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RESEARCH

Evaluation of the Recovery Rate of Different Swabs for Microbial Environmental Monitoring

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ABSTRACT: Contact plates, dipslides, and swabs are used for the microbiological monitoring of surfaces in controlled environments such as pharmaceutical clean rooms. In the present study, three different swab types using two different methods (direct streaking on agar versus elution followed by membrane filtration) were evaluated. In a first study, representative surfaces in pharmaceutical clean rooms were artificially inoculated using three different environmental strains (in vitro study). In a second study, a naturally inoculated floor was swabbed with the same three swab types, again using the two different recovery methods (in situ study). With the in vitro study, clear differences were found between the three swab types as well as between the two recovery methods. In addition, recovery rate of the swab type was dependent on the recovery method (interactive effect). One swab type showed a higher recovery rate with direct streaking on agar, while the other swab type showed better results using the elution/membrane filtration method. This difference can be explained by the fact that both swabs were each developed for their specific application. The type of surface also had a highly significant effect on the recovery rates. Recovery on stainless steel was better than for the other surfaces, while lexan had the lowest recovery rate. From the three different strains applied in the in vitro study, *Micrococcus luteus* had significantly higher recovery results compared to the other two strains (*Bacillus thuringiensis*, *Aspergillus brasiliensis*). The differences in recovery between the swab type and recovery method were less pronounced in the in situ study. In particular, the recovery of the swab type depending on the recovery method was not found. In conclusion, if swabs are to be used for environmental monitoring, their suitability should first be evaluated. This can be approached with artificially inoculated surfaces. However, naturally inoculated surfaces might be more realistic and might better reflect what is found in pharmaceutical clean rooms.

KEYWORDS: Microbiological environmental monitoring, Recovery rate, Swab, Membrane filtration, Streak on agar, In situ study.

LAY ABSTRACT: Environmental microbiological monitoring provides information on the hygiene condition of pharmaceutical clean rooms and equipment for manufacturing of drug products. Different methods can be used to recover microorganisms. For surfaces, normally contact plates (e.g., RODAC or dipslides) are used; however, when surfaces are uneven, swabs should be used. In the present study three different swabs were evaluated for their ability to recover microorganisms from different surfaces. Thereby two methods and two approaches were evaluated. Swab samples were either directly stroked on agar or the swab was eluted, membrane-filtrated, and the filter placed on an agar plate. Experimentally, artificial inoculated surfaces typically found in clean rooms (in vitro study) and naturally inoculated floors (in situ study) were sampled. Thus with this approach the most convenient swab and the most suitable recovery methods under laboratory as well as real clean room conditions were evaluated. With this set-up, we found the most suitable swab for our environmental monitoring not only by using artificial inoculated surfaces but also under more realistic clean room conditions, which is most important for microbiological environmental monitoring sampling.

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1. Introduction

To control the environment of clean rooms, microbiological monitoring needs to be established. In general the air and various surfaces (floor, tables, equipment, and product-contacting surfaces) are monitored. For air monitoring, various well-established air samplers are available. However, each device has certain advantages and disadvantages (1, 2, 3, 4). For flat surfaces in general, contact plates or dipslides are used. These methods are established and their recovery rates have been evaluated in different studies. Depending on the set-up, recovery rates of 0–59% are found (see reference *5* for an overview). In several unpublished studies in which pharmaceutical companies evaluated the recovery rate of contact plates on facility surfaces applying different in-house microorganisms, recovery rates of approximately 40–60% were found (M. Goverde and A. Staerk, unpublished data). This is in line with the result from one of the first studies published on this topic, in which Angelotti et al. investigated the recovery rates of contact plates in 1964 (6). In this study a recovery of 41% for contact plates was found compared to 47% for cotton swabs using the rinse method.

