EUROPEAN JOURNAL OF PARENTERAL AND PHARMACEUTICAL SCIENCES

Peer Review Article | Open Access | Published 11th July 2025

To Determine if the Surface Finish from Different Materials Influences the Microbial Recovery when Sampled with a Contact Plate

T Eaton, K Capper, M Ward, C Tobin, J Bright - AstraZeneca, Macclesfield, UK | EJPPS | 302 (2025)| https://doi.org/10.37521/ejpps30201 | Click to download pdf

Corresponding Author: Tim Eaton, Sterile Manufacturing Specialist
AstraZeneca,
UK Operations,
Silk Road Business Park,
Macclesfield
Cheshire. SK10 2NA
England

Email: tim.eaton@astrazeneca.com
Telephone: +44(0) 1625 514916

To Determine if the Surface Finish from Different Materials Influences the Microbial Recovery when Sampled with a Contact Plate

T Eaton[1] , K Capper[1], M Ward[1], C Tobin[1] and J Bright[1]

[1] AstraZeneca, Macclesfield, UK

Summary

The recovery of naturally occurring surface microbial contamination with sterile tryptone soya agar 55 mm diameter RODAC plates had previously been evaluated for five different typical cleanroom related materials. The recoveries were shown to vary and to understand the influence that the surfaces have on the recovery efficiency, further investigation of the surface finishes and roughness values was completed and correlated with the recovery efficiencies determined for the different materials. Overall, it has been concluded that the surface recovery of naturally occurring microbe-carrying particles (MCPs) from the different cleanroom related surfaces is influenced by the surface roughness of the material under consideration and as the roughness values increase, the plate recovery efficiencies are reduced.

Key words: Microbiological surface contamination, RODAC contact plates, microbe-carrying particles (MCPs), recovery efficiency, surface finish, surface roughness value.

1. INTRODUCTION

For sterile products manufacturing, it is a requirement of Annex 1 of the European Union Guide to Good Manufacturing Practice (EU GGMP) [1] that microbiological monitoring of cleanrooms includes the use of 55 mm diameter contact plates for sampling defined surface locations. Typically, circular RODAC (replicate organism detection and counting) plates (55 mm diameter, 24 cm^2 surface area) containing nutrient agar (between 15.5 and 16 ml) are used for sampling surfaces that are relatively flat. Viable particles removed from the surface adhere to the agar and the lidded plates are then incubated and the number of colony forming units (CFU) and types of micro-organisms recovered are reported, and the results expressed as the number of CFU per plate. The material and the associated finish of the surfaces to be sampled are reported to be one of many factors that influence the recovery efficiency [2]. Previous experimental work, using the same contact plate sampling procedure, was completed to determine the recovery associated with different cleanroom related materials that are subjected to routine monitoring [3]. The results recorded varying levels of recovery for the different materials and this variation may be influenced by the surface finishes of the materials under consideration. Consequently, although the materials themselves are different, the topography of the surfaces has been further investigated to determine if this is a factor that influences the recovery.

2. PREVIOUS INVESTIGATIONS TO DETERMINE SURFACE MICROBIAL COLLECTION EFFICIENCIES FROM DIFFERENT SURFACES

Approach to collection efficiency determinations

The surfaces of different materials, which are typically monitored in cleanrooms, were used. These surfaces were polyester garment, stainless steel, cleanroom latex gloves, workstation barrier ethylene propylene diene monomer (EPDM) gauntlets and cleanroom goggles copolyester lens. To ensure the surfaces had sufficient microbial contamination, they were exposed in a microbiological testing laboratory that was used daily by numerous people and continually contaminated throughout the exposure period with naturally occurring microbe-carrying particles (MCPs), predominantly dispersed from personnel in relatively large numbers.

Determination of collection efficiencies

A mathematical model is described which may be used to assess the efficiency and consistency of a surface sampling method [4]. This was based on a two stage sequential sampling which is a convenient method if the counts on the surface following the second sampling are relatively low. The recovery efficiency for the two stage sampling can be determined using equation 1.

