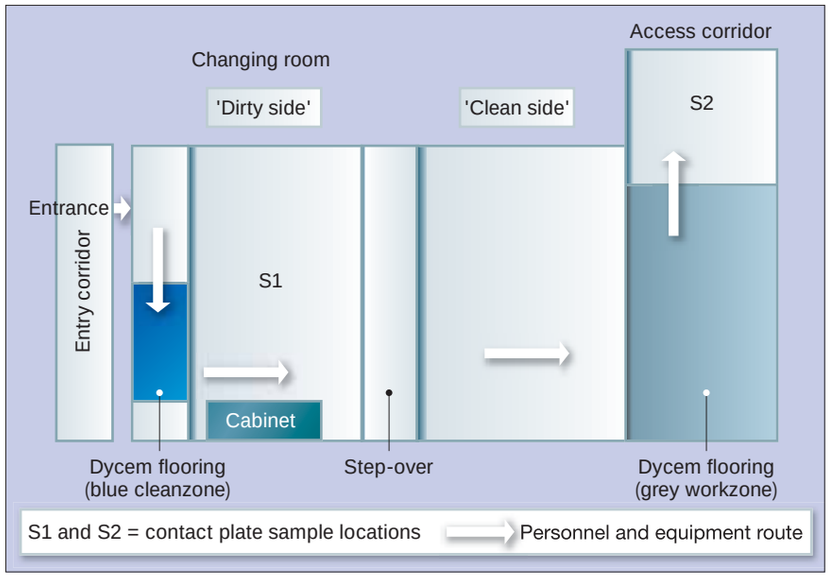
Microbiologist Tim Sandle presents a recent study on the use of polymeric flooring in reducing contamination in cleanrooms and hospital changing areas

The transfer of people and trolleys is major contamination source in hospitals

A final floor show for bugs



The reduction of contamination from personnel and movable objects is a key requirement for any healthcare facility. Such contamination consists of viable (bacteria and fungi) and non-viable particles (dirt and debris); the former is particularly important when reducing the risk of infection acquired in a hospital.

The main source of contamination is the transfer of people within a facility. A second, – and equally problematic – source of risk is from trolley wheels and truck traffic.[1]

The association of micro-organisms and dust particles has long been documented and dates back to the practices of coating hospital floors with oil to attract particles and thereby minimise bacterial transfer.[2]

It is on this same basis that flooring can be used to attract dirt and dust, capturing micro-organisms and preventing them from being transmitted to a new location, or disseminated into the air when the surface is disrupted by walking across it, moving equipment or cleaning it.

This study concerns a cleanroom changing facility where there is a high personnel throughput, making it prone to the transfer of contamination due to air disturbance. It involved examining the counts on surfaces prior to entering the changing room and from the corridor leading out of the changing room. A variable was introduced whereby the cleanroom floor was fitted with polymeric flooring (Clean-Zone and Work Zone*). The counts pre- and post- the fitting of the polymeric flooring were compared using a statistical test for significance.

Micro-organisms will move around a clean area depending upon the design of the facility and the effectiveness of HVAC operational parameters, such as the number of air-changes per hour. The dispersal of micro-organisms in non-laminar flow zones occurs relatively easily.[3] The transportation of a microbial cell to a surface is either by direct physical contact or as a result of gravity, convection or diffusion.[4] Once contact has been made with a surface the microbial cell can adhere to the surface either reversibly or irreversibly through a combination of chemical or electrostatic forces. Irreversible attachment is more commonly associated with water systems in relation to the ▶

Table 1: Summary of descriptive statistics

Measure	Pre-Dycem Flooring	Post-Dycem Flooring
Mean count	26.09 CFU	4.73 CFU
Median count	21 CFU	4 CFU
Modal count	23 CFU	0 CFU
Standard deviation	20.23	4.01
Range	0 – 89	0 - 17

Table 2: Student's t-test result:

Hypothesized Mean Difference	=	0
t-statistic	=	10.4138
One-sided significance level	=	0.0000
Two-sided significance level	=	0.0000
PRE: Mean = 26.099	SD =	20.2255
POST: Mean = 4.73267	SD =	4.01221
Difference of the Means	=	21.3663
Std. Error of the Difference	=	2.05173
Approximate 95% C.I.		[17.3205, 25.4122]
Pooled SD	=	14.5803
Degrees of Freedom	=	200

◀secretion of glycocalyx and the formation of biofilm communities[5] and is outside the scope of this paper.

