Millipore®
Preparation, Separation,
Filtration & Monitoring Products

Application Note

Detection of Low Bacterial Contamination from Neoprene® Gloves with ICR Swabs

Contact plates and swabs are common methods for surface monitoring in aseptic filling lines for pharmaceuticals. Both methods are recommended by the current European and US GMP guidance.

The published recovery rates for several swabs and contact plates vary widely due to differences in chosen methods, surfaces and test microorganisms. This variability is also reported by the USP chapter <1116>, but the methods should be able to detect low levels of microorganisms, especially in grade A cleanrooms or isolators.

The manufacturing of drugs in isolators is an obvious trend in the pharmaceutical industry and therefore, microorganisms must be detected from surfaces commonly used in isolators, such as isolator gloves, e.g. made from Neoprene®.

This study was designed to prove the suitability of ICR Swabs (Ref. No. 146529) and lockable TSA w. LTHThio contact - ICR+ (Ref. No. 146783) contact plates, to detect low numbers of different bacterial test strains from Neoprene® gloves. The ICR Swab is designed for presence/absence tests on dry and hard-to-access surfaces, whereas ICR contact plates are suitable for the enumeration of microorganisms on flat, dry surfaces in cleanrooms and isolators. As a control an "Agar Overlay" method was chosen to determine the number of surviving microorganisms.

Material & Methods

Neoprene® glove pieces (5 cm x 5 cm) were cleaned and autoclaved. The surfaces were inoculated with 10 x 10 µL drops of two different dilutions of 4 microbial suspensions to obtain a homogeneous dispersion on the highly hydrophobic surface. Table 1 lists the chosen microorganisms and the total number of CFU used to inoculate the test surfaces. A higher inoculum level is used for Gram-negative bacteria because they are expected to be more sensitive towards the drying procedure of the inoculated surface material.

The number of CFU used to inoculate the test surface was controlled using the spread plate method on 90 mm TSA plates.

Table 1: Selected Test Stains and CFU inoculated by applying 100 µl of suspension

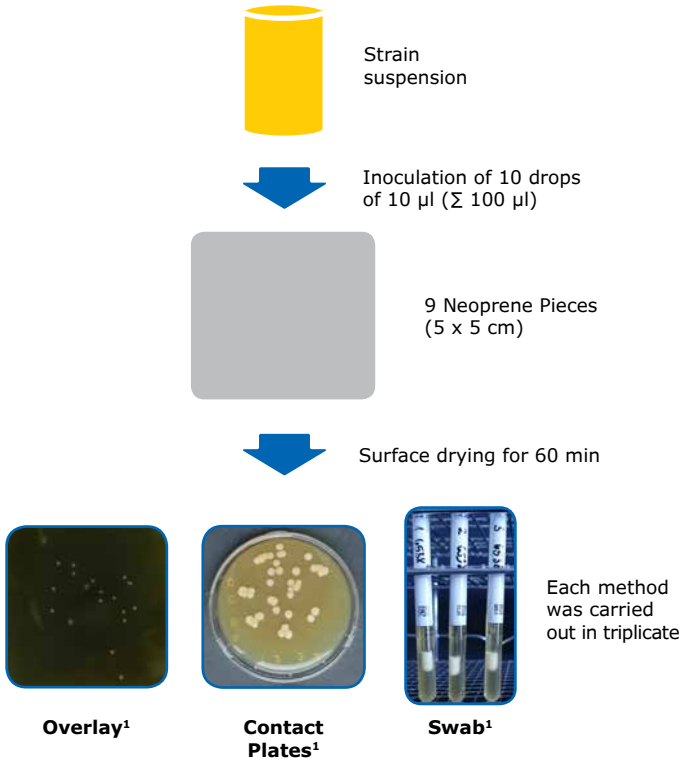
Test Strain	Dilution 1	Dilution 2
Staphylococcus aureus ATCC® 6538	93	24
Bacillus subtilis ATCC® 6633 (spores)	26	6
Escherichia coli ATCC® 8739	650	130
Pseudomonas aeruginosa ATCC® 9027	228	32

Each surface coupon was dried for 60 min under a laminar flow hood without air stream. The detection of the microorganisms from the dry surface was performed in parallel using 3 different methods and each test was performed in triplicate. The procedures are described as follows and shown in Figure 1.