Recovery efficiency (%) $= [1 - (B/A)] \times 100$ **Equation 1**

Where,

B = total count from second sample

A = total count from first sample

Sampling method and results

All plates used were Becton Dickenson, BD BBL™ IC-XT Trypticase™ Soy Agar medium with lecithin and polysorbate 80 surface neutralising agents, 55mm diameter RODAC™ LL. The plates have locking lid features and are gamma irradiated and sealed in triplicate polythene bags, sourced from an approved supplier and are routinely tested for their ability to recover microbial contamination. Following sampling, all plates were immediately and simultaneously incubated, in the same validated incubator, at 30- 35^{0}C for 5 days and the number of CFUs counted.

To minimise any variability associated with the sampling, the plates were rolled over the different surfaces in a single motion, lasting 1 second, with firm force and all performed by the same person and had previously been reported to be an efficient sampling procedure [5]. Twenty samples of each material were tested and the collection efficiencies for each of the twenty samples determined using equation 1 and the average collection efficiencies calculated. The results are shown in table 1.

Table 1 Summary of microbial contact plates test results

Surface Material	Total A[a] (CFU) [Average/plate]	Total B[a] (CFU) [Average/plate]	Total A and B[a] (CFU)	Average Recovery Efficiency[b] (%)	Standard Deviation (%)
Polyester garment	789 [39.5]	278 [13.9]	1067	65.8	20.0
Stainless steel tray	1384 [69.2]	255 [12.8]	1639	79.8	16.0
Copolyester lens goggles	797 [39.9]	133 [6.7]	930	81.7	10.7
Latex gloves	789 [39.5]	234 [11.7]	1023	69.2	12.7
EPDM barrier gauntlet	1324 [66.2]	409 [20.5]	1733	68.5	11.6

Notes

a. Combined bacteria and mould counts

b. Average of all the individually calculated recoveries

3. INVESTIGATION OF MATERIALS SURFACE FINISHES AND ROUGHNESS

Two sample coupons of each of the five cleanroom materials (stainless steel, latex, polyester, EPDM, and copolyester) were examined for surface finish and roughness measurement. The surface finish was determined using a Keyence digital optical microscope and the surface roughness was measured with a calibrated Mitutoyo SJ-210 tester (resolution of 0.002µm at a measurement range of 25µm) on coupons of each of the materials.

4. RESULTS

Surface Finish

The optical microscopy examinations of the different surface coupons are shown in Figures 1-5.