Swabs are normally used for sampling from non-flat surfaces such as tubes, seals, and valves. Before use, the swab needs to be moistened. Dry swabs result in a very low recovery rate (*7,* M. Goverde, unpublished data). After swabbing the surface, the swab can either be directly streaked onto the surface of an agar (e.g., contact agar plate; see reference 8) or it can be rinsed with a buffer, after which the rinsing solution is filtered and the filter is placed on an agar plate to grow colonies trapped on the filter surface (8, 9). In both cases the numbers of colony-forming units (cfu) are counted after incubation. Depending on the method or the swab type used, the recovery of the microorganisms collected by the sampling can differ significantly (e.g., *7, 10, 11, 12, 13*). However, this has mainly been shown with artificially inoculated swabs or surfaces.

In the present study the recovery rate of three different swab types using both the direct streak method and the rinsing method were evaluated using two different approaches. In a laboratory experiment (in vitro study), different surfaces typically used in pharmaceutical production clean rooms were inoculated with three different microbial strains. The surfaces were then sampled using three different swabs that were either streaked onto an agar plate or rinsed using a sodium chloride peptone buffer followed by enumeration with the membrane filtration method. Although such experiments are helpful to evaluate the efficacy of the recovery rate, their design is different to what we find in a routinely sampled clean room where the surfaces are contaminated with environmental strains in a natural way. Differences between in vitro studies and naturally occurring contaminations were shown in other studies (14, 15). To overcome this problem, in the second study an in situ approach was used, that is, both methods and all three swabs were used to sample a defined surface—in this case a microbiology laboratory polyvinyl chloride (PVC) floor, which is also used in production. Here it is not the recovery rate itself that can be calculated because the real number of microorganisms is unknown. However, using appropriate statistics the difference between the swab types, the applied recovery method, and their interactions can be fitted.

2. Methods

For both studies described hereafter three commonly used swabs were evaluated:

- Swab 1: Nylon-flocked Quantiswab® from Copan, bioMérieux, Ref: 43801. According to the supplier this swab is seen as superior to others due to its hydraulic capillary action. It shows a higher recovery and a better release rate than other swabs.

- Swab 2: Sterile Swab for Environmental Monitoring, Becton, Dickinson and Company, Catalog No. 220518. This is a ready-to-use, sterile Dacron™ swab in a pre-filled tube of rinse solution (10 mL) for surface and equipment sampling.

- Swab 3: heipha ICR swab from Merck Millipore, Art. model 146529. This knitted polyester, pre-moistened swab has its own culture broth reservoir to minimize the risk of secondary contamination due to handling.

2.1. In Vitro Study

For the in vitro study, four typical types of surface in the production facility were chosen. These were glass, stainless steel, polycarbonate (lexan®) and Teflon (APSOplast® PTFE virgin). The samples were cut into 25 cm^2 squares, thereby representing the size of contact agar plate used for microbial environmental monitoring.

The following three different species, which are most commonly found in the environmental monitoring, were selected as microbial strains:

- *Micrococcus luteus*: Gram-positive coccus, typical species of human skin flora; in-house isolate.

- *Bacillus thuringiensis*: Gram-positive rod, typical species from the environment; used as spore suspension; in-house isolate.

- *Aspergillus brasiliensis*: mold; typical species from the environment, fungal representative, used as a spore solution; because no in-house isolate was available, the ATCC 16404 strain was used.

For *M. luteus* a suspension of 20–200 cfu/10 µL was prepared by using three colonies from a CASO (casein soya bean digest agar, Oxoid Code CM0131 using 40 g in 1 L purified water) agar plate incubated for 3 days at 30–35 °C. These colonies were suspended in 500 µL PBPS (buffered sodium chloride peptone broth, Merck, No. 110582 using 16.1 g in 1 L purified water) + 1% BSA (albumin bovine fraction V pH 7, Acros organics, No. 240401000 using 10 g in 1 L purified water) and diluted until the correct cfu/mL was reached. For *B. thuringiensis* and *A. brasiliensis*, spore suspensions were directly diluted in PBPS + 1% BSA until 20–200 cfu/10 µL was reached. From these suspensions, 10 µL were used for all four different surfaces. For glass, steel, and lexan, two droplets of 5 µL each were placed on the opposite corners of the surface and were spread over the surface using a glass slide while leaving a dry, 5 mm border to ensure none of the suspension was lost. Due to the high surface tension of Teflon, the two 5 µL droplets were placed on the same side of the surface and spread to the other side using a glass slide. This was repeated in each of the other directions, that is, a total of four times per droplet, to ensure that the suspension was distributed over the surface. For drying, the surfaces were placed in a laminar air flow hood.

