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Published in:
European Journal of Hospital Pharmacy: Science and Practice

DOI:
10.1136/ejhpharm-2018-001672

Improving the aseptic transfer procedures in hospital pharmacies part A: methods for the determination of the surface bioburden on ampoules and vials

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Received 13 July 2018
Revised 27 March 2019
Accepted 15 April 2019

EAHP Statement 3: Production and Compounding.

ABSTRACT

Objectives To develop methods for surface bioburden determination of ampoules and vials to be used in the validation of the disinfection procedures and in routine monitoring of ampoules and vials.

Methods The surface bioburdens of ampoules and vials are determined before and after disinfection by contact plates and total immersion.

Results The mean surface bioburdens of non-disinfected ampoules and vials taken straight from the original boxes are 2.4 and 5.01 cfu (total immersion; n = 20), and 0.97 and 0.94 cfu (contact plates; n = 60). The mean surface bioburdens of ampules and vials after disinfection by wiping are 1.15 and 7.50 cfu (total immersion; n = 20), and 0.12 and 0.10 cfu (contact plates; n = 60). The high number of cfu on vials (total immersion) indicate hidden cfu around the neck not removable by wiping and not detected by contact plates. Total immersion needs special laboratory facilities and is expensive (about €50 a sample). Therefore, it is less appropriate for use in routine monitoring. However, because of the high recovery, it is the method of choice for the validation of the disinfection procedure. Surface bioburden determination by contact plates is relatively simple. Non-flat surfaces cannot be reached, but the recovery from the touched flat part of the surface is high (around 50%). The recovery from swabs is low (around 10%). Another disadvantage of swabs is the laboratory work after sampling. We therefore advise contact plates for routine monitoring. To get a reliable value of the mean surface bioburden at least 30 samples need to be examined.

Conclusion Total immersion is the method of choice for the determination of the effectiveness of a disinfection procedure for ampoules and vials. Contact plate is the method of choice for routine monitoring of the surfaces of ampoules and vials.

INTRODUCTION

Aseptic handling is the process enabling sterile products to be made ready to administer using closed systems.[1 2] The starting materials are sterile and must be kept so during the process.[2] Aseptic handling is performed in a laminar airflow cabinet (LAF), a safety cabinet (SC) or in an isolator (I). The background area is the room in which the LAF, the SC or the I are housed.

During aseptic handling many materials are used. They can be divided into materials with a sterile surface, like sterile medical devices and infusion bags, and materials with a non-sterile surface, like ampoules, injection and infusion vials. The transfer of these materials into the LAF/SC/I is a critical process. If executed without enough precaution micro-organisms can be dragged with the materials into the LAF/SC/I.

Information about the surface bioburden of materials used in aseptic handling is scarce. This is remarkable because, once inside the LAF/SC/I, materials are touched by the operators' hands and these hands can easily touch critical spots like needles, syringe tips and connection points. This makes materials critical items. According to the principles of microbiological monitoring, critical items have to be monitored regularly.[3] This can be done by contact plates, swabs or total immersion.

For flat surfaces, 55 mm diameter agar contact plates are recommended. The surface is above the edges of the dish, which makes direct contact between the agar and an object possible. During sampling enough contact time and pressure are important to get a good transfer of the micro-organisms from the surface to the agar. The exact efficiency of the recovery is difficult to estimate because micro-organisms adhere differently to surfaces of different materials.[4] Besides, wetness of the plates may have an influence on the recovery.[5] On facility surfaces recovery rates of approximately 40%–60% were found in the pharmaceutical industry.[6]

Swabs are recommended for non-flat surfaces. They are made from cotton, rayon or polyester. Before use, the swab needs to be moistened. After swabbing, the swab can either be directly streaked onto the surface of an agar plate or can be rinsed with a buffer, after which colonies are counted by the membrane filtration technique. The recovery from traditional cotton, rayon or polyester swabs is around 10%.[7] If more expensive high-recovery swabs are used this percentage increases to 60%.[8]

Total immersion is the method with the highest recovery. Doing so, materials are submerged in a rinsing fluid and shaken for a predetermined time. After shaking, the rinsing fluid is filtrated and colonies are counted by the membrane filtration technique.

