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Assessment of the disinfection of impaction air sampler heads using 70% IPA, as part of cleanroom environmental monitoring

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Active (volumetric) air-sampling is an important component of the environmental monitoring of cleanrooms. It is important that the results of such monitoring are accurate. One aspect of ensuring that the result is 'valid' is through minimising cross-contamination. The 'at risk' part of the sampler is the head. There are three alternatives to control cross-contamination during active air sampling contamination control: using multiple air samplers, autoclaving the sampler head in-between samples, or disinfecting the sampler head intermittently. This paper summarises a study where a disinfectant (70% isopropyl alcohol) was used to disinfect the head of an impaction air sampler between sampling sessions (spray-and-wipe technique). The study examined two factors: disinfectant decontamination effectiveness and the potential for the inhibition of microbial growth. With decontamination effectiveness, successive operations of an air sampler were examined within different cleanroom grades; with microbial growth inhibition studies, different disinfection time points were assessed. The paper concludes that this method of contamination control is effective and applicable to most cleanroom monitoring situations: it is unlikely to allow carry-over of microbial contamination and it is not shown to cause inhibition of microbial growth.

Key words: Environmental monitoring, active air sampling, biocontamination control, disinfection, cleaning, sanitisation, alcohol, culture media, microbiology.

Introduction

Contamination control is a necessary part of pharmaceutical processing to avoid microbial contamination of the environment or product[1]. This is achieved through good cleanroom design (air filtration, air movement, air changes, and pressure differentials) and through the correct gowning and training of personnel[2]. Once contamination control has been established, physical and microbiological tests can be undertaken to verify cleanroom cleanliness. These tests range from formal classification (primarily the assessment of airborne particulates) of cleanrooms to scheduled environmental monitoring.

An effective environmental monitoring programme will be risk based, with the locations selected and frequency of sampling based on some form of assessment; moreover, the programme will include a range of sampling methods. While rapid microbiological methods are promising (such as spectrophotometric particle counters)[3], the majority of methods are comprised of the classic culture-based techniques: settle plates, active air sampling, surface sampling by contact plates and swabs; with personnel monitoring by finger dabs and gown plates, depending upon the cleanroom grade and nature of operations.

Each of the environmental monitoring methods is variable and, despite a long history of use, the meteorology has a wide range of tolerance and is, to an extent, poorly understood. One of the greatest variabilities is with active air samplers.

Microbial air samplers are used to collect a predetermined volume of air and operate in a way to capture the microorganisms onto an agar-based growth medium. There are different models of active air sampler available for the achievement of this task. These are, in terms of widespread use, impaction, centrifugal or filtration[4]. An air sampler with a sieve-like impaction head functions by the aspiration of air through small holes on the sieve. Below the sampler head is a mechanical fan which draws air in through the lid. The air is forced to directly impact onto a Petri dish containing microbiological agar. Any microorganisms in the air stream are deposited onto the surface of the agar. A centrifugal air sampler draws air into the sampler head through a rotating vane mechanism. The vane causes microorganisms to be thrown out of the air and onto the agar through the effect of the centrifugal force. For filtration air samplers, air is sucked

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through a filter and any microorganisms are captured onto a membrane filter, which is then transferred to the surface of a culture medium and this is then incubated[5].

Variables that affect the collection efficiency relate to the sampler itself; the environment within which it operates, the culture medium used and incubation conditions, and the distribution of microorganisms in the environment. These factors form a necessary part of the qualification of environmental monitoring sampling methods[6].

Differences between samplers are expressed in terms of collection efficiency, which is a factor of the physical parameters of the sampler and the d50-value (cut-off size for particle collection)[7,8]. Collection efficiency relates to variables: inlet (or extraction efficiency), which relates to representative collection and transport of particles to the growth medium; and separation efficiency (separation and collection of the particles drawn into the device)[9]. The focus on particles is important given that few microorganisms in cleanrooms are found 'free floating'; the majority originate from personnel and they are attached to rafts of skin detritus or dust[10,11]. With the model selection, although no single model or type has universal acceptance and each model has strengths and weaknesses[12], in this study was a Merck-Millipore MAS - 100 NT air sampler. This is an impaction sampler, with technology traceable to the pioneering Anderson Disposable Sampler[13].

