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Science and Technology Feature

Microbial transfer by surface contact in cleanrooms

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Experiments were carried out to ascertain the proportion of microbes that would be transferred from a contaminated surface to a receiving surface in a cleanroom. To simulate transfers, microbe-carrying particles (MCPs) were sampled from the skin onto donating sterile surfaces, which were latex gloves, stainless steel and clothing fabric. A contact was made between these surfaces and a sterile receiving surface of stainless steel, and the proportion of MCPs transferred ascertained. The proportion of MCPs transferred, i.e. the transfer coefficient, was 0.19 for gloves, 0.10 for stainless steel, and 0.06 for clothing fabric. These transfer coefficients would vary in different conditions and the reasons are discussed.

Key words: Microbial transfer, surface contact, cleanrooms.

Introduction

A major route of transfer of microbial contamination in cleanrooms is by surface contact. This occurs when a contaminated donating surface, such as a gloved hand, comes into contact with a receiving surface, and microbes are transferred. By this means, microbial contamination can be transferred between various surfaces, or directly to a product being manufactured in a cleanroom. The proportion of microbes transferred from a donating to a receiving surface is the microbial surface transfer coefficient (MSTC) that is calculated using Equation 1.

Equation 1

$$\text{MSTC} = \frac{\text{Concentration of microbes donated to receiving surface}}{\text{Concentration of microbes on donating surface}}$$

The MSTC of materials used in hospital operating rooms has been investigated by Knobben *et al*[1], who showed that they varied according to (a) the type of microbes, (b) surface properties that included roughness and hydrophobicity, (c) whether the contact was dry or moist, and (d) whether the surfaces were rubbed together or not. Their experiments were carried out on surfaces that were artificially seeded with suspensions of different species of microbes. The transfer coefficients of contacts that are directly applicable to this investigation, i.e. simple dry contacts made between gloves, clothing, metal, and plastic surfaces, were found to vary from about 0.05 to 0.45.

Cleanrooms are supplied with filtered air and there should normally be no external source of airborne microbes. The main source of airborne microbes is the people in the room who disperse microbes carried on skin cells and, to a lesser extent, on particles of clothing fabric. These are known as microbe-carrying particles (MCPs). Macintosh *et al*[2] studied the dimensions of skin cells and skin fragments dispersed during activity of people. It was reported that the outer layer of skin is made up of cells that have a flake-like shape with a maximum length of about 44 μm, a minimum length of about 33 μm, and about 4 μm thick. These MCPs are dispersed into the air as microbes carried on whole skin cells, or on fragments of cells. The number of MCPs dispersed varies between individuals, their activity, and the type of clothing they wear, but is in the region of 1–230/s[3,4] and account for most, if not all, of the MCPs found in the air of cleanrooms.

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Due to their size, and the effect of gravity, MCPs easily deposit from the air onto surfaces. When deposited, they firmly adhere to surfaces, and are not removed by the air currents found within ventilated rooms, but are transferred by contact with surfaces such as gloves.

MCPs on surfaces in cleanrooms and operating theatres differ in size and shape from the unicellular microbes deposited from suspensions and studied by Knobben. Also, skin has a mixed microbial flora, and surface transfers are likely to involve a variety of microbial species. It would, therefore, be interesting to find if the transfer coefficients of MCPs gave similar results to those determined by Knobben. In addition, we wished to find the values of surface transfer coefficients in the following three situations to advance our research into the degree of risk associated with the various microbial sources in cleanrooms[5,6].

a. Contact between a gloved finger, and a hard surface such as stainless steel.
b. Contact between two hard surfaces, such as between stainless steel and stainless steel.
c. Contact between clothing, and a hard surface such as stainless steel.

Experimental methods

The overall approach to these experiments was to contaminate a donating surface with MCPs from human skin, and then press the surface onto a sterile receiving surface. The number of microbes on the donating and receiving surfaces was then determined and the transfer coefficient calculated. The transfer surfaces used, and the methods of measuring and calculating surface concentrations and transfer coefficients, are now described.

