European Journal of Parenteral Sciences 1997; **2**(4): 109–111
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A comparative study of two floor-cover materials in control of foot- and wheel-borne contamination

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It is recognised that the interfaces between classified cleanrooms and less clean areas form a fragile barrier to the ingress of particles and micro-organisms into cleanrooms. In many cases, operators and materials are transferred into airlocks, subsequently entering the cleanroom via a simple 'step-over' bench barrier. The principles of quality assurance, in the manufacture of sterile medicinal products, include the requirement that particle and microbiological contamination levels should be minimised, both in the cleanroom and surrounding areas, to reduce the probability of such contamination entering the product.[1,2] In this context, foot- and wheel-borne contamination represent two potential sources of viable and non-viable particles. This paper describes the relative reduction in these two potential sources achieved by two different, commonly used types of floor cover. Polymeric floor cover was found to be more effective in the reduction of foot- and wheel-borne contamination, over a wide range of particle levels, than was the surface of a 'peel-off' or 'tacky' mat.

The entry to cleanroom areas should be protected so as to minimise the access of both viable and non-viable contamination. Airborne contamination can be limited to acceptably low levels by selecting and correctly installing appropriate filters and airflow arrangements, and by balancing pressurisation over the rooms within the cleanroom suite.

Operator-borne contamination can be minimised by the selection of particle-retentive gown materials, appropriate gowning procedures and correct wear of the garments provided, while various types of particle-retentive floor cover can be used to assist with the removal of foot- and wheel-borne contamination at the entry to gowning, preparation and product manufacture areas. However, few comparative studies of the effectiveness of such floor covers have been published.[3-7]

The work described here was carried out at the Centre for Drug Formulation Studies at the University of Bath in the UK, utilising its Class 10,000 cleanroom suite. This cleanroom suite is, in effect, a 'living laboratory' intended for cleanroom research studies and for the manufacture of small batches of sterile products for clinical trials.

The suite comprises a conventional turbulent vertical-flow room with vertical-flow unidirectional Class 100 cabinets. Air is extracted from the room at low level for recirculation. Access to the cleanroom is via a gowning area and there are also separate viewing and preparation areas. The viewing area is used in addition for operators to put on overshoes and for removal of outdoor garments. The area wall finishes are epoxy-coated steel panels, and flooring is welded PVC coved at floor-to-wall and wall-to-ceiling joints.

In this type of area, both viable and non-viable particle levels are expected to be relatively high near floor level, but adequately controlled at bench or workstation height and where operators carry out their working manipulations.

Scope

This paper describes procedures for evaluating the reduction in the amount of contamination on operators' footwear and on trolley wheels at the entry, the gowning, and the preparation areas of a cleanroom suite. These studies compare the efficacy in reducing the numbers of viable and non-viable particles of a polymeric floor cover with that of a peel-off ('tacky') mat. The mechanism of particle retention is not assessed or described, since this information can be found elsewhere.[3]

Materials

All measuring equipment used in the studies was calibrated against traceable national or international standards. The major equipment used was an airborne particle counter, model ULPC (Particle Measuring Systems Ltd), a liquid-borne particle counter, model ULPS (Particle Measuring Systems Ltd), an air-to-agar sampler, model RCS (Biotest Ltd), and a filtration manifold (Millipore Ltd).

Laboratory disposable items included sterile cottonwool swabs (Western Laboratory Service Ltd), low-shedding sterile plastic foam swabs (Dage Ltd), Milliflex filter funnels (Millipore Ltd), Hycheck samplers (Difco Ltd), and agar strips (Biotest Ltd). Sterile Water for Injection, and Sterile

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0.9% Sodium Chloride Solution (Steripak Ltd) were used as diluents.

Two floor coverings were tested. One was a polymeric floor cover and the second a peel-off 'tacky' mat. Both were commercially available items.

Methods: viable particle tests

To carry out the viable particle tests, sterile cottonwool swabs were moistened with Sterile 0.9% Saline Solution and used to take samples at the entry to the cleanroom suite from an area of 5 sq. cm. on the soles of operators' footwear, employing overlapping strokes to obtain maximum recovery. Swabs were plated out systematically: one on the surface of a tryptone soya agar plate followed by one on the surface of a Sabouraud dextrose agar plate, using classical microbiological techniques and ensuring that the swab was rotated to make contact on the plate with the entire swab surface.

The procedure was repeated with four trolleys, and the swabs were plated out as before.

Methods: non-viable particle tests

For the non-viable particulate tests, plastic foam swab samples were taken as previously described for the viable samples. After sampling, any attached particles were dispersed in a sterile particle-free universal container holding 25 ml of sterile particle-free distilled water. The particles were counted using the liquid-borne particle counter system.

The above procedures were repeated after operators had walked across either polymeric flooring or peel-off mat, making four foot falls, as representative of best industrial practice, on either type of floor covering. Swabs were taken from fresh 5 sq. cm. areas on the soles of footwear, and were plated out as before.

All plates were identified with suitable descriptions. The tryptone soya agar plates were incubated at 32-35°C. The Sabouraud dextrose agar plates were incubated at 20-25°C.

Sterile cottonwool swabs were moistened with Sterile 0.9% Saline Solution and samples obtained from an area of 10 sq. cm. on the floor of the entrance to the cleanroom suite. Two swabs were used for each location and the swabs were treated as before.