In relation to the environment, some samplers perform less well within unidirectional airflow. With this, some samplers are less effective at collection or the sampler itself can disrupt the airflow, triggering a potential contamination control risk as well as resulting in the collected data being unrepresentative[14]. With culture media, the medium itself will be limited by what it can recover, even with a 'universal' medium like tryptone soy agar; a secondary variable is the temperature range selected for incubation and the time that plates remain in the incubator for[15].

With distribution, there will always be a degree of inaccuracy because microorganisms are not evenly distributed in air and several factors, such as moisture, temperature, electrostatic charge, light, air movement, and so on, influence the distribution[16,17].

Whilst the issue pertaining to sampler selection and operation are important, ensuring that the results obtained are valid is also equally important. Here 'valid' is discussed in the context of the result obtained reflecting a proportion of the microorganisms collected from the cleanroom air rather than a facet of contamination being carried over between sampling sessions.

The use of an air sampler for multiple samples within a given sampling session is commonplace. When an operator undertakes environmental monitoring, it is typical to sample more than one location within a given cleanroom and for more than one cleanroom to be sampled. Here, it is important to ensure that the head of the sampler, especially the region within that has made contact with microbiological culture media, is decontaminated between each sample. The design of the 'head' varies with the model of sampler. A sieve-like head, for example, is commonly used with impaction samplers; whereas a rotor-head is used with a centrifugal sampler.

Most impaction air samplers are fitted with metal heads (typically fashioned from stainless steel or aluminium, with stainless steel necessary where autoclaving is required). An alternative is irradiated antistatic resin plastic disposable heads, although these are not suitable for all models of air sampler and they are relatively expensive compared with re-using a head. The use of metal heads allows the heads to be sterilised via autoclaving. Regular autoclaving of sampler heads is important, especially when an air sampler is transferred between areas of different cleanroom classes, and it is a sensible practice when monitoring areas where the expected recovery of airborne microorganisms is minimal (such as an EU good manufacturing practice (GMP) Grade A/ISO class 4.8 environment, as would be used for the aseptic filling of medicinal products).

Outside of this, the autoclaving of heads is relatively impractical, especially when two or more samples need to be taken from the same cleanroom. An alternative practice is to decontaminate the head between sampling sessions through the use of disinfectant. The choice of disinfectant is important. Some disinfectants will be unsuitable for this task: they will corrode or causes 'pitting' of the metal (such as chlorine-based chemicals) or they might present a safety concern to the operator (as with chlorine and hydrogen peroxide). A safer alternative is 70% isopropyl alcohol (IPA) or IUPAC 2-propanol with water, which are non-toxic to the operator and do not present a skin or inhalation risk. Moreover, IPA is commonly used for the disinfection of cleanroom gloves. A 70% volume/volume solution in water is the recommended concentration for use as a hand sanitiser[18]. Water is necessary to open up membrane pores of bacteria; the opening of the pores functions as a gateway to allow the ingress of isopropyl, which is toxic to many bacteria[19].

It is important to establish that the method of decontamination is effective at eliminating common cleanroom microbiota[20] and that it does not lead to the inhibition of microbial growth. This paper presents the results of such an examination.

Materials and methods

For the study, a Merck-Millipore MAS – 100 NT model air sampler was used. With this device, air is aspirated through a perforated lid and impacted onto the surface of growth media. The sieve-like head of the sampler has 300 x 0.6 mm holes. This type of sampler uses 90 mm agar plates.

The first part of the study was to determine that the decontamination method was effective. With this, active air samples were taken within a Grade D cleanroom. This cleanroom formed part of a wash-bay within a pharmaceutical facility. Previous experience had shown that this area contained a level of microorganisms with a mean recovery of 50 colony forming units (CFU) per cubic metre of air (below the EU GMP maximal value of 200 CFU/m^3, but sufficiently high to allow the decontamination method to be assessed).

In order to assess the decontamination, samples were taken individually within the Grade D area. For this, 10 samples of one cubic metre were collected onto microbiological culture media (tryptone soy agar with 1% glycerol) and incubated for 3 days at 20–25°C followed by 2 days at 30–35°C (a dual incubation regime designed to recover bacteria and fungi).

The formulation of the agar was:

- pancreatic digest of casein (15.0 g),
- enzymatic digest of soya bean (5.0 g),
- sodium chloride (5.0 g),
- agar (15.0 g),
- glycerol (1g),
- pH adjusted to 7.3 ± 0.2 @ 25°C.