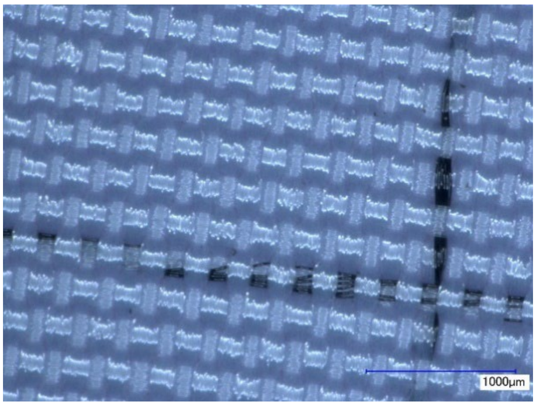

Figure 1 Polyester

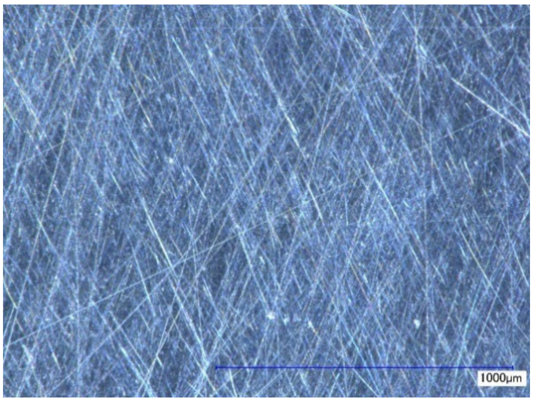

Figure 2 Stainless steel

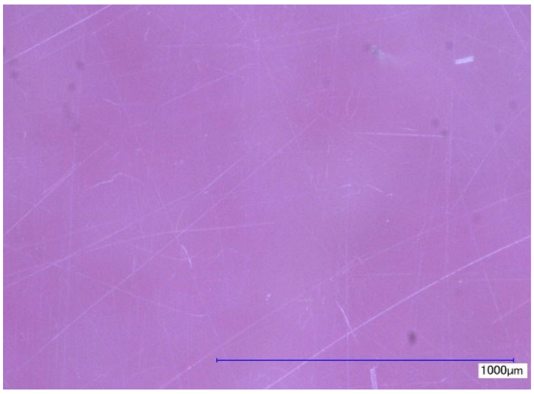

Figure 3 Copolyester

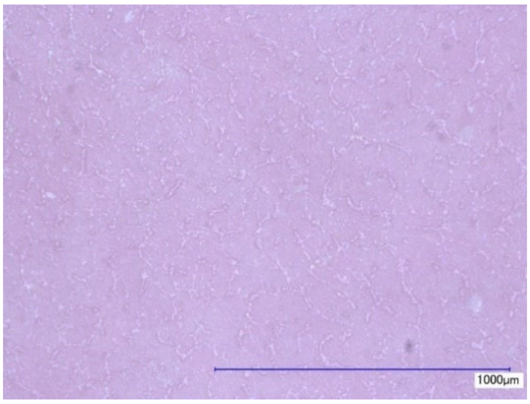

Figure 4 Latex

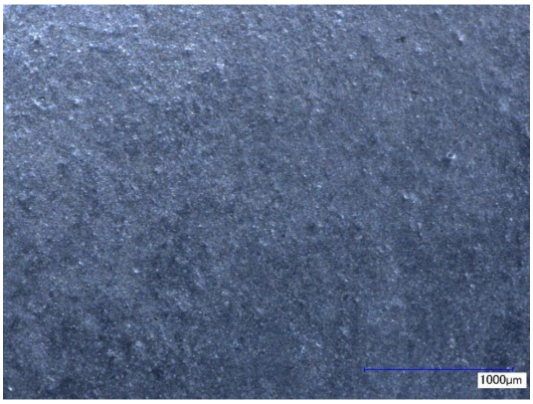

Figure 5 EPDM

Surface Roughness Measurements

Five roughness measurements were conducted on each side of the two provided coupons, for each material. The roughness measurements, reported as Ra (Arithmetical Mean Roughness) values, are summarised in table 2.

Table 2 Materials roughness measurements

Material	Coupon	Side	Roughness, RA (µm)							
			Measurements					Side Average	Coupon Average	Surface Average
Polyester	1	1	10.490	10.159	11.483	10.280	9.900	10.462	10.845	10.973
		2	10.394	11.674	11.293	10.699	12.074	11.227		
	2	1	12.498	12.247	11.527	11.452	12.047	11.954	11.102	
		2	10.125	10.061	10.294	10.109	10.659	10.250		
Stainless steel	1	1	0.551	0.460	0.460	0.472	0.478	0.484	0.489	0.496
		2	0.361	0.428	0.512	0.543	0.621	0.493		
	2	1	0.531	0.442	0.440	0.532	0.542	0.497	0.504	
		2	0.547	0.456	0.566	0.532	0.455	0.511		
Copolyester	1	1	0.139	0.172	0.090	0.027	0.168	0.119	0.106	0.153
		2	0.031	0.246	0.113	0.047	0.031	0.094		
	2	1	0.254	0.393	0.258	0.316	0.301	0.304	0.200	
		2	0.037	0.033	0.301	0.058	0.046	0.095		
Latex	1	1	4.893	4.897	4.554	3.953	4.672	4.594	3.128	2.650 (3.705)[a]
		2	1.859	1.918	1.136	1.647	1.751	1.662		
	2	1	3.419	2.225	2.612	2.977	2.851	2.817	2.172	
		2	1.253	1.576	1.308	1.898	1.600	1.527		
EPDM	1	1	0.588	0.640	0.725	0.428	0.571	0.590	0.568	0.580
		2	0.685	0.485	0.531	0.492	0.533	0.545		
	2	1	0.483	0.547	0.517	0.503	0.555	0.521	0.593	
		2	0.587	0.694	0.672	0.684	0.684	0.664		

