

Inspection and Evaluation of Antimicrobial Effects of Liquid Handwash and Bath Gel

1. Principles

By means of simulated tests, let the hands of the subject become polluted by experimental germs. Apply some tested product to wash the hands of the subject according to the standardized methods. Finally, observe the removal of the experimental germs on the artificially polluted hands so as to evaluate the removal effects of temporary germs by the tested product. This method is applicable to the evaluation of antimicrobial effects of liquid handwash and bath gel which are claimed to have antimicrobial functions(including the removal of and reduction in germs) .

2. Experimental Apparatuses

- 2.1 Experimental Samples: 25 samples of liquid handwash (or bath gel) products
- 2.2 Experimental Germs: When *Serratia marcescens* (ATCC14756) is grown on typtic soy agar (TSA), the germ appears to be pink.
- 2.3 Culture Media: Typtic soy broth (TSB)
Typtic soy agar (TSA)
- 2.4 Cleansing Fluids: 0.4g of KH_2PO_4 , 10.1g of Na_2HPO_4 , 1.0g of Triton X-100 and neutralizer (having inspected to be passed) are dissolved in 1 litre of distilled water. Use 0.1mol/L of HCL or 0.1mol/L of NaOH to adjust the pH to 7.8.
- 2.5 Diluent: 0.075mol/L of phosphate buffer solution (PBS) with its pH adjusted to 7.2.
- 2.6 Vibration Culture Box: At the temperature range of $25\pm 2^\circ\text{C}$ and the rotation of 100rpm (revolutions/min).
- 2.7 Tap Water Heater: Heat the tap water to $40\pm 2^\circ\text{C}$ for washing hands.
- 2.8 Culture Box: At the temperature range of $25\pm 2^\circ\text{C}$
- 2.9 Germ Meter
- 2.10 Glass Rod for Pasting Germs: At 121°C for removal of germs for 20 minutes.
- 2.11 Plastic bag (25 × 35cm)
- 2.12 70% isopropylalcohol or 75% ethanol.

3. Experimental Methods

3.1 Adjustment Stage

7 to 14 days before testing, the subject uses soap, shampoo and bath gel with no antimicrobial ingredient to wash hands and take baths every day. This stage must be sustained for at least 7 days.

3.2 Experimental Stage

3.2.1 Preparation of *Serratia Marcescens*

Select the typical individual germs on the plate, and plant them on 10ml of TSB for 24 ± 4 hours at a temperature of $25\pm 2^\circ\text{C}$. The germs are increased to form the *serratia marcescens* culture. The TSB culture is transmitted for two consecutive generations. Take out 0.1ml of TSB, and plant it in a triangular flask containing 100ml of TSB, for 24 ± 4 hours at the vibration speed of 100rpm at a temperature of $25\pm 2^\circ\text{C}$. While in use, the germ suspension liquid should be adjusted to between 1×10^8 and 1×10^9 cfu/ml, and the time of use should not exceed 8 hours.

3.2.2 Experimental Method of Comparison Group

Wet both hands of the subject with warm water at $40\pm 2^\circ\text{C}$ for 5 seconds. Apply 1.8 to 2.0ml of liquid handwash (or bath gel) containing no antimicrobial ingredient to the hands. Rub the hands and wrists for 30 seconds. Wash both hands from the finger tips to the elbows with warm water for 30 seconds (during this time do not touch the basin and other objects so as to prevent both hands from being polluted). Use paper towel to wipe the hands of the subject using a gentle patting technique. Let 70% isopropylalcohol stay in the palms and the back of hands for 10 seconds. Rub both hands for 15 seconds, and then wash them with warm water for 15 seconds. Wipe the hands with a paper towel.

Artificial Germ Spreading: Apply a total of 4.5ml of the suspension liquid of *serratia marcescens* on both hands at 3 different times, 1.5ml each. After the germ fluid is applied onto the palms, the subject slightly spreads it all over the hands up to the arms for 20 seconds each time, but must avoid touching the fingernail beds. The hands applied with germ fluid should not touch the body. After application of germ fluid for the first and second times, let the hands air dry for 30 seconds. After application of germ fluid for the third time, leave the hands to air dry for 90 seconds.

Way of Sampling: Wrap the left and right hands of the subject in clean plastic bags. Fill the bags with 75ml of cleanser (both the comparison group and testing group use the same cleanser) which contains a neutralizing agent. After the air inside the plastic bags is exhausted, tie both wrists tightly with

nylon strings. Two working staff massage the left and right hands of the subject for 1 minute respectively. After that, by means of germ-free operation, take out a 5ml of sample from the plastic bag and pour into a test tube (do not let the sample touch the hand), which serves as the sample for the comparison group.

