA plates for bacteria colonies to determine the bacteria load per cm$^2$ of surface area. TSA is a non-selective medium that will support the growth of a wide variety of bacteria.

MSA is selective and differentiating. It contains salt, which makes it selective for Staphylococcus spp. Some species of Staphylococcus such as Staphylococcus aureus are also capable of fermenting mannitol, which produces acid waste products. The acidification of the mannitol causes the red indicator dye in the medium to turn yellow around the colony. Thus, colonies surrounded by the yellow color in the medium could be S. aureus. It is important to examine the plates as early as possible (within 12 –16 hours from the time you collected the samples) because sometimes the yellow coloration will extend over large sections of the plate making it very difficult to determine which colonies are mannitol fermenting. In other cases the yellow coloration dissipates after a few hours and you will not be able to determine if any of the colonies were mannitol fermenters (false negatives).
A mannitol salt agar (MSA) plate inoculated with \textit{S. aureus} (right side), which ferments mannitol, causing a pH-directed color change in the medium. On the left is a non-fermenter that causes no color change.

We will monitor the plates for you and refrigerate them so that you have an opportunity to observe them.