Note

a. The latex gloves exhibited roughness variation between the internal and external surfaces of the glove and the value shown in parenthesis is for the outer surface only that relates to the data recorded in table 1.

5. DISCUSSION AND CONCLUSION

Surface Roughness

Surface roughness testing is a complex subject, but it can be considered to describe the properties of a surface. Specifically, it defines the distance between the valleys and peaks on the surface created by ridges, grooves, marks and scuffs made by the manufacturing process and, if determined following a period of use, those resulting from any wear during use. The roughness is the distance between the peaks and troughs on the surface and an average of the measurements is presented as the roughness average, or Ra value (the Arithmetical Mean Roughness) as shown in figure 6 and a higher Ra value is an indication of greater surface roughness. Waviness is another factor that influences roughness and refers to the larger-scale variations of roughness defined as the irregularities where the spacing is greater than for the roughness sampling length, as illustrated in figure 7.

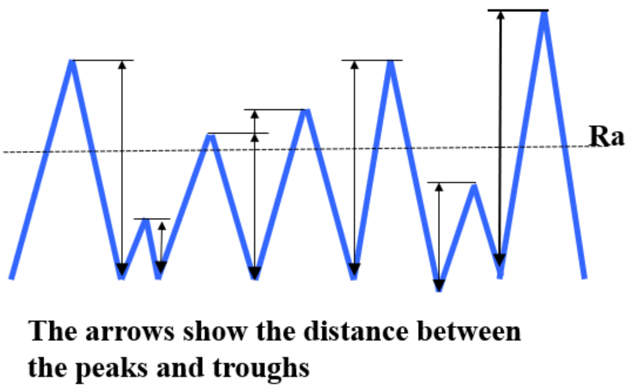

Figure 6 Surface roughness

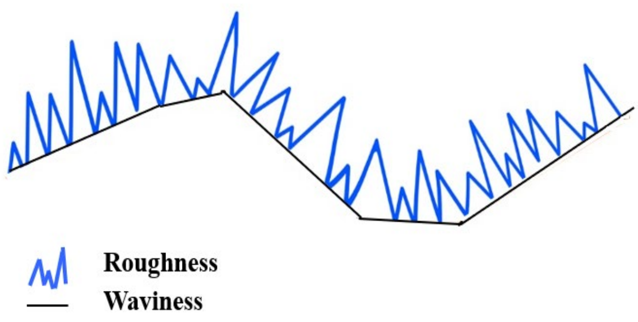

Figure 7 Surface waviness

Micro-organisms used to determine plate recovery efficiencies

The determination of the contact plate recovery efficiencies that are shown in table 1 used naturally occurring microbe-carrying particles (MCPs) that were predominantly dispersed from the skin cells of personnel onto the different surfaces under consideration in a busy laboratory environment. These are representative of the majority of the microbes recovered from cleanroom environments and usually have a maximum length of about 44 µm, a minimum length of about 33 µm, with a thickness of about 4 µm [6]. They will readily deposit by gravity onto surfaces where they become firmly adhered and are not removed by air currents but transferred by contact. The use of MCPs also avoids issues resulting from the utilisation of standard commercial test organisms with a carrier medium to deposit suspensions of micro-organisms onto the test surfaces. On evaporation of the carrier medium, sub-micron unicellular microbes, about 1 to 10 µm in length and 0.2 to 1 µm in width, are deposited across the surfaces and are not representative of the much larger MCPs. These unicellular microbes may be transferred to the plate with different efficiencies compared to the much larger naturally occurring MCPs and so present data that is not representative of surface sampling within cleanrooms.

