Examination of air and surface particulate levels from cleanroom mats and polymeric flooring

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This paper describes a study undertaken in a biopharmaceutical manufacturing facility, which examined particle levels from the footwear of personnel entering a cleanroom and after stepping onto a cleanroom mat. The study compared six adhesive cleanroom mats and polymeric flooring and considered the change in the number of particles on footwear (uncovered shoes and shoes covered with an overshoe) before and after personnel had traversed cleanroom flooring. From this comparison, the level of reduction was greatest from the footwear of staff who had walked across the polymeric flooring. The study also assessed the level of particles produced when the top layer of a cleanroom mat was removed, and these data are presented for information purposes.

Key words: Contamination control, cleanroom mat, polymeric flooring, cleanroom, particle counts.

Introduction

The control of materials and personnel into and out of cleanrooms and other controlled areas is an important part of contamination control. The main source of contamination in cleanrooms is from people and this risk is increased by the transfer of people within a facility1. A second, and equally problematic source of risk is from trolley wheels and truck traffic2. Both activities can release particles, which will be deposited into the air-stream, and some of which will settle onto surfaces depending upon air distribution3. For a cleanroom to function properly, particle levels need to be controlled below the classification of the area. Cleanrooms and clean zones in critical environments are typically classified according to their use (the main activity within each room or zone), controlled through the physical operation of heating, ventilation and air conditioning (HVAC), with the classification confirmed by the measurement of the airborne particle concentration4.

The dispersal of particles, including microorganisms, in turbulent flow clean zones occurs relatively easily5. Eventually any particles present will be either removed from a clean area, through the function of the air-handling system, or deposited onto a surface as a result of gravity, convection or diffusion. Once contact has been made with a surface particles will adhere to the surface either “reversibly” (i.e. temporarily) or “irreversibly” (i.e. permanently) through a combination of chemical or electrostatic forces6. For contamination control, reversible attachment is a concern. Particles on the floor that are not completely contained or trapped onto a surface will eventually, through air movement and vortices, become re-suspended into the air. Previous studies have shown that transfer of contamination by people walking across floors has one of the highest re-dispersal factors7.

One of the measures commonly employed to reduce the level of contamination entering cleanrooms through the transit of materials and people is the use of special flooring. Such flooring, which is normally in the form of temporary or permanent mats, is designed to remove a number of visible and sub-visible particulates and, therefore, reduce the likelihood of the transfer of contamination and thus minimise the associated risk of re-dispersal. Given that the interfaces between classified cleanrooms or changing rooms and unclassified areas are potentially vulnerable areas, cleanroom mats are commonly situated in these areas8. Environments like changing rooms, areas where there is a high personnel presence and throughput, are arguably more prone to the transfer of contamination due to air disturbance. The reduction of contamination within changing rooms is of importance in terms of reducing any potential contamination that may be carried into a processing area9.

Special flooring designed to control contamination in cleanrooms commonly takes one of two forms: adhesive mats or polymeric flooring. Given the range of adhesive-based, peel-off disposable flooring produced by different companies, it is likely that the adhesive capabilities, and hence the ability of the flooring to reduce the number of particle carried on footwear, will vary. There have been few published studies into the effectiveness of cleanroom flooring, despite the long
history and widespread use of adhesive mats. The most comprehensive study conducted by Whyte et al. identified that the removal efficiency of particles by cleanroom mats was generally found to be greater where the mat was of a softer type, the particles smaller, the particle size distribution more homogeneous, the distance between the particles greater, and the adhesive strength of the mat surface greater. The number of steps taken on a mat was also shown to be a factor for particle removal, with up to four steps required. A further variable will be the weight that each operator places on each foot as it is pressed onto the mat. The type and design of the shoe worn can also be a factor, particularly smooth-soled shoes compared with shoes with ridges.

The ability of polymeric flooring to reduce the numbers of microorganisms carried on footwear has previously been studied, with one study showing that an 80% reduction in microbial contamination can be achieved. Earlier research has suggested that polymeric flooring removes more particles than a conventional cleanroom mat. In contrast to the microbiological examination, there have been no major studies into the particulate removal of the polymeric flooring. The study described in this paper is an attempt to redress this.