2.1 Donating and receiving surfaces

Metal surfaces

Two types of metal 'dabbers' were used as donating and receiving surfaces, and a photograph of these is shown in **Figure 1**.

(a) The two dabbers in the foreground of **Figure 1** were used to transfer microbes between a latex glove and stainless steel, and between two stainless steel surfaces, and were made from 316 L stainless steel. The contact area was a 2 cm x 2 cm square with a thickness of 3 mm, and the handle was 6.5 cm long. To obtain a smooth and flat contact surface, the dabber was manufactured in a lathe from a solid piece of metal. The contact face was then smoothed and electropolished. The surface roughness and flatness of the contact surfaces were measured by a Taylor Hobson TalySurf Form Intra 50 by scanning across the two diagonals in each dabber. This showed that the surface roughness, as measured by the Ra, gave an average distance between the peaks and valleys of the surface of about 0.15 μm.

The flatness across the 28 mm of the two diagonals of each dabber was also measured using the Talysurf and the result from one of the diagonals is shown in **Figure 2**; the other three diagonal profiles were very similar.

Figure 2 shows that the flatness of the contact surface fell away about 1.5 mm from the outer edge, and dropped by about 8 μm. However, as shown in **Figure 2**, the main part of the surface (about 80%) had a surface in which the peaks and valleys of the surface lay within boundaries of about 2 μm.

(b) For use with clothing fabric, a slightly larger and circular dabber was used, which was easier to wrap, tape, and present an unwrinkled fabric surface. This is shown in the background of **Figure 1**. It had a round stainless steel surface of 2.5 cm diameter that was 1 cm thick and attached to a handle 8 cm long. When the fabric was fitted to the dabber, the fabric's surface contact area was 5 cm^2.

The contact surfaces of the dabbers were placed, prior to the tests, in 70% isopropyl alcohol (IPA). When needed, a dabber was removed and, to finalise the sterilisation and ensure a completely dry surface, the 70% IPA was 'flamed off' and the dabber allowed to cool.

Gloved finger

The tip of the first finger of a fresh sterile glove was used as the donor surface. The gloves were powder-

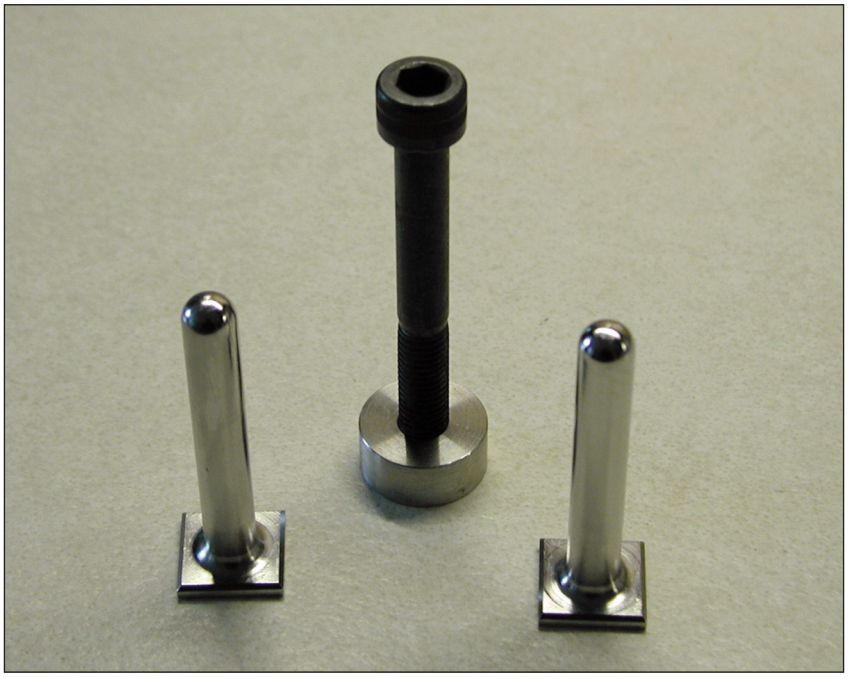

Figure 1. Dabbers used for transferring MCPs.