In preliminary studies the time for drying was visually defined for each surface type. To keep the handling easier, the next higher minute was chosen. This was 3 min for steel and glass, 4 min for Teflon, and 5 min for lexan. All the surfaces were cleaned with 70% ethanol and autoclaved at standard conditions (121 °C at 2 bar for 15 min) before use.

Each strain was independently tested three times for each treatment. For each run, the following treatments were carried out:

- Inoculum control: 10 µL of the suspension was spread on CASO.

- Pour plate control: After distributing 10 µL suspension on the corresponding surface and drying (see above), the entire surface was placed in a petri dish and approximately 20 mL of liquefied CASO agar was poured in.

- Swab for streak on agar: After distributing 10 µL suspension and drying (see above), the surface was swabbed with the corresponding swab and this swab was then streaked on a CASO+LTHTh agar (heipha Merck Millipore, article number ICR 0308260e) that is routinely used for environmental monitoring.

- Swab for membrane filtration: After distributing 10 µL suspension and drying (see above), the surface was swabbed with the corresponding swab and this swab was then suspended in 9 mL of PBPS by vortexing the suspension for approximately 10 s. Afterwards the suspension was filtered and the filter was placed on a CASO+LTHTh agar plate for incubation.

All the plates were incubated at 25–30 °C for 2–7 days depending on the microorganism according to the standard procedure used and validated at the site (14). The recovery rate was then calculated, that is, the cfu from the swab was divided by the cfu from the control. For the control, the mean of three different replicates was used.

To avoid any systematic effects, the order of the different treatments was randomized (pour plate control, swab for streak on agar, and swab for membrane filtration) per strain, and the surface was defined by throwing a dice.

Because in the present study the suspension was applied and dried on the surface the same way for both the control and treatment, no correction for loss of drying was needed. Nevertheless, in a preliminary study the mortality rate was determined, that is, the loss of viable cells due to the drying process was evaluated. An overall loss of 42% was found. This degree of loss depended on the strain and surface used. The highest mortality rate was found on Teflon (54%) and the lowest on stainless steel and lexan (37% and

TABLE I
Reduced Full-Factorial ANOVA for the Recovery Rate of the In Vitro Study

Parameter	d.f.	Sum of Squares	Mean Square	F-Ratio	*P*-Value
Run (R)	2	104.27290	52.13645	30.7626	<0.0001
Swab Type (S)	2	320.58169	160.290845	94.5780	<0.0001
Method (M)	1	7.78339	7.78339	4.5925	0.0334
Surface (O)	3	22.96575	7.65525	4.5169	0.0044
Microorganism (MO)	2	417.79789	208.898945	123.2587	<0.0001
S × M	2	30.85641	15.428205	9.1033	0.0002
S × O	6	9.82021	1.63670167	0.9657	0.4499
S × MO	4	11.32249	2.8306225	1.6702	0.1588
M × O	3	2.53508	0.84502667	0.4986	0.6837
M × MO	2	17.19085	8.595425	5.0716	0.0072
O × MO	6	15.72260	2.62043333	1.5462	0.1655
Error	182	308.4537	1.69480055		
N total	216				

To reach normal distribution, the data were square root–transformed. The factor "Run" was used as a co-variable. Higher interactions were not significant and therefore they were omitted from the analysis (for details see section 2.3. Statistical Analysis). d.f. = degree of freedom.