**Types of cleanroom mat**

Adhesive (or “tacky”) mats consist of layers (normally 30–60cm) of polymeric sheet or film, each coated with an adhesive, which means that when walked across the mat is “sticky” when the foot comes into contact with the mat surface (often acrylic adhesives are used, layered onto polyethylene film). The mats are intended to be disposable and, after a period of use, possibly once per day or per shift although practices may vary between organisations, the top layer is removed from the stack attached to the floor and discarded. To facilitate the removal of the top layer most brands of mat have a tapered end. The removal of this top layer generates a level of airborne particles as particles are dislodged from the surface of the mat.

Polymeric flooring consists of a polymeric surface manufactured from a non-toxic, plasticised material. The polymeric molecules decrease the surface resistivity of the material and are deposited onto a non-conductive substrate surface. The mat remains permanently tacky. The flooring is designed to retain particulate contamination (viable and non-viable) that comes into contact with its surface, and electrostatic forces bind particles to the surface. The 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 through cleaning and disinfection).

**Experimental design**

With consideration of the concern for minimising particle levels in cleanrooms and in light of the little information regarding the efficiencies of different types of mats to reduce particle levels, a study was set up to compare different types of cleanroom mats alongside polymeric flooring.

The study set out to compare several variables.

(a) What level of particles is typically found on cleanroom shoes and shoes covered with overshoes?
(b) What level of particles is captured by cleanroom mats?
(c) What level of particles typically remain on shoes and overshoes after a person has stepped onto and then off the cleanroom mat?

With (b) and (c) above, consideration was given to an individual stepping onto a portion of a mat previously untouched and onto an area of a mat which had previously been walked on (an “overstrike”). It was recognised when designing this part of the study that, in practice, an individual crossing a cleanroom mat will step onto the mat more than once and that particle removal will be greater, up to some saturation point, the more steps an individual takes on the surface of mat. For reasons of study time, and in relation to some observed practices where some cleanroom operators do not take many steps along the surface of a mat, the study was designed to investigate when one imprint was made onto a mat’s surface.

Given that the layers of mats are generally changed each working day whereas polymeric flooring can remain in place for longer periods, a new piece of polymeric flooring was compared with a piece that had been in place for 2 years (and subjected to a daily detergent clean). The adhesive mats consist of layers and, in order for the mat to continue to be effective, a layer needs to be removed periodically, therefore, the study also set out to examine the following.

(d) How many particles are released into the air when a layer of an adhesive cleanroom mat is removed?

This was examined to see if mats which are visibly different in terms of how dirty they appear produce different levels of particles. This part of the study was not applicable to the polymeric flooring because layers are not removed (instead the flooring is normally subjected to a daily detergent clean).

With this examination of airborne particle counts, sub-variables were also considered.

(i) To what extent do the particle levels vary if the mat is moderately dirty (10 footprints) compared with a mat that is very dirty (20 footprints)?
(ii) To what extent do the particle levels vary when the top layer of mat is peeled off slowly or rapidly?

For the study, 10 people were used for stepping onto the mats. This was to allow the data to be replicated. For the analysis, the mean particles from shoes, overshoes or footsteps on the mats of the 10 people were analysed.

**Methods**

The study was undertaken in an EU GMP Grade C (ISO 14644 Class 8 in-operational state) cleanroom. The study was conducted between November 2011 and January 2012. In the cleanroom, cleanroom mats from six
different manufacturers and polymeric flooring (Dycem Clean-Zone) were individually examined. New (not previously used) and old (2-year-old) polymeric flooring was examined.

Between assessments of each mat and between surface and air studies, the cleanroom was allowed to “rest” for 20 minutes to ensure that particulate build-up did not occur.

For the surface assessments (Analysis 1 and 2), 10 different staff were used for each mat in order to generate 10 footsteps. This was undertaken for both uncovered shoes (shoes of a smooth-soled design, captive to cleanrooms) and shoes covered with a plastic overshoe. The step onto the mat was undertaken at normal walking pace, that is, the foot had contact with the mat for about 2 seconds. For consistency, the right foot was used for each surface particle reading. For this study, 960 surface particle count measurements were taken, as shown in Table 1.