Contamination prevention

Various studies have shown that transfer of contamination by people walking across floors has one of the highest re-dispersal factors.[6] There are four possible approaches to minimising this contamination risk:

- preventing contamination from entering the critical area in the first place
- the physical removal of contamination
- the reduction of viability
- its destruction.[9]

This paper is concerned with the first of these. The removal of such contaminants prior to entering a critical area is an important step in a risk-based contamination control programme.[10] The use of polymeric flooring, such as Clean-Zone and Work-Zone mats, can be effectively used at key points in a HACCP or FMEA process. The mats will reduce the soiling of footwear and thus minimise the entry of contaminants into the cleanrooms.

Polymeric flooring is manufactured from a non-toxic, plasticised material and is designed to retain particulate contamination (viable and non-viable) that comes into contact with its surface. A function of polymeric flooring is to attract particles to its surface and retain them for long periods of time (until such a time when they can be removed, totally, through cleaning and disinfection).[11]

Of central concern to this study was the effect of reversible adhesion of viable micro-organisms to cleanroom surfaces. The area studied was the main, first stage changing area in a biopharmaceutical manufacturing facility based in the south-east of England (EU GMP Grade C), which is part of the UK's National Blood Service operating within the National Health Service. The changing room studied led to a second, final stage changing room via an access corridor. The concern was to identify a mechanism to reduce the number of contaminants carried by the personnel, who exited from the first change area, on their footwear, thereby lowering the level of contamination entering the second stage changing area.

The changing area is entered by up to 30 personnel per shift. The personnel enter wearing outdoor clothing, then change into cleanroom garments, put on captive shoes and then exit through an access corridor into the final change area. The aim of the study was to determine if the fitting of polymeric flooring reduced the number of contaminants carried on the footwear of staff as they moved from one change area to another.

The study involved sampling the following conditions within the changing area:

A) Prior to the fitting polymeric flooring

- Samples of the entry area into the changing room
- Samples in the access corridor after exiting from the changing room (on standard flooring)

B) After the fitting polymeric flooring

- Samples of the entry area into the changing room
- Samples exiting in the access corridor after exiting from the changing room (post-polymeric flooring)

One hundred samples were taken from each condition in two locations (800 samples in total). The samples were taken over approximately a three-month period in the same locations according to a sampling plan. The locations selected were areas of high personnel transit, near the entrance and at the approximate centre of the area. The samples were taken at varying times during the working day to allow a variety of different sampling times to be studied, although no time was considered to be 'worst case'. The area of polymeric flooring fitted was 1.2m x 4m and the flooring was cleaned using an approved detergent (Dygiene plus) once a day. The polymeric flooring was secured to the floor.

The results of each condition were statistically examined. The method for analysis selected was Student's unpaired t-test for significance. An unpaired sample t-test was used to determine whether there was a significant difference between the mean values of the different measurements made under two different conditions. Therefore this approach involved comparing the mean count for each sample, rather than the individual results. It is acknowledged that individual sample results can be variable.

The unpaired t-test was selected due to the introduction of a variable (the polymeric flooring). The t-test method was chosen so that the general difference between the different conditions could be analysed. The null hypothesis was: "That the fitting of the polymeric flooring would not result in a significant difference to the mean count.[11] The study set out to disprove this hypothesis.

The micro-organisms detected were identified for some plates. This was for

Polymeric flooring will trap particulate contamination and prevent its transfer elsewhere

informational purposes only, although a check was made to determine if the micro-organisms were typical of cleanroom flora.