1) Agar Overlay: As a control method an "Agar Overlay" method was chosen. Under the assumption that a high number of microorganisms are killed by the drying process on the surface, this test method was used to determine how many survived on the surface. The molten TSA Agar was poured onto the inoculated and dried surface. All plates were incubated for up to seven days at 30–35 °C. The colonies were counted every day.

2) Contact plate Method: The dried surface was sampled using a TSA w. LTHThio contact - ICR+ contact plate, which was pressed on the test surface for 10 seconds with a standardized pressure of 500 g. All plates were incubated for up to seven days at 30–35 °C. The colonies were counted every day.

3) Swab Method: The dried surface was carefully sampled with the premoistened swab tip of ICR Swab in longitudinal and cross directions. The broth medium from the reservoir was squeezed onto the swab tip afterwards, and the swabs were incubated at 30–35 °C for up to seven days to take into account a prolonged lag phase due to desiccation. The tubes were inspected for turbidity every day.



Incubation for up to 7 days at 30–35 °C and daily evaluation (CFU on overlay and ICR contact plates, turbidity of Swab media)

[1] = e.g. *S. aureus* (low inoculum) after 48 h incubation

Figure 1. Test procedure for microbial detection Neoprene® glove pieces by "Agar Overlay" method, TSA w. LTHThio contact- ICR+ plates and ICR Swabs:

Results and Discussion

1) "Agar Overlay" Method vs. ICR Contact Plates

The survival rate of microorganisms after dehydration on the surface materials was determined by the "Agar Overlay" method.

The detected CFU per method and test strain are indicated in Figure 2, whereas table 2 shows the approximate recovery rates by percent of inoculum. The recovery rates (Table 2) were calculated for each dilution. The listed amounts illustrate the spectrum of both dilutions compared to the inoculum.

The recovery of surviving microorganisms was dependent on the type of microorganism. The "Agar Overlay" method recovered fewer CFU than the ICR contact plate in most cases, but the results were still comparable.

Nevertheless, it can clearly be shown that spores of *B. subtilis* are most resistant towards dehydration, as the recovery was high for ICR contact plates with 70% to 85%, followed by *S. aureus* with 25% to 30%. The Gram-negative bacteria *E. coli* and *P. aeruginosa* are much more sensitive towards the dehydration of the test surface and resulted in recovery rates of less than 10% with the ICR Contact plate.

All positive results were achieved after an incubation time of ≤3 days. The prolongation of incubation up to 7 days did not improve the detection.