Following sampling, the plates were removed and transported for incubation. The air sampler head was then subjected to the decontamination step using 70% IPA. The decontamination step involved spraying the inside and outside of the head with the disinfectant and then wiping down the surfaces with a sterile lint-free cleanroom wipe. The wiping process took approximately 5 seconds; with a 30-second contact time applied.

The sampler was then transported into a Grade A (ISO class 4.8) environment within an isolator. Experience has shown that the mean recovery of microorganisms was 0 CFU/m^3. The aim here was to determine if microorganisms within the isolator were recovered and, if this was the case, whether there was similarity between the species recovered by comparing the isolates using microbiological identification with those found in the Grade D area. The inference here was that if the Grade A results were 0 or 1 CFU/m^3, then this is indicative of the decontamination process being successful; however, if the results were higher and the recovered organisms were similar, then this was indicative that the decontamination procedure was unsuccessful. For this assessment, the activity was undertaken 10 times in order for the results to be reproducible. The method used to identify the microorganisms was an automated phenotypic system called OmniLog®[21].

The second part of the study examined whether the process of decontamination affected the validity of the results in terms of microbial inhibition. The risk with the decontamination process was that residues of the 70% IPA could remain and this could inhibit microbial growth, leading to the results obtained from the second use of the air sampler being invalid. This was assessed through growth promotion.

For this study, the air sampler was decontaminated using the same procedure for the decontamination study: spraying the inside and outside of the head with the disinfectant and then wiping down the surfaces with a sterile lint-free cleanroom wipe. The wiping process took approximately 5 seconds. In order to assess the effect of prolonged exposure to the disinfectant and to assess evaporation rates, the sampler was run for a standard volume after different time points. At different time points, agar plates were taken and subjected to growth promotion testing. The time points selected were 30 seconds, 60 seconds and 120 seconds.

To determine inhibition, the following microorganisms were used for growth promotion:

- *Staphylococcus aureus* (ATCC 6538)
- *Bacillus subtilis* (ATCC 6633)
- *Pseudomonas aeruginosa* (ATCC 9027)
- *Candida albicans* (ATCC 10231)
- *Aspergillus brasiliensis* (ATCC 16404)
- *Escherichia coli* (ATCC 8739)
- *Staphylococcus epidermidis* (Environmental isolate)

ATCC is a reference to the American Type Culture Collection. These microorganisms were selected because they are commonly used for the quality control testing of culture media. They also form part of the recommended US and European Pharmacopoeia panel for method suitability testing for the sterility test and the qualification of media used for the Microbial Limits Test. The organisms also covered a wide range of morphological types: Gram-positive coccus, Gram-positive rods, Gram-negative rods, yeast-like fungus, and filamentous fungus. In addition to these, the panel was complemented by an environmental isolate. This was *S. epidermidis*. This organism was recovered from a cleanroom and it is a human skin commensurable.

With the growth promotion, agar plates at the different time points were challenged with not more than 100 CFU of each organism. The method used to inoculate the agar was the spread plate method where 0.1 mL of the challenge is deposited onto the agar plate and evenly spread using a sterile implement across the agar surface. Control plates using agar not used in an air sampler were prepared at the same time to verify the challenge count. A negative control plate was performed with a sterilised sieve. All agar plates were incubated at 20–25°C for 3 days followed by 30–35°C for 2 days. The acceptance criteria were as follows.

- No inhibition – test plates to be recovered within 50–200% of the control plates.
- Inhibition – failure of the test plates to recover the microbial challenge within 50–200% of the control plates.
- Negative control – no growth should be observed.

Results

Decontamination study

The results from the decontamination study are shown in **Table 1**. The microbial counts obtained are summarised in **Table 2**. The results from the study show that the decontamination method was effective. No microorganisms were recovered from the air samplers run within the Grade A environment within the isolator. Because no microorganisms were recovered from the Grade A environment, no comparative identifications were required.

Table 1. Results of the decontamination study.

Sample number	Result from Grade D cleanroom (CFU/m³)	Identified microorganisms	Result from Grade A (ISO class 4.8) isolator environment (CFU/m³)
1	55	*Kocuria rhizophilia, Micrococcus luteus, Staphylococcus arlettae, Staphylococcus warneri*	0
2	40	*M. luteus, Staphylococcus cohnii ss cohnii*	0
3	12	*M. luteus, Staphylococcus saprophyticus, Cladosporium spp.*	0
4	20	*K. rhizophilia, Rothia mucilaginosa*	0
5	67	*S. warneri, Staphylococcus epidermidis*	0
6	101	*M. luteus, S. arlettae, Micrococcus lylae, S. epidermidis*	0
7	63	*M. luteus, S. cohnii ss cohnii, Microbacterium maritypicum*	0
8	42	*M. lylae, Corynebacterium spp.*	0
9	71	*M. luteus, K. rhizophilia*	0
10	17	*M. luteus, M. lylae, S. epidermidis*	0

Table 2. Descriptive statistics for the microbial counts obtained.