Surface bioburden determination is not only needed in routine monitoring, but also to determine the effectiveness of the applied disinfection procedure (validation). Monitoring and validation might demand different determination techniques. The aim of this study is to answer this question and to give advice for validation and routine monitoring in daily practice.

Two subsequent articles deal with the disinfection of materials with a non-sterile surface and the transfer of materials from outside the background area into the LAF/SC.

To cite: Boom FA, Le Brun PPH, Boehringer S, *et al*. *Eur J Hosp Pharm Sci Pract* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/ejhpharm-2018-001672

MATERIALS AND METHODS

Sample preparation

Sampling was executed in the background area. Operators wore clean room clothes, face masks and disposable gloves.

Non disinfected materials

Samples of plastic ampoules (Suplivent 10 mL, Fresenius Kabi), glass ampoules (Vitintra adult 10 mL, Fresenius-Kabi), injection vials (Soluvit N, Fresenius-Kabi) and infusion vials (water 100 mL, Fresenius-Kabi) were collected aseptically straight from the original boxes and placed in smooth sterile stainless steel trays. The trays with samples were placed in a LAF cabinet.

Disinfected materials

The same samples as mentioned above were collected aseptically straight from their original boxes and placed in clean and disinfected smooth plastic trays. Plastic flip-off caps were removed from vials. Samples were disinfected by thoroughly wiping with sterile polypropylene wipes impregnated with 85% isopropylalcohol and 15% demineralised water (227×279 mm, Prosat) and directly placed in smooth sterile stainless steel trays. The trays with samples were placed in a LAF cabinet.

Surface bioburden determination

All experiments were executed in a LAF cabinet. Operators wore clean room clothes, face masks and sterile gloves.

Contact plates

- The ampoule or vial was taken in the dominant hand and the contact plate in the other hand. The ampoule or vial was rolled slowly and with light pressure from left to right and back again (each in around 3 s) over the surface of a contact plate (Tryptone Soya Agar 55 mm diameter, Biotrading Benelux). The contact plate was turned and slow rolling was repeated twice (see figure 1). Care was taken not to touch the agar surface with the operator's gloved fingers.
- For infusion vials, only the label was monitored (about 15% of the surface of a 100 mL vial). The contact plate was put on the label surface for 10 s. To promote full contact the plate had to be rolled a little with light pressure from left to right.
- After sampling, the agar residues on the ampoules and vials were removed by wiping with Prosat wipes.
- Contact plates were incubated for 7 days at 30±1°C.
- Cfu were counted after 3 and 7 days.

Total immersion in accordance with ISO 11737-1:2006[9]

- Total immersion experiments were executed by Bactimm (Nijmegen, The Netherlands), a microbiological contract laboratory with a focus on the pharmaceutical and medical device industry. Ampoules or vials were put into sodium chloride peptone buffer with tween (NaCl 4.3 g; peptone 1.0 g; KH_2PO_4 1.5 g; $Na_2HPO_4.2H_2O$ 3.5 g; polysorbate 80 1.0 g; demineralised water 1000 mL), shaken for 60 min (200 RPM) at 35–37°C in a shake incubator and filtered through a filtration funnel (Milliflex, Merck Millipore).
- After filtration the membrane filter (mixed ester cellulose) was put on a cassette, prefilled with Tryptic Soya Agar (Milliflex, Merck Millipore) and incubated for 7 days at 30±1°C.
- Cfu were counted after 3 and 7 days.

RESULTS

Tables 1 and 2 show the mean bioburden on ampoules and vials determined by total immersion and contact plates before and after disinfection respectively.