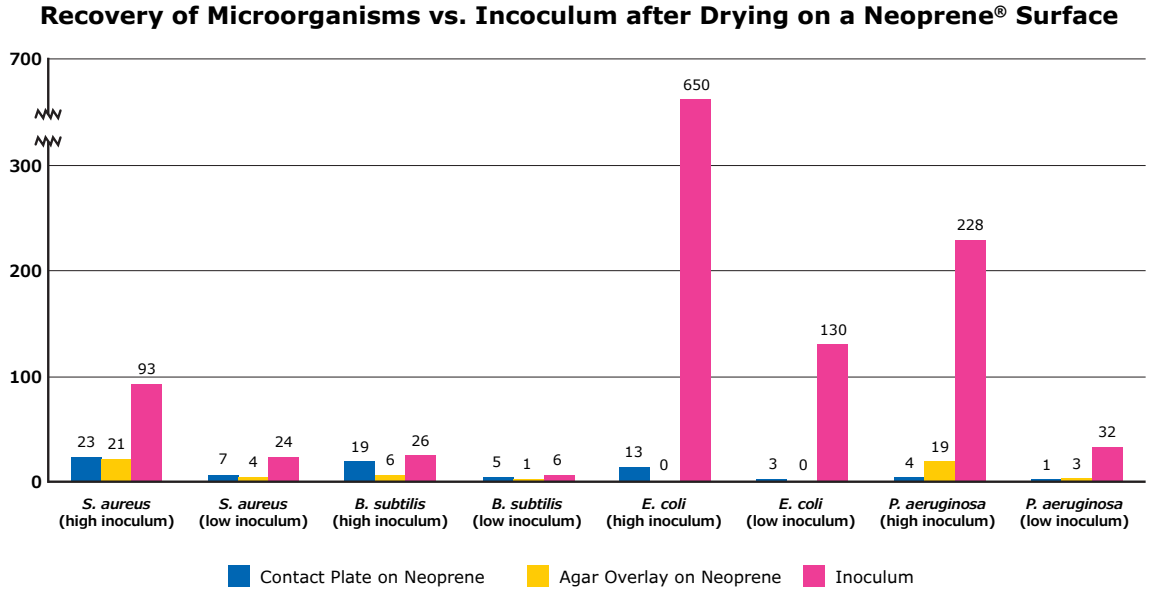


Figure 2. Survival of Test Strains on Neoprene® after a drying period of 60 minutes in CFU (Average of 3 samples each)

Surface Material	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*
Agar Overlay	15–25%	15–25%	0%	< 10%
ICR Contact Plate	25–30%	70–85%	< 10%	< 10%

Table 2: Approximate Recovery rates using ICR Contact Plates after 60 min of dehydration on the Neoprene® surface coupon or the "Agar Overlay" method

2) Detection Rates of ICR Swabs vs. ICR Contact Plates from Neoprene® Surfaces

According to the first part of this study the "Agar Overlay" was not the best method to use to determine the number of viable microorganisms on the surface following dehydration of the microbial suspensions. The CFU numbers derived using the contact plates were higher in most cases, and so were chosen to determine the suitability of ICR Swabs to detect low numbers of microorganisms on Neoprene® surfaces.

To check if the ICR Swab could be capable of detecting low numbers of microorganisms on Neoprene® surfaces, the overall rate of positive ICR samples was compared to the ICR contact plate. As indicated in figure 3, all bacteria were detected at low levels from the surface using either TSA+LTHThio cont. - ICR+ contact plates or ICR Swabs.

The total number of positive and negative samples (22 and 4, respectively) were identical for both ICR Swabs and ICR contact plates.

In all cases where the TSA w. LTHThio cont.-ICR+ contact plate detected ≥ 2 CFU in all 3 samples for the specified microbial suspension, the ICR Swab was also positive (see table 3).

When a minimum 1 out of the 3 contact plate samples either show no growth or ≤ 1 CFU single negative, the ICR Swab samples showed a positive turbidity result, as in case of *P. aeruginosa*. In addition, an average of 1 CFU for all contact plates, such as for the detection of *E. coli* results in 3 positive samples for the ICR Swab.