Sampling condition	Range (CFU/m³)	Mean count (CFU/m³)	Standard deviation
Grade D cleanroom	12–101	49	28.1
Grade A (ISO class 4.8) isolator environment	0	0	0

Growth promotion study

Table 3 shows that each of the challenge microorganisms was recovered at each time point. The microbial control counts fell within the not more than 100 CFU target range. The recovery of the challenge count for each organism was not affected by the waiting time, as shown in **Figure 1**.

Discussion

From the results of the study, two things can be concluded.

a) The decontamination of impaction model air sampler heads with 70% IPA is effective in that contamination is not transferred between sampling sessions.
b) The act of decontamination does not lead to the inhibition of microbial growth.

With the results obtained, considering the decontamination part first, it should be noted that while 0 counts were recorded within the Grade A area, the data relates to a given cleanroom and to the variation of microorganisms in the air at a particular time point. Past experience has shown that the cleanroom had a typical airborne concentration of microorganisms of 50 CFU/m³. This was borne out with the results collected (mean count 49 CFU/m³). What is not known is whether the same decontamination effect would occur if higher counts were recovered. A second limitation is with the types of microorganisms recovered (n = 27). With this study, the overwhelming majority of microorganisms were those transient or residential to human skin (see **Table 4**).

Although the profile is generally consistent with which will commonly be detected from cleanrooms[20], the decontamination step is limited by the types of microorganisms recovered. Had, for example, high number of *Bacillus* species been recovered, then the

Table 3. Results of the inhibition study, table displaying growth promotion counts (mean of two replicate tests).

Time	Microorganism (CFU)						
	E. coli	***P. aeruginosa***	***B. subtilis***	***S. aureus***	***A. brasiliensis***	***C. albicans***	**Environmental isolate *(S. epidermidis)***
30 seconds	41	43	44	40	38	44	45
60 seconds	44	45	46	44	42	41	48
120 seconds	42	45	50	46	39	46	42
Control plate count	**43**	**46**	**45**	**44**	**40**	**43**	**46**