3.2.3 Experimental Method of Experimental Group

Having finished the experiment of the comparison group, the subject washes both hands in warm water at $40\pm 2^{\circ}\text{C}$ for 30 seconds. Apply 70% isopropylalcohol to the palms and the back of hands for 10 seconds, and rub both hands for 15 seconds. Then, wash both hands in warm water for 30 seconds, and wipe them with a paper towel. Apply a total of 4.5ml of the suspension liquid of *serratia marcescens* on both hands at 3 different times, 1.5ml each. Follow the same procedures as the comparison group.

Hand Washing by Experimental Sample: Wet the germ-spread hands of the subject for 5 seconds. Apply 1.8 to 2.0ml of experimental sample on both hands. Rub both hands and wrists for 30 seconds, and wash them in warm water for 30 seconds.

Way of Sampling: Wrap the left and right hands of the subject in clean plastic bags. The sampling procedures are the same as the comparison group. After that, by means of germ-free operation, take out a 5ml sample from the plastic bag and pour into a test tube, which serves as the sample of experimental group.

3.2.4 Germ removal of both Hands after Testing

After the test, both hands of the subject, with finger tips pointing upwards, are sprayed with 70% isopropylalcohol for 10 seconds. Rub both hands and wrists for 15 seconds. Wash them with running warm water for 15 seconds, and then wipe them with a paper towel. Finally, soak both hands in 70% isopropylalcohol or other qualified hand sterilizing agent for more than 1 minute to sterilize both hands and wrists according to the application details.

3.2.5 Germ Plantation and Cultivation

Plant germs on plate by the way of pasting. Take out a 0.5ml sample from the comparison group. Use a diluent to conduct a series of 10-time dilution. Take out 0.1ml from each of the samples at 3 different dilution degrees, 10^{-3} , 10^{-4} and 10^{-5} , and plant them on two flat plates respectively.

Take out 0.1ml from the undiluted sample of the experimental group, and plant it on two flat plates. Then take out 0.5ml from the undiluted sample of the experimental group, and use a diluent to conduct a series of 10-time dilution. Take out the samples at the dilution degrees of $10(1)$, $10(2)$, $10(3)$ and

10(4, and plant them on two flat plates respectively. After plantation, place the plates under the temperature of 25(2C to perform cultivation for 48(4 hours. Count the number of germs on the plates at a range between 15cfu and 300cfu, and calculate the number of removed germs in each sample.

3.2.6 Calculation of Degerming Logarithmic Value

Calculate the average number of germs fallen on the left and right hands of both experimental and comparison groups, and take the logarithmic value of the average values.

Degerming Logarithmic Value = Logarithmic Value of Average Number of Fallen Germs of Comparison Group - Logarithmic Value of Average Number of Fallen Germs of Experimental Group

4. Standard of Evaluation

The number of subjects shall not exceed 16 man-times. A product is judged to have an antimicrobial function when its degerming logarithmic value ≥ 2.00 .

5. Notes

5.1 Requirements of Subject

5.1.1 Male or female at the age of 18 ~ 65.

5.1.2 Sign on a written letter of agreement.

5.1.3 There should be no skin disease or wound on both hands and wrists, and no cracked skin at the edges of fingernails, or other skin diseases.

5.1.4 During the testing period of hands, the subject is prohibited from applying any antibiotics, medical ointment or cream, sterilizing soap, anti-acne medicine and anti-dandruff shampoo on any part of the body or the whole body. In addition, for any contact with chloride, home-use cleaning detergent, organic solvents or any irritating chemical, the subject must wear rubber gloves. To ensure the strictness of the test, all subjects must use the same germ-free soap, shampoo and bath gel provided.

5.2 Standards for Rejection of Subject

Anybody with any one of the following situations is not allowed to participate in the test:

5.2.1 Participated recently in other clinical research .

5.2.2 Having received a hand or arm cleaning test within the last 14 days.

5.2.3 Having wounds, scars or any other skin injury on either hands or wrists.

5.2.4 Being allergic to soap, detergent and/or perfume.

5.2.5 Having eczema, psoriasis or other skin diseases on hands or wrists.

5.2.6 Being pregnant.

5.2.7 Breastfeeding.

5.2.8 Having diabetes, hepatitis, AIDS (HIV negative), or having undergone an organ transplantation.

5.2.9 Having any disease which is thought by researcher as being not suitable for inclusion in the test.

5.3 Other Restrictions

5.3.1 During the testing period, the subject shall not use any other personal cleaning product.

5.3.2 During the testing period, the subject shall not swim and take a hot water bath.

5.3.3 During the testing period, the subject shall not have contact with any irritating detergent, chloride or organic solvent.