36%, respectively). Of the three different species used, the highest mortality rate was found for *M. luteus* (55%), while the two others strains showed a loss of 35%. This difference is not surprising, as a spore suspension was used for *B. thuringiensis* and *A. brasiliensis*.

2.2. In Situ Study

To verify the results of the in vitro study, an in situ study was performed with the same three types of swabs. The sampling was performed on three different microbiology quality control laboratory floors made of PVC, which is also used in some areas of the production facility. The floor of these labs were swabbed, and the swabs were then either directly smeared on agar surfaces or the swab was suspended in PBPS (both methods as described above), resulting in 6 treatments per replicate: 3 different swabs + 2 different methods (streak on agar; suspension in buffer with concurrent membrane filtration). In each lab, 10 replicates of the treatment series were performed. For the in situ study no recovery rate was calculated because the real bioload of the floor is unknown, that is, the colony-forming units (cfu) per 25 cm^2 was enumerated and only the differences between the methods and swab types were statistically compared.

A 25 cm^2 square made of Teflon was used as a template to ensure the same surface area monitored for each swab. As with the in vitro study, the order of the different treatments was randomized to avoid any systematic effects and was defined using random numbers.

2.3. Statistical Analysis

Both studies were analyzed by a full-factorial analysis of variance (ANOVA) using JMP 5.1.2 (SAS Institute Inc.). For the in vitro study the following main factors were fitted: "Microorganism" (*M. luteus, B. thuringiensis, A. brasiliensis*), "Surface" (glass, lexan, stainless steel, Teflon), "Swab Type" (three different swabs), and "Method" (streak on agar, membrane filtration). The factor "Run" (1, 2, and 3) was fitted as a co-variable. To control for normal distribution, the residuals of this model were tested using JMP 5.1.2. (SAS Institute Inc.). To achieve normal distribution and improve homoscedasticity, the data were square root–transformed. This model reached a $R^2 = 0.757$. Because high interactions were not significant, a stepwise model reduction was applied whereby the highest interaction with the lowest significance was omitted (16). Model reduction ceased when the two-fold interactions were reached. Using this approach the model in Table I was fitted.

For the in situ study, an ANOVA with the two co-variables "Replicate" (10 replicates per lab) and "Lab"

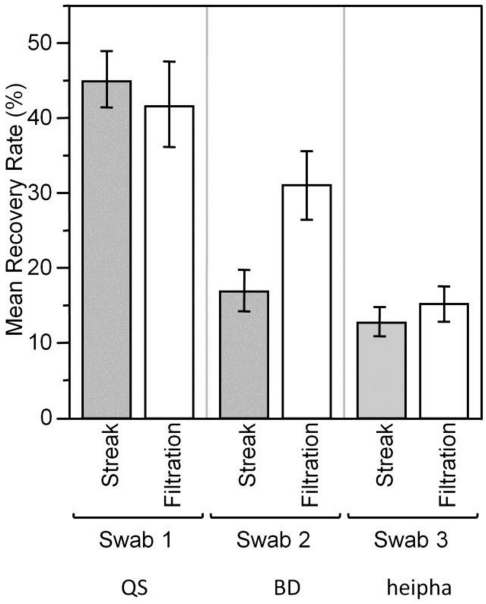

Figure 1

Differences in the mean recovery rate for the three swabs as a function of the two methods (streak on agar versus membrane filtration) found in the in vitro study. Mean ± standard error of the mean; N = 216.

(3 different rooms), and the main factor "Method" (3 different swab types either streak on agar or membrane filtration) was fitted. To reach normal distribution the data needed to be log-transformed [log(X+1); X+1 is needed because some recovery results were 0]. For this study all data above 300 cfu were not used because a correct enumeration above 300 cfu per plate is difficult and inaccurate. If not otherwise stated, throughout this article we give the arithmetic mean as measure of central tendency and the standard error of the mean as measure of variability.