For the airborne particle assessments, the number of particles released into the room (within the range of the counter) was measured. The counter was placed on a trolley, positioned approximately 2 metres from the floor and approximately 1 metre from the mat. For each activity, the counter was started 5 seconds prior to the mat being peeled and run so that a 5-minute sample was taken. The counts recorded were converted to counts per cubic metre.

The counter was located in the same position for each mat and for each removal activity. It is unknown how many of the particles released were within the range of the counter, although the proximal location of the counter was relatively close to the mat and provided an indication of particle generation within the cleanroom. Given that the same location was used for each mat, the results are considered to be comparative.

For the mat removal activity, the following conditions were assessed:

1. Clean mat, slow peel;
2. Clean mat, fast peel;
3. Semi-dirty mat, slow peel;
4. Semi-dirty mat, fast peel;
5. Dirty mat, slow peel;
6. Dirty mat, fast peel.

The clean mat (conditions 1 and 2) was a mat that had not been stepped on. For the semi-dirty conditions, mats were used with 10 footprints from uncovered shoes on them. The dirty mats had 20 footprints on them. For the mat removal, slow peel and fast peel were qualitatively assessed. A degree of consistency was added by using the same person to peel the mat in each instance. The fast peel was intended to be representative of how a layer would be removed in practice, whereas the slow peel was intended to capture the removal of a layer at about half the speed that would normally be undertaken in practice.

Slow peeling involved holding the tab on the corner of the mat and folding it towards the centre. Following this, the opposite corner was folded towards the centre, and the procedure was repeated until the top layer was removed. For the fast peeling, the outer layer was ripped off from the layer below and crumpled up. For both paces of removal, the layer was folded and placed into a cleanroom waste bin. In practice, some layers were easier to remove from

<table>
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<tbody>
<tr>
<td>Six adhesive mats</td>
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<td>6 x 10 measurements (n=60)</td>
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some brands of mat than others and there were noticeable variations in the “stickiness”. For six conditions and with six adhesive mats, 36 samples were taken.

The particles were assessed using a Lighthouse Solair 3100+ particle counter (calibrated against a traceable international standard). The counter functioned by detecting the effect of particles scattering light as they passed through a laser diode. The counter was set to measure cumulative particles of different sizes. The cumulative particle sizes of most interest within pharmaceutical and healthcare facilities are those ≥0.5µm and ≥5.0µm and the counter was set to measure these sizes.

The counter was fitted with a surface particle probe to measure particles from footwear and from the surface of cleanroom mats and flooring. Between each measurement, the probe was cleaned using a 70% isopropanol cleanroom wipe. For the surface measurements, a 10-second sample was taken. For airborne particle counting, the counter was fitted with an isokinetic probe. The particles counted were converted to particles per cubic metre by the instrument software to enable comparison to the particle limits for the cleanroom class.

Results

The results analysis is divided into three sections: the level of surface particles measured from shoes and overshoes before walking across a mat and afterwards (Analysis 1); the level of surface particles remaining on a mat after it had been walked across (Analysis 2); and the level of particles generated when a mat is peeled (Analysis 3). The ≥0.5µm particle data is presented in graphical form.

Analysis 1: surface particle count measurements from shoes and overshoes

For the first part of the study, the level of surface particles remaining on shoes and overshoes was examined before and after personnel had walked across each type of mat.

Figure 1 displays the results of the analysis of particle measurements from shoes before an individual has walked across a mat and then afterwards. The graph displays the mean result for each of the 10 right foot shoes measured from the 10 personnel for each mat type. The adhesive mat types are coded 1 to 6.

Figure 1 indicates that the level of particles from the shoes reduced after the individual had stepped onto the mat. The graph indicates that a greater reduction was seen for the polymeric flooring compared with the six adhesive mats. The average level of surface particles from shoes was 2690 particles. The reduction in particles for the shoes in relation to each mat is shown in Table 2. Table 2 indicates that the adhesive mats gave a reduction in particles between 20% and 52%, whereas far larger reductions in particle levels from shoes were shown from the polymeric flooring, with the previously unused flooring producing the greatest reduction at 85%.