The same procedure was carried out at the end of the polymeric flooring nearest to the entry door of the cleanroom suite, and the end of the peel-off mat furthest from the point of entry to the cleanroom suite. It was further repeated on the polymeric flooring mat at the entry to the gowning room and the preparation room, as well as on both the 'grey' and 'white' sides of the gowning room step-over bench. Three tests were carried out by each operator at each location.

Swab samples, using sterile cottonwool swabs moistened with Sterile 0.9% Saline, were also taken from two wheels of each trolley in the area near the entry to the cleanroom suite (each sample was approximately one half of a wheel circumference). To simulate actual use, each trolley was weighted with approximately 25 kg.

The trolley was pushed across the polymeric flooring or the peel-off mat, and then the remaining half of trolley wheel was swabbed.

Results

The results as shown in **Tables 1–5** were averaged for ease of handling the data. Full individual raw data is available for each participant.

Discussion and conclusions

The results confirm the high degree of efficiency of the polymeric flooring in particulate removal across a wide range of particle sizes. By comparison, the peel-off mats

Table 1: Foot- and wheel-borne viable particulate collection. Comparison of polymeric flooring with peel-off mats: total viable count.

Viable counts Before	Viable counts After Polymeric flooring	Viable counts After Peel-off mats	% Reduction Polymeric flooring	% Reduction Peel-off mats
Foot-borne >1,000*	569	967	43	3
Wheel-borne >1,000*	17	764	98.3	23

*Confluent or near-confluent growth on plate

Table 2: Foot-borne viable particles collection as a function of culture type.

	Viable counts Before	After Polymeric	After Peel-off	% Reduction Polymeric	% Reduction Peel-off
Gram-positive bacteria	1,750	21	970	98.8	44.6
Gram-negative bacteria	59	8	35	86.5	40.7
Yeast and moulds	290	26	139	91.0	52.1

Table 3: Wheel-borne particles collection as a function of culture type.

	Viable counts Before	After Polymeric	After Peel-off	% Reduction Polymeric	% Reduction Peel-off
Gram-positive bacteria	2,884	30	755	99.0	72.4
Gram-negative bacteria	1,041	8	496	99.3	52.4
Yeast and moulds	3,532	21	1,824	99.4	48.4

Table 4: Foot-borne non-viable particulate collection as a function of particle size

Particle size (micron)	Particle count Before	Particle count After		% Reduction	
		Polymeric	Peel-off	Polymeric	Peel-off
2	12,301	5,648	9,395	54.1	23.6
10	526	219	362	58.4	31.1
25	32	20	26	62.0	18.8
100	NS	NS			NC

NS = not significant number counted; NC = not calculable

Table 5: Wheel-borne non-viable particulate collection as a function of particle size

Particle size (micron)	Particle count Before	Particle count After		% Reduction	
		Polymeric	Peel-off	Polymeric	Peel-off
2	5,880	3,328	4,183	43.4	28.9
10	113	38	82	66.5	27.4
25	10	3	6	70.0	40.0
100	NS	NS			

NS = not significant number counted; NC = not calculable

demonstrated a very much lower level of efficiency in particulate removal, particularly for smaller particle sizes, allowing literally thousands of additional 2- and 5-micron particles to pass into the cleanroom from the gowning area.

This has a major bearing on the control of micro-organisms, since the majority attach to small particulates within this size range and any of the particles allowed to pass in this way may be biologically active; this is borne out by the study on viable particles.

The efficiency of particulate collection, as measured by the percentage of particles removed, increases with particle size. Of greater significance, however, is the very large number of 2-micron particles collected by the polymeric flooring. The effectiveness of the polymeric flooring in controlling viable foot-borne contamination is clearly demonstrated.

Trolley wheels can be a very significant carrier of microbial contamination and here again the polymeric flooring is highly effective in the control of a range of micro-organisms. As with foot-borne contamination, the efficiency of particulate collection from trolley wheels increases with particle size. Again of greater significance, however, is the very large number of 2-micron particles collected.

Overall, polymeric flooring is demonstrated to be highly effective as a means of control of microbiological contamination, both for foot-borne and wheel-borne contamination. By comparison, the results suggest that peel-off mats are largely ineffective.

This is an important conclusion for all cleanroom operators, particularly those engaged in the production of pharmaceuticals, medical devices and associated industries where the control of microbiological contamination is important. However, any product for control of foot- and wheel-borne contamination must be used as part of a disciplined management regime directed to contamination control as a whole.

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Acknowledgements

The work described in this study was funded by Dycem Ltd, Bristol UK. The author acknowledges the assistance and encouragement of Mr Mark Dalziel (Dycem Ltd) and Dr Geoffrey Barrett (Corporate Development Consultants Ltd) in the preparation of this paper. Acknowledgement is also made for permission to use data shown in the tables. Thanks are also due for the laboratory work carried out by Mrs Muriel Davies and Mrs Renate Schiller in the Kennet Bioservices Laboratory, and to the volunteers from Bath University.

Based on a paper presented to the Ninth Annual Congress of the Association pour les Produits Parentéraux et Stériles (A3P), Arcachon, France, 1996.