3. Results

3.1. In Vitro Study

The results of the in vitro study are presented in Figures 1–3 and Table I. The swab type has a strong influence on the recovery rate (Figure 1, $P < 0.0001$ in Table I). The highest mean recovery rate of 43.6% was found for swab 1, while swab 2 recovered 24.1% and swab 3 14.1%. The method (streak on agar versus membrane filtration) showed a significant effect (Figure 1, $P = 0.0334$ in Table I), with a higher recovery rate for the membrane filtration method (29.4%) than

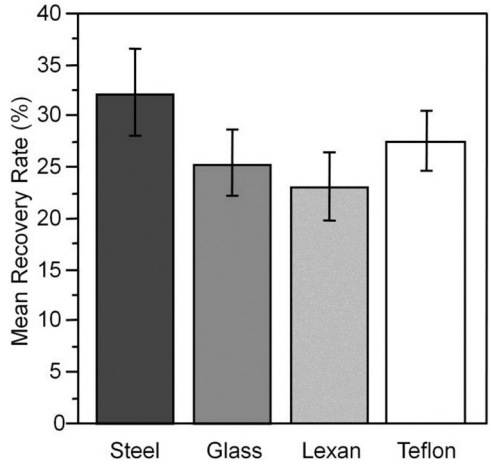

Figure 2

Overal mean recovery rate found for the four different surfaces used in the in vitro study. Mean ± standard error of the mean; N = 216.

the streak-on-agar method (24.0%). Much more important is the interactive effect found for "Swab Type" by "Method" ($p = 0.0002$ in Table I). For swab 2, the recovery rate using membrane filtration is better than the recovery rate using the streak-on-agar method,

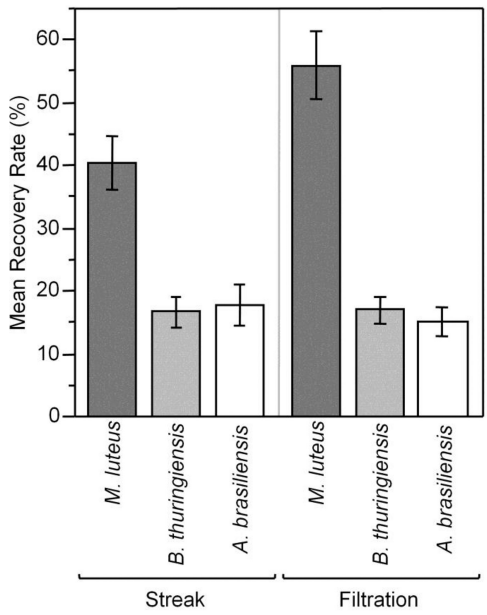

Figure 3

Recovery rate for the three different species used in the in vitro study depending on the method used (streak on agar versus membrane filtration). Mean ± standard error of the mean; N = 216.

TABLE II
Full-Factorial ANOVA for the Colony-Forming Units (cfu) Found in the In Situ Study

Parameter	d.f.	Sum of Squares	Mean Square	F-Ratio	*P*-value
Replicate	9	10.87618	1.20846444	1.0553	0.3992
Laboratory	2	143.88766	71.94383	62.8245	<0.0001
Method (M)	1	2.47870	2.4787	2.1645	0.1433
Swab Type (S)	2	15.79859	7.899295	6.8980	0.0014
M x S	2	2.85044	1.42522	1.2446	0.2910
Error	153	175.20894	1.14515647	1.1452	
N total	170				

To reach normal distribution the data were log(X+1) transformed. The factor "Replicate" and "Laboratory" were used as co-variables. A total of 10 values were >300 cfu and were omitted because a correct enumeration was not possible. These 10 values were randomly distributed over the treatments. d.f. = degree of freedom.

while for the other two swabs no effect or the opposite is true (Figure 1).

Figure 2 shows the recovery rate on the four different surfaces. The highest recovery was found on stainless steel with 32.4%, while the lowest was found for lexan with 23.3%. The factor "Surface" resulted in a highly statistically significant difference ($P = 0.0044$) and was independent of the other main factors, that is, none of the interactions showed significance (Table I).