Table 3 shows the results from table 2 expressed as mean cfu and percentage of samples contaminated with ≥1 cfu.

DISCUSSION

Recovery on contact plates

Surface bioburden shows considerable variety within one kind of material, as shown by the relatively large standard deviations (tables 1 and 2). This variability reflects heterogeneity in the samples analysed and variability induced by the measurement process, which will make recovery calculations less reliable. In spite of this limitation, the following remarks can be made.

The recovery from glass ampoules compared with plastic ampoules is higher (see table 1). Differences in packaging materials such as glass and plastic and the fact that the surface of the plastic ampoules is not completely smooth are the most plausible explanations for this. Injection vials have the lowest recovery (9.7 %, see table 1). The most likely reason is that the surfaces round the neck and crimp cap are hard for the contact plates to reach. These surfaces are also not easily reachable during disinfection by wiping, which explains the even lower recovery after disinfection (see table 2).

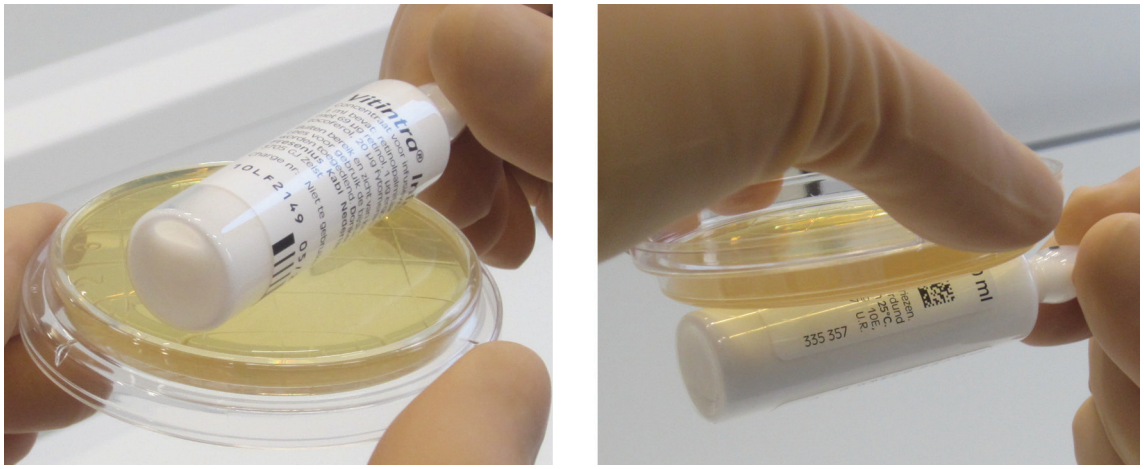

Figure 1 Ampoule surface sampling by contact plate.

Table 1 Surface bioburden before disinfection expressed as mean cfu per ampoule or vial

	Plastic ampoules		Glass ampoules		Injection vials		Infusion vials	
	Mean cfu	SD	Mean cfu	SD	Mean cfu	SD	Mean cfu	SD
Total immersion	2.50 (n=10)	3.14	2.30 (n=10)	1.88	5.90 (n=10)	3.14	4.11 (n=9)	4.62
Contact plate	0.76 (n=30)	1.01	1.17 (n=30)	1.60	0.57 (n=30)	1.07	1.30 (n=30)	1.47
Recovery (%)	30.4	–	50.9	–	9.7		*	–

Recovery is the number of cfu found on contact plate as a percentage of the number at total immersion.
*Recovery is not determined because of the small part of the surface which is monitored by a contact plate (see Methods).
n, number of samples examined.

Total immersion

The ISO 11737 method, used for total immersion in this study, is developed for sterile medical devices.[9] It is comparable to a method used by Cockcroft *et al* for surface bioburden determinations of ampoules, vials and syringes.[10] Because of the required laboratory facilities as well as knowledge and experience with the ISO 11737 method we outsourced it to a specialised laboratory.