Samples were taken using contact plates containing tryptone soya agar (TSA, equivalent to soya-bean case in digest medium). The TSA also contained a disinfectant neutraliser effective against a wide spectrum of biocides. The contact plates were Petri-dishes filled with micro-biological agar. The plate is filled to a level above the rim of the plate so that the agar surface extends upwards when dry. The plate has a typical diameter of 50-55mm and a surface area of 25cm^2. The raised surface allows the agar to be pressed onto a surface. The design of the contact plate is therefore different from the standard Petri-dish, where the agar is contained within the Petri-dish. The Petri-dishes were subjected to a standard micro-biological incubation regime: 30-35°C for up to three days followed by 20-25°C for up to six days.

Microbial count

The contact plate is a quantifiable method, because the contact between the plate and the surface provides a "mirror image" of the surface. Following incubation, this image transfer provides information relating to the number of microbial colonies and their relative position. The quantification is derived from recording the number of colony forming units (cfu) per square centimetre.

The repeatability and reproducibility of contact plates is generally superior to that of other surface sampling techniques, such as swabs.[12,13] The counts obtained on TSA contact plates are not the absolute numbers present, but represent those culturable micro-organisms that will grow under the defined temperature and time conditions.

A summary of the results for both test conditions is shown in table 1. The data indicates that the measures of average were different, with the post-polymeric flooring showing an 80% decrease in the microbial counts. The range of the data indicates a greater spread for the pre-polymeric flooring counts, as noted by the standard deviation. The fitting of the polymeric flooring has reduced the count. The next step was to determine the significance of this.

Samples were analysed using a statistical software package (NWA Quality Analyst version 5.1) for Student's t-test. The unpaired t-test was used for the comparison of each condition. The results are shown in table 2.

At a level of significance of p=0.05, the value of the t-distribution table, for a one sided level of significance, is 1.671. This is less than the calculated value of t (10.41). Therefore the null hypothesis can be rejected and there was a significant difference between the results of the two types of contact plate. This difference affirms the reduction seen in the mean microbial count of approximately 80%.

Table 3: Micro-organisms recovered

Taxanomic Group	Percentage
Gram-positive cocci	75%
Gram-positive non-sporing rod	10%
Gram-positive sporing rod	8%
Gram-negative rod	4%
Fungi	3%

A review of the main types of micro-organisms recovered inidcated *Staphylococcus epidermidis, Micrococcus luteus, Bacillus spp., Staphylococcus capitis* and *Micrococcus lyale*. These species represented >90% of all isolates and are typical microflora from footwear from a first stage clean area, based on an historical examination of microflora (see table 3).

The study demonstrated that the fitting of polymeric flooring reduced the microbial counts on the footwear of staff moving between one cleanroom and another. This reduced the potential for the transfer of contamination out of the cleanroom and into process areas. The brief review of the detected microflora has indicated that they were typical to a standard cleanroom with no water present. Therefore, the results maybe applicable to other cleanroom and hospital environments where standard microbial flora and similar ranges of microbial count occur.

Strategic locations

The use of polymeric flooring could be applied to airlocks and other cleanroom interfaces, and a study such as this one could be replicated by other cleanroom users wishing to achieve a level of contamination control. The use of such flooring can be located at strategic locations through the use of a risk assessment tool (such as HACCP).

There are alternatives to the fitting of polymeric flooring. These could include revisions to cleaning and disinfection regimes; restriction of staff numbers accessing a room; a change to the type of cleanroom clothing and a review of other equipment entering the area.

However, some of these other approaches, like increasing the frequency of disinfection, are both labour intensive and increases the risk of human error through inadequate techniques.

Additional studies looking at the use of polymeric flooring could include mechanisms for the cleaning of the flooring using detergents and mechanical aids; the frequency and time of cleaning in relation to degree of personnel transit and the most effective length of flooring in relation to the number of footsteps required to reduce microbial contaminants. ■

Mats can be effective in a HACCP plan

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Footnote

* Work-Zone and Clean-Zone are branded proprietary products manufactured by Dycem Limited, Bristol, UK

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