Finally, the species of microorganism also influenced the recovery rate significantly ($P < 0.0001$ in Table I). *M. luteus* was recovered with 48.2%, while recovery of 16.9% for *B. thuringiensis* and 16.6 for *A. brasiliensis* was found (Figure 3). This effect was independent of the swab type or surface (no interactive effect in Table I), but an interactive effect with the method was found that is related to the fact that the method did not influence recovery for *B. thuringiensis* and *A. brasiliensis,* but it did for *M. luteus.* Higher recovery of *M. luteus* was achieved by the membrane filtration method than the streak-on-agar method (Figure 3).

3.2. In Situ Study

For the in situ study, the method used (streak on agar versus membrane filtration) did not have a significant effect on the number of microorganisms recovered; neither did it interact with the swab type (Table II). The recovery for the streak method was 49 ± 7 cfu/25 cm^2 and for the membrane filtration method 41 ± 6 cfu/25 cm^2. A highly significant effect on the number of microorganisms recovered was found in regard to the swab type (Figure 4, Table II). This was mainly due to the poorer recovery of swab 3, which confirms the results of the in vitro study.

The highly significant difference between the laboratories (Table II) can be explained by the much higher frequency of people coming into laboratory 1 and the missing sticky mat to reduce microbial contamination

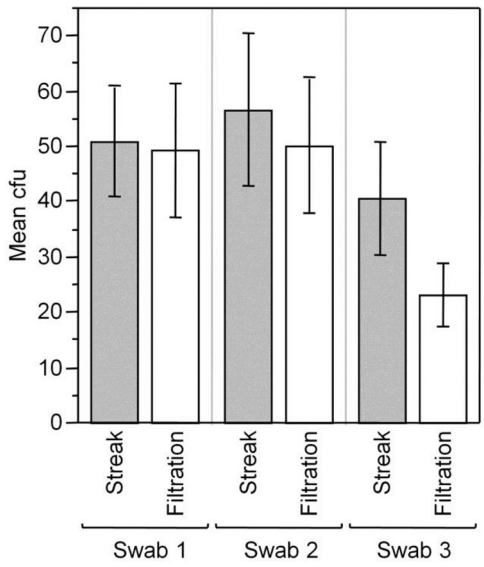

Figure 4

Differences in the mean colony-forming units (cfu) for the three swabs as a function of the two methods (streak on agar versus membrane filtration) found in the in vivo study. Mean ± standard error of the mean; N = 170 (The original N was 180, but a total of 10 values were >300 cfu and were omitted from the analysis. These 10 values were randomly distributed over the treatments).

on the floor at the entrance. Therefore higher counts in this lab are the logical consequence [mean values ± standard error of the mean (SEM) of the three laboratories: 95 ± 10 cfu versus 29 ± 4 cfu versus 16 ± 4 cfu)]. The replicate did not have any statistical significant effect, which shows the reproducibility of the data collected.

4. Discussion

4.1. Effects of Swab Type and Method Used

With the in vitro study, highly significant differences in the recovery rate of the three tested swabs were found. The low recovery rate of swab 3 is not surprising. This swab is designed for a Grade A area with a yes/no answer and not for enumeration. For swabs 1 and 2, a similar recovery rate was expected because both are designed for quantitative recovery. However, the results show that there is a higher recovery for swab 1 compared to swab 2. This difference might be due to the different materials of the swab head. Swab 1 is a nylon-flocked swab, while swab 2 is made of Dacron™ on a polypropylene applicator.

A significant difference was found for the method of recovery. In general, membrane filtration was better than streaking on agar. This difference is not surprising, as it can be suggested that recovery of microorganisms from the swab should be better if the swab is first eluted in a liquid and the elution then filtered. However, with 13.6% the difference is small in terms of microbiological recovery methods (12 ± 1 cfu for the streak on agar versus 14 ± 1 cfu for the membrane filtration; mean ± SEM) where often 30–50% is seen as no difference (17, 18). A comparable difference in recovery rate between direct plating (streak on agar) and filtrating was found by Dalmaso et al. (12). In this study an increased recovery of 6% was found using the filtrating method compared to streak on agar. Unfortunately no statistical comparison was performed, and therefore it cannot be judged if this difference is significant or not.