Figure 2 displays the results of the analysis of particle measurements from overshoes before an individual walked across a mat and then afterwards. The graph displays the mean result for each of the 10 right foot overshoes measured from the 10 personnel for each mat type.

Figure 1. Surface particle counts (≥0.5µm) from shoes measured before walking across a mat and afterwards.
Figure 2 indicates that the level of particles from the overshoes reduced after the individual had stepped onto the mat. The levels of particles, as would be expected from overshoes, was lower than those recorded from uncovered shoes. The graph indicates that a greater reduction was seen for the polymeric flooring compared with the six adhesive mats.

The average level of surface particles from overshoes was 451 particles. The reduction in particles for the overshoes in relation to each mat is shown in Table 3.

Table 3 indicates that the adhesive mats gave a reduction in particles between 14% and 45%. This was a lower reduction than those seen from shoes, although the starting level of particles was far lower. Nonetheless, the reduction in particle levels achieved by the polymeric flooring remained at similar levels for the overshoes compared with the uncovered shoes (as indicated in Table 2). Of the different conditions of polymeric flooring, the new flooring gave a marginally higher reduction of particles.

Figure 3 displays the results of the analysis of particle measurements from shoes before an individual walked across a mat and then afterwards. The difference with these data compared with the data presented in Figure 1 is that each time an individual stepped onto the mat they did so in the same location, thereby creating an “overstrike”. The graph displays the mean result for each of the 10 right foot shoes measured from the 10 personnel for each mat type.

Figure 3 indicates that the level of particles from the shoes measured before an individual walked across the mat increased after the individual had stepped onto the adhesive mats and decreased after stepping on the polymeric flooring. The data for the adhesive mats thereby showed a reversal of the trend seen in Figure 1, where individuals stepped onto previously untouched areas of the mat. The inference here is that overstrikes lead to more particles being deposited onto shoes than are actually removed from shoes.

The average level of surface particles from shoes was slightly higher than previously measured at 4147 particles.
The change in the level of particles for the shoes in relation to each mat is shown in Table 4. Table 4 indicates that the adhesive mats led to an increase in particles between 11% and 482%, whereas significant reductions in particle levels from shoes was achieved by the polymeric flooring, with reductions of more than 80% observed (and with the new flooring producing the greatest reduction of all).

Figure 4 displays the results of the analysis of particle measurements from overshoes before an individual walked across a mat and then afterwards. The difference with these data compared with the data presented in Figure 3 indicates the change in the level of particles for the shoes in relation to each mat is shown in Table 4. Table 4 shows that the adhesive mats led to an increase in particles between 11% and 482%, whereas significant reductions in particle levels from shoes was achieved by the polymeric flooring, with reductions of more than 80% observed (and with the new flooring producing the greatest reduction of all).

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across the mat. The analysis assessed uncovered shoes and overshoes. Figure 5 displays the data from the examination of each type of mat after 10 personnel had walked across the mat in uncovered shoes. This was in relation to the overstrike activity (in relation to Figures 3 and 4) above, as this was considered to be the worst case. Due to the variation in particle retention, the counts were converted to logarithms at base 10.

Figure 5 indicates that the polymeric flooring retained a 2 to 3 log10 greater number of particles compared with the adhesive mats. The mean particle count retention by the six adhesive mats was 1105 particles of 0.5µm size, whereas the polymeric flooring retained a mean of 87,300 particles. There was little difference between the new and old polymeric flooring.

Analysis 2: surface particle retention by mats

The second part of the study examined the level of 0.5µm particles retained by each mat after 10 people had walked across the mat. The analysis assessed uncovered shoes and overshoes. Figure 5 displays the data from the examination of each type of mat after 10 personnel had walked across the mat in uncovered shoes. This was in relation to the overstrike activity (in relation to Figures 3 and 4) above, as this was considered to be the worst case. Due to the variation in particle retention, the counts were converted to logarithms at base 10.