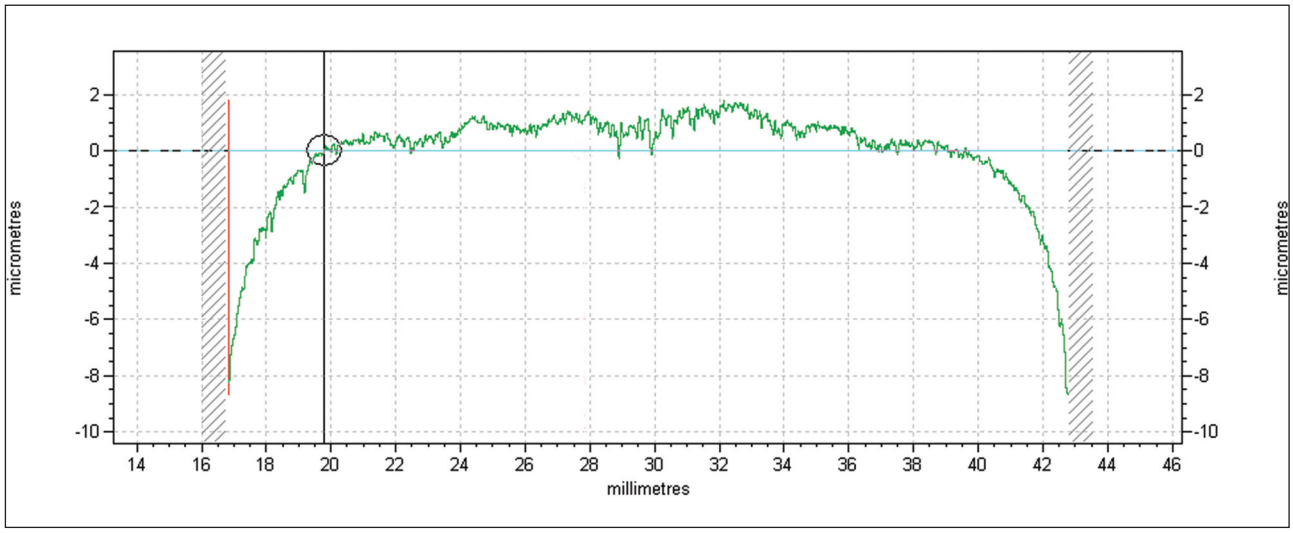

Figure 2. Surface flatness of a dabber.

free latex surgical gloves (Monlycke 'Biogel'). The roughness (Ra) of the finger of a glove was measured by the Talysurf Form Intra 50 and found to be 1.3 µm.

Cleanroom fabric

The cleanroom fabric was a woven Selguard polyester fabric used in the manufacture of cleanroom garments. The cleanroom fabric was sterilised prior to the start of the experiments by gamma radiation, and its sterility maintained throughout the tests by careful aseptic technique and storage in a sterile plastic bag.

2.2 Contact transfer method

To prepare the stainless steel dabber, latex glove, and clothing fabric surface, as a donating surface, they were contaminated with skin microbes by rubbing their surface 10 times over the face (cheek) of the person who carried out the experiments (WW). The contaminated surface was then pressed against the sterile receiving surface. It has been reported by Knobben *et al*[1] that a typical pressure when people touch a surface is 1 kg/cm^2. However, experiments carried out with a simple kitchen scale showed that a pressure of 1 kg was reasonable but when this pressure was transferred to a surface of 4 cm^2, a pressure of 250 g/cm^2 seemed more appropriate, and this pressure was used. The application time was 2 seconds.

When using a finger, it is not possible to reproduce an identical pressure on each experiment. However, variability occurs in normal situations in a cleanroom, and this experimental variability was considered acceptable. To provide confidence in the results, 10 tests were carried out on each type of surface contact.