Hiom developed a total immersion procedure for ampoules and vials by using sterile EVA bags and called it an in-house procedure.[11] We experimented with the EVA bag method, but found it difficult to get an ampoule or vial into the bag without touching it. Also, it is a labour-intensive method, using a Milliflex Filtration System and expensive Milliflex disposables, which makes the overall costs comparable to the outsourced ISO 11737 method (around €50).

The experiments by total immersion were executed in 2017 using ISO 11737-1:2006.[9] Currently the 2018 version is in use.[12] This new version however has no influence on the determination method itself.

The high costs of total immersion makes it less appropriate for routine monitoring. However, the high recovery makes it the method of choice for the validation of the disinfection procedure of materials. Because of differences in difficult to reach surfaces, which influences the effectiveness of the applied disinfection process, these validation studies have to be done with different kinds of materials.

Table 2 Surface bioburden after disinfection expressed as mean cfu per ampoule or vial

	Plastic ampoules		Glass ampoules		Injection vials		Infusion vials	
	Mean cfu	SD	Mean cfu	SD	Mean cfu	SD	Mean cfu	SD
Total immersion	1.60 (n=10)	2.91	0.70 (n=10)	0.95	6.20 (n=10)	6.44	8.80 (n=10)	11.35
Contact plate	0.03 (n=40)	0.16	0.20 (n=30)	0.41	0.13 (n=30)	0.43	0.07 (n=29)	0.26
Recovery (%)	1.9	–	28.6	–	2.1	–	*	–

Recovery is the number of cfu found on contact plate as a percentage of the number at total immersion.
*Recovery is not determined because of the small part of the surface which is monitored by a contact plate (see methods).
n, number of samples examined.

Table 3 Surface bioburden determined by total immersion and contact plates after disinfection (for clarification see text)

	Plastic ampoules		Glass ampoules		Injection vials	
	TI	CP	TI	CP	TI	CP
n	10	40	10	30	10	30
Positive	4	1	5	6	10	3
Negative	6	39	5	24	0	27
Mean cfu	1.60	0.03	0.70	0.20	6.20	0.13
Contaminated (%)	40	2.5	50	20	100	10

CP, contact plate; n, number of samples examined; negative, number of samples without growth; positive, number of samples with ≥1 cfu; TI, total immersion.

The next question is how many samples need to be examined? As mentioned above, the surface bioburden within one kind of material varies considerably from sample to sample. For example, after disinfection, the lowest and highest bioburden on the 10 injection vials (table 2, total immersion) were 1 and 20 cfu respectively. The number of high results has to be proportional to the sample size; therefore, in further experiments, the number of samples has to be increased.

Contact plates

Using contact plates, the contact pressure and contact time are important for a good transfer of micro-organisms to the agar surface. The advised pressure is 500 g/25 cm^2.[13] Consistency in sampling time and pressure can be ensured by a mechanical applicator.[4] This applicator, however, is not usable on the round surfaces of ampoules and vials. For manual sampling 'the weight of a single finger resting on the plate while the seconds are counted' is mentioned.[7] However, this is also not possible because the contact pressure is caused by pressing ampoules and vials on the agar surface (see figure 1). Nevertheless, a low pressure is important because the agar will break when the pressure is too high.

The longer the contact time, the better the transfer of micro-organisms[14]; 10 s is mentioned as realistic in daily practice.[5] These 10 s are converted into slowly rolling on the agar surface four times (see Methods). This, as well as the turning of the contact plate, will take around 20 s.

Slow rolling on the agar surface, without touching the agar surface with the gloved fingers and using the right pressure, makes monitoring of materials by contact plates more complicated than sampling of flat services. However, after training it can be done by an operator skilled in performing aseptic handling. Samples can be put into the incubator without further treatment. The costs are low: around €3 including sampling time.