Review of results of investigation of materials surface finishes and roughness

The surface finishes recorded by the digital optical microscope, and shown in figures 1 to 5, revealed significant differences in the surface topography of the five materials. The topography of polyester showed the most variation and of the other two soft materials, EPDM appeared to show more variation than for latex. For the hard surfaces, surface scratching resulting from use, is very evident for surface stainless steel (some of this scratching was imparted during the coupon preparation activities) and also present but less evident for copolyester. However, these optical images did not fully align with the surface roughness measurements obtained by testing.

The surface roughness measurements determined that the polyester had the highest average surface roughness (10.973 µm), followed by latex (2.650 µm), EPDM (0.580 µm), stainless steel (0.496 µm) and copolyester (0.153 µm). It should be noted that latex exhibited some roughness variation between the two sides (3.705 µm on the outer surface and 1.595 µm on the inner surface), likely because the outer side is intentionally roughened for better grip. The correlation of contact plate microbial recovery efficiencies (summarised in table 1) and the associated material surface roughness (detailed in table 2) is shown in table 3, in ascending order of recovery efficiency and, for simplification, also includes the relative surface roughness values (determined by dividing each average material roughness values by the lowest, copolyester, value). The plate contact recoveries and the associated relative surface roughness values (log scale) are shown in graphical format in figure 8. No information regarding surface waviness is available and this is discussed later.

Table 3 Surface contact plate microbial recovery efficiency and material surface roughness

Material	Average Contact Plate Recovery Efficiency (%)	Average Materials Roughness, RA (µm)	Relative Surface Roughness
Polyester	65.8	10.972	71.7
EPDM	68.5	0.580	3.8
Latex	69.2	3.705[a]	24.2[a]
Stainless steel	79.8	0.496	3.2
Copolyester	81.7	0.153	1.0

Note

a. Outer glove surface only that relates to the data summarised in table 1.