2.3 Measuring the microbial concentration on surfaces

To calculate the MSTC, it was necessary to ascertain the concentration of microbes on both the donating and receiving surfaces. This was carried out by pressing these surfaces onto the surface of nutrient agar (Difco TSA) in a Petri dish and counting the microbial colonies after incubation.

If a surface was pressed once onto nutrient agar, the microbial count was too variable to be acceptable. To obtain an exact count, sequential pressings would have to continue until all the MCPs were deposited on the nutrient agar and a total count from all counts obtained; this would require a large number of nutrient agar plates and much tedious work in counting the microbial colonies. An alternative was investigated in which the two methods suggested by Whyte, Carson and Hambraeus[7] for obtaining the initial microbial count on a surface, were used. These employed either a two, or multiple-press, sequential sampling method. The former method did not give sufficiently accurate results, and the latter method could not be used

Table 1. MSTC between different surfaces.

Transfer type	Individual transfer coefficients	Mean	Standard deviation	Range
Glove to electropolished stainless steel	0.29, 0.11, 0.27, 0.24, 0.10, 0.14, 0.19, 0.18, 0.19, 0.23	0.19	0.06	0.10 to 0.29
Electroplated stainless steel to electropolished stainless steel	0.15, 0.10, 0.09, 0.10, 0.04, 0.06, 0.05, 0.14, 0.03, 0.19	0.10	0.05	0.03 to 0.19
Clothing to electropolished stainless steel	0.08, 0.14, 0.04, 0.06, 0.13, 0.10, 0.05, 0.02, 0.03, 0.03	0.06	0.04	0.02 to 0.14

when there were zero counts. However, it was found that a single 140 mm diameter nutrient agar plate could accommodate seven sequential pressings from both the donor and receiving surfaces (total of 14 pressings), while still leaving sufficient space between the contact areas to ensure the microbial colonies were attributed to the correct contact area. This method was used and a total count from the seven sequential counts from both donor and receiving surfaces was obtained, and the MSTC calculated.

All nutrient agar plates used to measure the microbes on the donating and receiving surfaces were incubated at 37ºC and the microbial colonies counted. The commonly accepted maximum concentration of microbial colonies on an agar plate that allows easy and accurate counting is 5/cm^2. However, the concentration of MCPs on the donor surface had to be higher to ensure suitably high counts were obtained on the receiving surfaces. The acceptable concentration on the donating surfaces was, therefore, increased to about 20/cm^2. To ensure that the colonies did not crowd each other out, a short incubation time of 24 hours was used, and the colonies viewed under good illumination with 2.5 magnification. Any concentrations above 25/cm^2 were discarded. The concentration was calculated from the 4 cm^2 stainless steel dabber surface, and with the fabric surface (5 cm^2) the count was adjusted proportionally downwards. Also, the area of a glove finger that made contact with the agar surface varied and, therefore, the microbial colonies within an area of 2 cm^2 was ascertained and recalculated for 4 cm^2.

From these donating and receiving surface counts, the MSTC was calculated as follows:

Equation 2

$$\text{MSTC} = \frac{\text{Number of microbes on receiving surface}}{\text{Number on donating surface + number remaining on receiving surface}}$$

Results

Shown in **Table 1** are the MSTCs from each individual test result, along with the mean, median, standard deviation and range of the counts from each type of surface contact.

The difference between the results from the three transfer types were analysed statistically by means of Tukey's pairwise comparison. This showed that, at a 95% confidence level, the glove-to-stainless steel transfer results were statistically different from the other two transfer results. However, no statistical difference was found between the results of the stainless steel-to-stainless steel transfers and the clothing fabric-to-stainless steel transfers.