The purpose of routine monitoring of non-sterile materials is to check the way disinfection of these materials is performed by

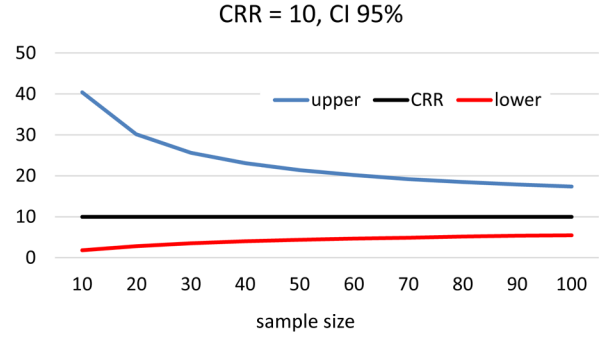

Figure 2 Upper and lower limit 95% CI at different sample sizes if contamination recovery rate (CRR) is 10.

the operators. However, taking a sample from the disinfected materials before use, rolling it on a contact plate, cleaning it and placing it back in the production environment disturbs the preparation activities. This is not allowed.[3 15] Glove prints and surface sampling from the worktop of LAF/SC/I will indirectly reflect the surface bioburden of materials. This makes daily monitoring of the disinfected materials less necessary. Therefore, checking the way disinfection is performed can be done at a different time, separate from the preparation activities.

How many samples are necessary to get a good impression of the surface bioburden? To answer this question the expected results have to be considered. As seen in table 3 column CP, many zero counts (negative) can be expected. When judging the results, they can be expressed better as a percentage of samples contaminated with ≥1 cfu.[16] In the US Pharmacopeia (USP) this is called the contamination recovery rate (CRR) and states: 'Because of the inherent variability of microbial sampling methods, contamination recovery rates are a more useful measure of trending results than is focusing on the number of colonies recovered from a given sample'.[17]

The CRR is defined in the USP as the total number of plates containing growth, divided by the total number of plates, times 100. If sufficient samples are taken, monitoring results expressed as CRR follow a normal distribution, which makes standard statistical analyses possible.[18] To judge whether a given CRR is lower than a given limit, the CI for the measurement has to be used which takes into account the uncertainty about CRR. For this CRR the expected upper and lower limit of the 95% CI can be calculated for different sample sizes.[19] The results for a constant CRR of 10 are shown in figure 2.

While using 30 samples the upper limit is 25 (see figure 2). Keeping in mind not only the accuracy, but also the amount of work required to determine surface bioburden, 30 samples of one kind of material seems to be a realistic number for a reasonably accurate value for the CRR.

Because of fewer zero counts (see table 3, column TI), the estimation of a representative sample size in total immersion by CRR is less valuable.

Swabs

Because of the extra laboratory work which has to be done after sampling (streaking onto an agar surface or rinsing and membrane filtration) and the poor recovery of the generally used cotton, rayon or polyester swabs, it is concluded without further experiments that swabs are not a useful method for routine monitoring of the surfaces of materials. For special applications, the use of expensive high-recovery swabs can be helpful.[8]

What this paper adds

What is already known on this subject

- Micro-organisms can be dragged with ampoules and vials into a laminar airflow cabinet, safety cabinet or isolator.
- Information about methods to determine the surface bioburden on these materials is scarce.

What this study adds

- A comparison of methods for surface bioburden determination on ampoules and vials before and after disinfection.
- Guidance on sample size and sampling methods for routine monitoring and for the determination of the effectiveness of a disinfection method.

CONCLUSION

Total immersion is the method of choice for the determination of the effectiveness of a disinfection procedure for ampoules and vials. Use of contact plates is recommended for routine monitoring of ampoules and vials.

Acknowledgements We thank Dr Annet van Merode, Bactimm (Nijmegen, The Netherlands), for her help in developing the procedure for total immersion of ampoules and vials as well as the interpretation of the results.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

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