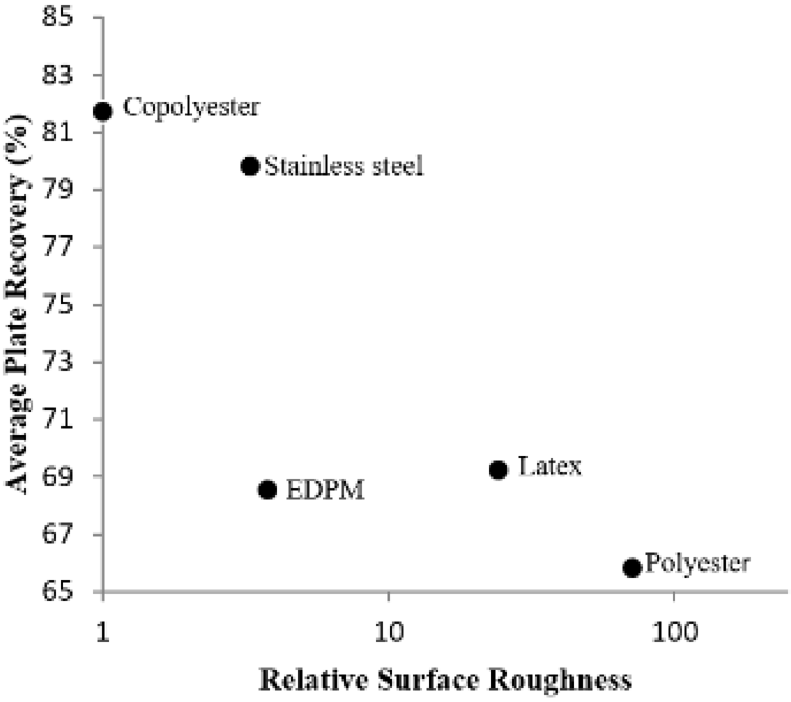

Figure 8 Average plate recovery efficiency and relative surface roughness

It is reasonable to assume that surfaces with the lowest roughness values would deliver the highest microbial recoveries as the large size MCPs have fewer opportunities to conceal themselves away from the surface and avoid transfer onto the contact plate nutrient agar. Consequently, the copolyester (0.153 µm) and stainless steel (0.496 µm) surfaces with the lowest roughness measurements recorded the highest recovery efficiencies of 81.7% and 79.8% and respectively. With consideration for the inaccuracies of the microbial method, these two recovery levels are effectively equivalent. It can be seen that the stainless steel surface roughness is 3.2 times greater than for the copolyester and so recovery from the stainless surface may be anticipated to be notably less. However, due to the much larger size of the contaminating MCPs compared to both surfaces roughness values, the flexible nature of the contact plate nutrient agar that enhances the contact with the surfaces and is also useful to minimise any differences in surface waviness, minimal differences between the recoveries may be expected, and this is the case.

Polyester, with the highest surface roughness measurement (10.973 µm) recorded the lowest recovery efficiency of 65.8% and it may be reasonable to propose that an even lower efficiency could be expected if it is assumed that when the fabric threads contact the plate nutrient agar, only the top of the threads makes a contact and not the entire top surface. The flexible agar surface however allows the thread to sink into and make good contact with the agar and help to address any significant waviness constraints and hence increase the recovery. The two other non-rigid surfaces, EPDM (surface roughness 0.580 µm) and latex (surface roughness 3.705 µm) both had similar plate recovery efficiencies of 68.5% and 69.2% respectively. As the roughness value of EPDM is 6.4 times less than for latex, the similar levels of recovery efficiencies is a little surprising when considering the increased surface roughness compared to copolyester and stainless steel. Even taking into account the size of the MCPS and the agar flexibility, as indicated in figure 8, this appears to be an anomaly and requires further consideration. The microbial recoveries that have been discussed have only considered surface roughness and have not taken into account any influence associated with the different types of materials. A simple touch assessment indicated that except for EDPM, which had a noticeably 'stickier' feel, all the other surfaces had no such characteristic, and this may therefore have had an influence on limiting the EDPM recovery efficiency from what may be anticipated. Overall, it is concluded that the surface recovery of naturally occurring MCPs is influenced by the surface roughness of the material under consideration and as the roughness value increases, the plate recovery efficiency is reduced.

REFERENCES

1. The rules governing medicinal products in the European Union –Volume 4 EU guidelines to good manufacturing practice - medicinal products for human and veterinary use - Annex 1- Manufacture of sterile medicinal products. European Commission, Brussels, 2022.
2. Werner, H-P., Swinke, U. and Werner, G. Development of a new test method for surface disinfection procedures. III. The impression method: influence of the test surface material and the types of microorganisms on the recovery rate. Zentralbl Bakteriol Orig B. 165 (1):20-42 (1977).
3. T Eaton, K Capper, M Ward, C Tobin, J Bright. To Determine the Microbial Recovery from Different Surfaces Using a Standard Contact Plate Sampling Method. European Journal of Parenteral and Pharmaceutical Sciences 29(4). 2024.
4. W. Whyte, W. Carson and A. Hambraeus. Methods for calculating the efficiency of bacterial surface sampling techniques. Journal of Hospital Infection (1989) 13, 3341.
5. Eaton T, Capper K, Ward M, Bright J. Evaluation of Three Different Contact Plate Methods for Microbial Surface Sampling of Naturally Occurring Human Borne Microbial Contamination. European Journal of Parenteral & Pharmaceutical Sciences 2024; 29 (1).
6. Mackintosh C, Lidwell OM, Towers AG and Marples RR. The dimensions of skin fragments dispersed into the air during activity. Journal of Hygiene 1978; 81:471–479.