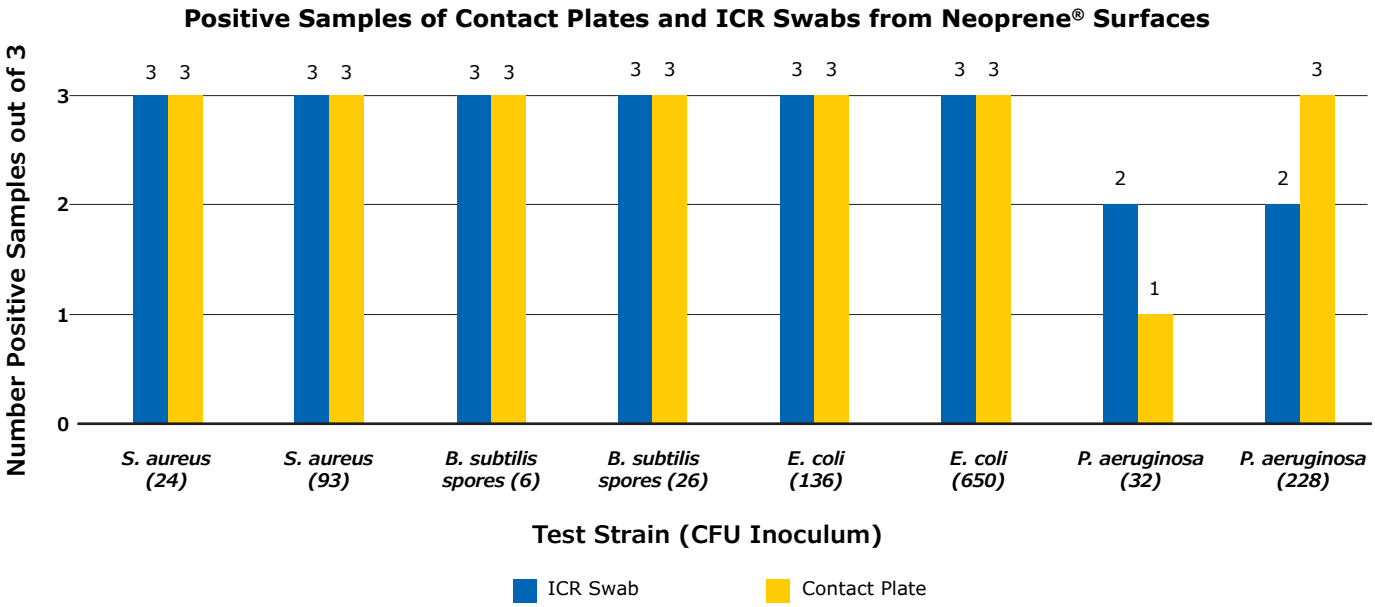


Figure 3: Detection rates in positive samples by ICR contact plates and ICR Swabs from Neoprene® surfaces with the average CFU detected from sample surface

Test Strain	Inoculated CFU to Surface	CFU ICR Contact Plate 1	CFU ICR Contact Plate 2	CFU ICR Contact Plate 3	Positive ICR Swabs
S. aureus	24	10	6	5	3 of 3
	93	22	26	22	3 of 3
B. subtilis (spores)	6	6	5	5	3 of 3
	26	20	21	16	3 of 3
E. coli	130	2	1	1	3 of 3
	650	14	11	13	3 of 3
P. aeruginosa	32	2	0	0	2 of 3
	228	4	8	1	2 of 3

Table 3: Recovery of ICR Contact Plates in CFU from Neoprene® compared to inoculated CFU and positive sample results of ICR Swabs

Conclusion

In summary, this study showed that ICR Swabs and ICR contact plates are both suitable for the detection of low numbers of Gram-positive and Gram-negative microorganisms from Neoprene® gloves and shows comparable growth results. It was shown that the overlay method is not the most appropriate method to detect microorganisms on Neoprene® surfaces. An explanation could be that the highly hydrophobic structure of Neoprene® surface could prevent homogeneous spreading of the microorganisms on the surface.

Literature

1. FDA Guidance for Industry (2004): Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice.
2. United States Pharmacopoeia 40 NF 35: <1116> Microbiological Control and Monitoring of Aseptic Processing Environments
3. EU GMP Guide (2008): Volume 4 EU Guidelines to Good Manufacturing Practice Medicinal Products for Human and Veterinary Use - Annex 1 Manufacture of Sterile Medicinal Products (corrected version)

MilliporeSigma
400 Summit Drive
Burlington, MA 01803

To place an order or receive technical assistance

In the U.S. and Canada, call toll-free 1-800-645-5476
For other countries across Europe, call +44 (0) 115 943 0840
For other countries across Europe and the world, please visit: **EMDMillipore.com/offices**
For Technical Service, please visit: **EMDMillipore.com/techservice**

EMDMillipore.com

Lit No. MS_AD4911EN Rev. 1.0
2019 - 24967
10/2019