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Figure 6 displays the examination of each type of mat after the 10 personnel had walked across the mat wearing overshoes. This was similarly in relation to the overstrike activity and the particle counts displayed have been increased. The average level of surface particles from overshoes was slightly higher than previously measured at 533 particles. The change in the level of particles for the overshoes in relation to each mat is shown in Table 5. Table 5 indicates that the adhesive mats led to an increase in particles of between 10% and 381%, whereas, in contrast, significant reductions in particle levels from overshoes occurred with the polymeric flooring, with reductions of more than 85% being achieved. For this part of the study, the in-use (older) flooring producing the greatest reduction of particle levels at 90%.

### Table 4. Percentage reduction of particles from overshoes after individuals had walked across the cleanroom mat (where footprints are overstrikes).

<table>
<thead>
<tr>
<th>Mat type</th>
<th>Percentage reduction</th>
</tr>
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<tbody>
<tr>
<td>Adhesive 1</td>
<td>Increase: 11.1%</td>
</tr>
<tr>
<td>Adhesive 2</td>
<td>Increase: 18.3%</td>
</tr>
<tr>
<td>Adhesive 3</td>
<td>Increase: 173.1%</td>
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<tr>
<td>Adhesive 4</td>
<td>Increase: 101.9%</td>
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<tr>
<td>Adhesive 5</td>
<td>Increase: 263.2%</td>
</tr>
<tr>
<td>Adhesive 6</td>
<td>Increase: 482.3%</td>
</tr>
<tr>
<td>Polymeric old</td>
<td>Decrease: 82.9%</td>
</tr>
<tr>
<td>Polymeric new</td>
<td>Decrease: 83.5%</td>
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<tr>
<td>Adhesive 1</td>
<td>Increase: 9.6%</td>
</tr>
<tr>
<td>Adhesive 2</td>
<td>Increase: 76.3%</td>
</tr>
<tr>
<td>Adhesive 3</td>
<td>Increase: 85.8%</td>
</tr>
<tr>
<td>Adhesive 4</td>
<td>Increase: 81.3%</td>
</tr>
<tr>
<td>Adhesive 5</td>
<td>Increase: 381.9%</td>
</tr>
<tr>
<td>Adhesive 6</td>
<td>Increase: 108.8%</td>
</tr>
<tr>
<td>Polymeric old</td>
<td>Decrease: 90.3%</td>
</tr>
<tr>
<td>Polymeric new</td>
<td>Decrease: 86.9%</td>
</tr>
</tbody>
</table>

Figure 5. Surface particle (0.5µm) retention by flooring type with uncovered shoes.
converted to logarithms at base 10. Figure 6 indicates that the polymeric flooring retained a 1 to 2 log10 greater number of particles compared with the adhesive mats. This was a smaller difference than seen for the uncovered shoes (Figure 5), although the level of particles from the overshoes was itself lower.

The mean particle count retention by the six adhesive mats was 573 particles of 0.5µm size, whereas the polymeric flooring retained a mean of 44,200 particles. In relation to the two conditions of polymeric flooring, there was a difference between the new and in-use material, with the new flooring retaining a higher number of surface particles.

Analysis 3: airborne particle count generation from mat removal

The third part of the study examined the level of particles released from removing the top layer from each adhesive mat. Airborne particles were measured and the results were converted to counts per cubic metre of air. Before the
mats were peeled the background level of particles was assessed. The figures displayed represent the airborne particles after mats were peeled less the standard room particle count.

Different conditions were examined for each mat. These were:

1. Clean mat, slow peel;
2. Clean mat, fast peel;
3. Semi-dirty mat, slow peel;
4. Semi-dirty mat, fast peel;
5. Dirty mat, slow peel;
6. Dirty mat, fast peel.

For this analysis, both 0.5µm and 5.0µm size particles were examined.

Figure 7 displays the airborne 0.5µm particle generation from the removal of the top layer from each of the six mats under the six different conditions. Figure 8 shows the airborne 5.0µm particle generation from the removal of the top layer from each of the six mats under the six different conditions. Figures 7 and 8 indicate that the level of particles increase in relation to the both the degree of dirt on the mat, that is, the clean mat generates fewer particles than the semi-dirty mat, and the semi-dirty mat generates fewer particles than the dirty mat. In addition, the levels of particles also rise if the mat is peeled away quickly compared with the mat being peeled away slowly.