The interactive effect between swab type and method is of interest. For swab 2 in particular, the recovery rate with the membrane filtration method was much better than with the streak method, while for the other two swabs no effect or the opposite effect is found (Figure 1). This difference can be explained by the different applications indicated for the swabs. Swab 1 was developed to be streaked directly onto an agar plate, while swab 2 is delivered with a tube containing a buffer solution to re-suspend it and then filter the suspension. This shows the high specificity of development work by the suppliers. The result found here underlines the relevance of evaluating different swabs for routine monitoring to have the best recovery rate.

Several studies show the importance of evaluating different swab types with regard to their effect on the viability of microorganisms. In a study using 14 different swabs for the recovery of *Chlamydia trachomatis,* high recovery was found for certain swabs while others were toxic to the bacteria (10). Österblad et al. (11) evaluated a swab with highly absorbent cellulose viscose sponge material compared to three traditional swabs. In clinical specimens they found no differences in the recovery of beta-hemolytic streptococci, but using the swabs for bacteria suspended in broth, the recovery was better for the cellulose viscose sponge swab compared to the other tree swabs. A comparison of nylon-flocked and rayon swabs was performed by Dalmaso et al. (12). They found a superior recovery rate of 55.4% for the flocked swabs compared to 19.7% for the rayon swab using experimentally inoculated surfaces. Also, a better recovery was found for nylon-flocked swabs compared with rayon swabs by Hedin et al. (19), while their recovery rate was comparable to ours, at 13–56%. In a further study, steel coupons artificially inoculated with *Bacillus anthracis* were swabbed with four different swabs: cotton, macrofoam, polyester, and rayon swabs (7). The number of recovered spores ranged significantly from 11.5% to 43.6%. Finally, Probst et al. (20) showed the difference between a well established National Aeronautics and Space Administration (NASA) recovery method for bacterial spores compared to a new protocol using flocked swabs. The traditional method recovered 13.2% spores, while the new swabs recovered 45.4% and 49.0%. All these findings show that the recovery rates of different swabs can differ significantly and therefore imply the importance of evaluation studies to find the best swab and method to be used for microbiological monitoring of clean rooms.

4.2. Effect of Surfaces

With the in vitro study, the difference in recovery rates on the four surfaces was found to be highly significant. The highest rate of recovery was found on steel, while the lowest was found on lexan. These differences are probably due to the different surface structures of the materials. For example, for Teflon it was very difficult

to inoculate the surface due to the hydrophobic properties of the material. It is important to note that for this surface the effects were independent of the swab type, method, or microbial species used (no interactive effects in the ANOVA).

Differences in recovery rate between surfaces have been demonstrated using more highly differentiated surface types. For example, Probst et al. (20) found recovery rates of 5.9% to 62.0% depending on the surface used. Compared to the present study, the only comparable surface used was stainless steel, for which a recovery rate of 45.2% was found. In our study on stainless steel, a mean recovery rate of 34.5% was found. Also, high variances among different surfaces were shown in the study of Maunz and Kanz (21). For example, the recovery from cotton tissue was 0.1%, from wood 2.8%, while for steel and tiles it was 46% and 47%, respectively.