Discussion and conclusions

The experiments described in this article were carried out to ascertain the proportion of microbes that were likely to be transferred from one surface to another in a cleanroom, i.e. the transfer coefficient. Knobben *et al*[1] had carried out experiments that showed the transfer coefficient was dependent on the type of bacteria, type of surface, whether surfaces were rubbed together, and whether the transfer occurred in moist or dry conditions. The experiments reported in this paper were confined to situations most likely to occur in a cleanroom, i.e. dry conditions with no rubbing. The transfers were between the following three surfaces (donating surface first): (a) latex gloves to stainless steel, (b) stainless steel to stainless steel, and (c) clothing to stainless steel. The experiments differed from Knobben's experiments in that microbe-carrying skin particles were used in place of suspensions of different species of bacteria.

To simulate the naturally-occurring MCPs found in occupied cleanrooms, the donor surface was rubbed on a person's face and pressed against a receiving surface. The microbial concentration on the donor and receiving surfaces were obtained by pressing the surfaces in a sequential manner onto nutrient agar surface, and ascertaining the total counts. The transfer coefficient was then calculated and the average values found to be as follows.

Gloves to stainless steel = 0.19
Stainless steel to stainless steel = 0.10
Clothing fabric to stainless steel = 0.06

Knobben *et al*[1] showed that during dry contact with no rubbing, the transfer coefficients of glove-to-stainless steel contact were between about 0.3 and 0.5, and in clothing-to-hard surfaces were between about 0.10 and 0.15. Our experiments, therefore, gave a transfer coefficient that was approximately half the value obtained by Knobben. No stainless steel-to-stainless steel experiments were reported by Knobben.

It was expected that the type of surface would influence the transfer coefficient found in the present experiments and this appears to be correct. The transfer coefficient between latex gloves and metal had the highest value, and it was assumed that this was caused by the softness of the latex glove allowing good contact with the stainless steel surface. The clothing fabric gave the lowest transfer coefficient. The reason for this is likely to be caused by the fabric being woven from threads of polyester and, as the threads are circular, it would be expected that only a small surface area would make contact with the stainless steel surface.

Hard surface-to-hard surface contacts between different surfaces were likely to give a variation in their transfer coefficient that would be related to the smoothness and overall flatness of the surface. The surface of the stainless steel used in the experiments was smooth with a Ra of about 0.15 μm. The deviation from perfect flatness across about 80% of the surface was shown to vary from the highest to lowest point by no

more than 2 μm. However, if two of these stainless steel surfaces were pressed together, there would be spaces between the two surfaces that could be 4 μm apart. If the particles were smaller than this size, as would occur if a microbe was present in a unicellular form and, therefore, in the size range of between about 0.5 μm to 2 μm, the microbe is less likely to be contacted and transferred. However, MCPs carried on skin cells, fragments of skin cells, or clothing fragments, could be transferred, depending on their size and position on the surfaces. Although it is not possible to predict from the information gathered, what proportion of the MCPs will be transferred, it is clear that surfaces that are rough and uneven are more likely to give low transfer coefficients.

Transfer experiments were carried out using MCP concentrations in the region of 5 to 20/cm^2, which is higher than the concentrations found on surfaces in cleanrooms[4], which might range from practically sterile to about 0.001/cm^2. If the experimental contact surfaces were so dirty that microbes and skin particles were piled on top of each other, or were close enough to influence the transfer, the transfer coefficient might differ from when contamination was sparse. However, the concentration of MCPs found on the donating surface, as determined by contact with nutrient agar, was not greater than about 25/cm^2 and this appeared too low to have an influence on the transfer coefficient. Also, a microscopic investigation of a slide drawn over the face in the same manner as the contact experiments showed that skin cells were present at a low concentration, and relatively difficult to find on the slide and a distance apart that was unlikely to influence the surface transfer.

Although the transfer coefficients found in these experiments were likely to be similar to those found in cleanrooms, to obtain an accurate transfer coefficient it would be necessary to carry out experiments of the type reported in this article. However, consideration of the experimental results, the differences between the results, and the variables that affect the magnitude of the transfer coefficients suggests that a value of 20% could be used as a general transfer coefficient for the types of transfers found in the situations studied in this article.

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