**Discussion**

The study demonstrated, from Analysis 2, that the levels of particles retained by the polymeric flooring were highest and levels of particles remaining on footwear (whether overshoes were worn or not) were fewer when compared with the range of different adhesive mats. The same data pattern was shown, with Analysis 1, when uncovered shoes and overshoes were measured, that the particle counts from shoes and shoes covered with overshoes after individuals had stepped onto adhesive mats were greater, indicating that fewer particles had been removed, than the levels of particles measured after individuals had stepped onto the polymeric flooring. Therefore, on the basis of this study, polymeric flooring is superior to an adhesive mat for the removal of surface particles. These findings suggest that the potential for the transfer of contamination into process areas can be lessened if polymeric flooring is used in place of adhesive mats.

A possible risk with the use of disposable adhesive cleanroom mats was shown through the removal of the outer layer of the mats (Analysis 3). The data showed that the act of peeling an adhesive mat generates a relatively high number of airborne particles and this number of particles is highest when the mat has been used on several occasions (visibly dirty, in this case from 20 footprints). The level of airborne particles increased further when the top surface of the mat was peeled quickly. For some cleanrooms, using adhesive mats for contamination control may be unsuitable due to the act of unpeeling the mat. This will relate to the cleanroom grade or class, and the level of particles generated under the dirtiest condition (mean count of 125,000 at ≥0.5µm) indicates that such mats are unsuited to the highest grade clean areas.

Although the particle levels varied according to the mat (and this would have additionally related to the uncontrolled variable of the level of dirt on each of the shoes worn by the personnel walking across the mats), the
levels were sufficiently high from either a “slow peel” or a “fast peel” to indicate that many of the trapped particles were likely to become re-suspended in the air and thus have the potential for re-contaminating more critical surfaces or being deposited onto personnel clothing. Although the fast peel activity, when the mat was dirty, gave the highest particle count, it was not much higher than that from the slow peel suggesting that the removal of a used top layer at the end of a shift will produce an elevated level of particles. It was also noteworthy that there were particles released from unused mats suggesting that some commercially available mats are not suitable for higher grade cleanrooms.

The findings in this study should be regarded as general trends. There were a number of variations which will affect the levels of particles recorded, although the direction of the trends and the differences observed between the adhesive mats and polymeric flooring is unlikely to alter. These variables include the differences between personnel behaviour, particularly how hard or soft people step onto the mat, and, with the overstrides, exactly how much of each footstep covered a previous footstep. A second variable is the amount of dirt on each shoe and overshoe. This was impossible to quantify and thus the levels of particles monitored will reflect the amount of dirt deposited. The use of an artificial substance, such as talc, could perhaps lend a greater degree of consistency for simulating ‘dirt’ should this study be replicated. However, when assessing the particle dispersion into the air, an artificial substance may not behave in the same way as the dust and debris that would adhere to shoes worn in cleanrooms.

A third variable is the speed at which the outer layer of each adhesive mat is peeled. This, again, cannot be quantified, although a level of consistency was maintained by using the same person. A fourth variation is the location of the particle counter and the sample of air taken. If another location had been sampled, the level of particles may have been different. Furthermore, a particle counter cannot measure the degree of dispersion of the airborne particles, and this is a phenomenon which would be particular to each cleanroom. This could be controlled should the study be replicated in an air dispersal chamber.

On the basis of the data presented in this paper, polymeric cleanroom flooring is superior at removing particles from uncovered and covered shoes compared with a range of different disposable adhesive cleanroom mats. Furthermore, it does not carry the risk of disseminating particles into the air stream through the removal of a top layer.

Finally, it should be noted that cleanroom mats are but one part of a cleanroom contamination control programme. Other factors, such as a functioning HVAC and a proven cleaning and disinfection regime, are essential aspects of contamination control. Further variables include control of staff numbers, control of personnel and material transit between cleanrooms, and re-design of cleanroom clothing. The efficiency of the cleanroom can be assessed through environmental monitoring. Nonetheless, cleanroom mats play a role as part of a cleanroom contamination control strategy and, where such flooring is required, the polymeric solution appears to offer advantages for particle level cleanliness in terms of removal of particles from footwear and avoiding the particle generation which arises from the peeling of adhesive mats.

References