4.3. Effect on Species Recovery

Looking at the different species used in the in vitro study, the recovery rate for *M. luteus* compared to the other two species was significantly better. Additionally an interactive effect with the method appears that again is mainly due to better recovery of *M. luteus* using the membrane filtration method compared to the streak-on-agar method (Figure 3). The higher recovery of *M. luteus* compared to the other two species could be linked to different factors. For *M. luteus* vegetative cells were used while for the other two strains spores were applied. Thus, for vegetative cells the isoelectric charges or their hydrophobicity could be different than for bacterial and fungal spores. For the interactive effect it can be assumed that recovery of *M. luteus* using the swab is higher by filtration than by the streak technique. Another possibility is a higher release rate of *M. luteus* compared to the other two strains. In in vitro studies, a release rate between 83.8% and 93.9% of the recovered microorganisms was found upon direct inoculation to the swab with concurrent plating on agar plates or vortexing and filtrating (7, 12). In an additional study, a strong difference (nearly factor 10) was found using inoculated nylon-flocked swabs compared to two other swab types. However, the membrane filtration method was used with the nylon-flocked swab while the streak-on-agar method was used for the other two swabs (13). Thus a correct direct comparison cannot be made.

4.4. In Situ Study

In contrast to the in vitro study, in the in situ study the recovery method did not show a significant difference. This missing effect could be due to the higher variability (e.g., different microbial species are found than in the in vitro study used) and higher complexity of the in situ study that are found in other biological contexts (e.g., *14, 22*). However, the swab type in itself did matter in the in situ study. While in the in vitro study swab 1 clearly showed superior recovery over swab types 2 and 3, in the in situ study swab types 1 and 2 were equal. The highly significant effect found was therefore due to swab type 3, especially with the filtration method, where the lowest recovery was found. Thus in contrast to the in vitro study, in the in situ study the recovery of the two swabs that are designed for quantitative recovery (swab 1 and 2) is equal, independent of the method used. The low recovery results of swab 3—as in the in vitro study—might result from its design for a yes/no answer, that is, it might recover the same amount or even more than the other swabs but its release rate is worse. This would not matter in a yes/no answer test, while it does matter for enumeration. The only study the authors are aware of using an in situ approach comparable to the present study is the one by Dalmaso et al. (12). They also found a less pronounced difference between the swabs tested compared to the in vitro study. These results show that the recovery of experimentally inoculated surfaces can be substantially different from that of naturally inoculated surfaces. These findings apply not only to swabs but also to contact plates used for environmental monitoring (*14,* Goverde unpublished data).

5. Conclusion

In the present study, significant differences in recovery rates between different swabs are demonstrated. Therefore, when swabs are used for routine microbiological monitoring in clean rooms, it might be advisable that their efficacy is evaluated, preferably in studies as described in the present article. However, at least a thorough evaluation of the suppliers' validation documents or scientific literature search should be performed. Performing practical studies brings up the question if this should be done with artificially inoculated surfaces (in vitro) or with naturally inoculated surfaces (in situ). Both approaches have their pros and cons. In vitro studies are very controlled and it is easier to show their effects, but how meaningful the results might be in the real world is questionable. In

situ studies, in contrast, reflect more faithfully what is found in clean rooms. However, here the inoculum is less controlled, so statistically correct study design and result evaluation are very important. In the present study, the in vitro results showed clear preferences for one swab type in association with the method used. However, as shown by the in situ study, these differences might be irrelevant to routine use.

Finally, one of the main questions is how well microorganisms are absorbed by swabs or contact plates. Several studies show that when repeatedly sampling the same spot, even after sampling 10 times, some microorganisms are still recovered (summarized in *5* and *21*, for swabs see also *19* and *23*). In general after sampling the same surface spot 3 times, approximately 80–90% of the microorganisms are recovered, while after sampling once approximately 40–50% are recovered. This recovery rate is in line with other data where a recovery of 40–60% was found for using contact plates and different artificially inoculated surfaces (*6,* M. Goverde and A. Staerk unpublished data). In conclusion, correct evaluation of methods and devices used for microbiological methods must be performed by the user. Thereby, depending on the questions to be answered, studies must be designed appropriately. These studies can be performed using clearly defined parameters, that is, standardized microbial inoculum, several surface types, and so forth. However, the results can significantly differ from what is found if naturally inoculated surfaces are used.

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Conflict of Interest Declaration

The authors declare that they have no competing interests.

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