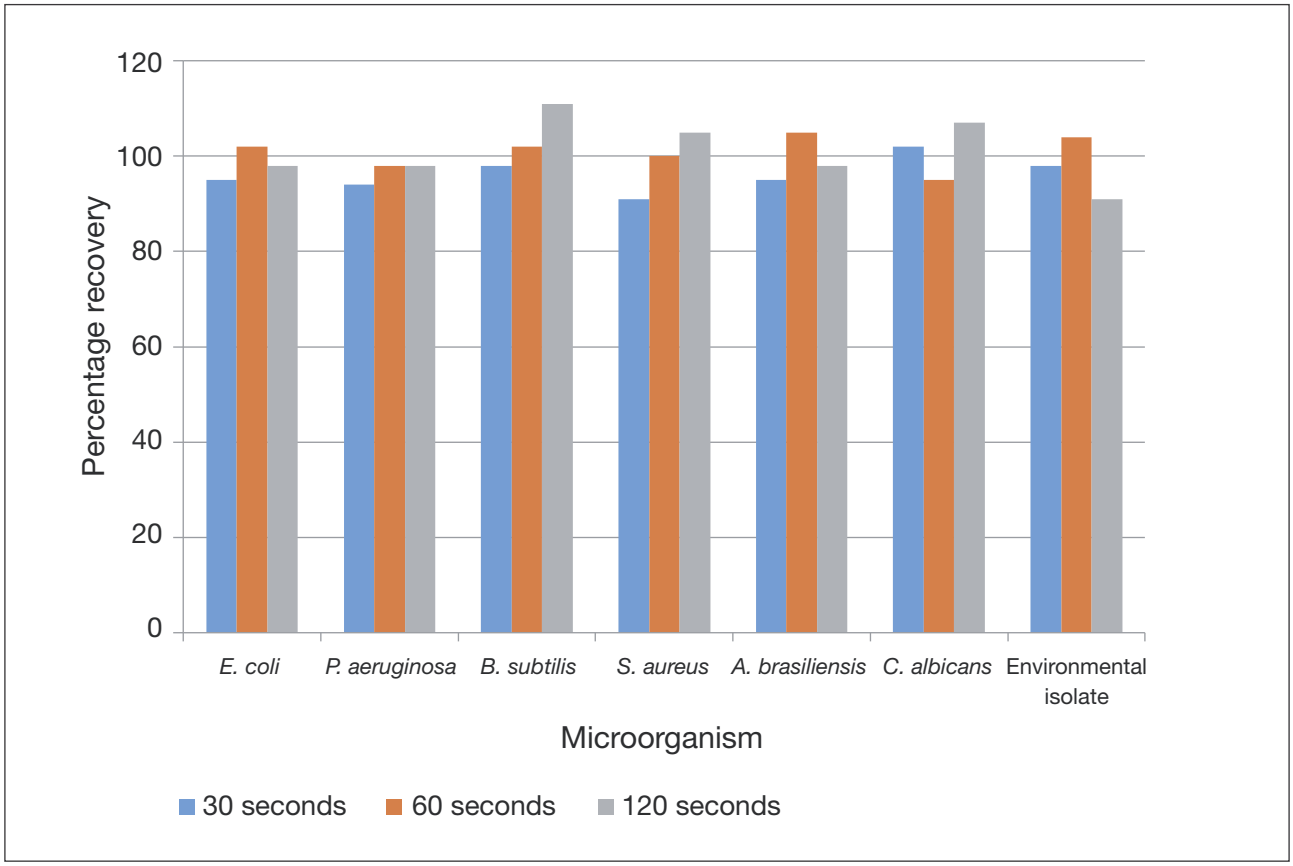

Figure 1. Chart showing percentage recovery of the challenge microorganisms across three time points.

decontamination process may not have been as effective. IPA is not a sporicidal disinfectant. Although the recovery of high numbers of *Bacillus* species would be a sign of a problematic cleanroom or production process, it would be prudent should this occur with regularity, to consider autoclaving the air sampler head.

With the inhibition study, the results showed that the usage of 70% IPA and the method of decontaminating the head did not cause microbial inhibition. Similar levels of recovery of the challenge organisms were observed across the three time points. While this part of the study was satisfactory and the results consistent, further studies could be attempted using more environmental isolates. In addition, other methods of decontamination could be attempted (such as the use of a pre-saturated cleanroom wipe). A further variable is the time taken for the 70% IPA to evaporate. The time points used in this study – 30 to 120 seconds – may need modifying in different cleanrooms with different ranges of relative humidity.

In drawing the two sets of observations together, the method of running the air sampler appears to be sufficient to decontaminate it and leaves the air sampler with little residue of disinfectant (or at least a sufficiently low level of disinfectant) so as not to cause inhibition of the growth of a range of microorganisms.

Whether the results would extend to other types of air sampler heads, such as centrifugal samplers, is unknown. The study described in this paper used impaction heads with sieve-like holes. Given the method of application (wiping) and the expected profile of microorganisms, there is a strong likelihood that similar results would be

Table 4. Microorganisms recovered that are transient or residential to human skin.

Recovered genera	Number recovered	Proportion of isolates (as a percentage)
Micrococcus	10	37%
Staphylococcus	10	37%
Kocuria	3	11%
Corynebacteria	1	4%
Cladosporium	1	4%
Microbacterium	1	4%
Rothia	1	4%

obtained. However, this would require separate assessment.

It also cannot be inferred that other alcohols, such as ethanol or IPA at different concentrations, would achieve the same level of microbial kill. In terms of these two types of alcohol, earlier studies have shown IPA to be slightly more bactericidal than ethanol when measured against some bacteria[22,23]. Based on this, the use of ethanol should be assessed separately. With concentration, the optimum bactericidal concentration, for both ethyl alcohol and IPA is 60%–90% solutions in water (volume/volume)[18]. A 70% concentration is a standard preparation for pharmaceuticals and healthcare.

Therefore, the method of decontamination is effective. In practicing such measures, it is important that there is consistency of practice, in terms of the volume of 70% IPA applied and the run time. It is also important that heads are autoclaved periodically between disinfection sessions. This interval will need to be determined by the user and such a practice is recommended if samplers are removed from cleanrooms for service or calibration, and when transferring samplers between different cleanroom grades.

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