European Journal of Parenteral & Pharmaceutical Sciences 2005; **10**(2): 3-7

Collection efficiency of microbial methods used to monitor cleanrooms

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Microbiological sampling methods used in pharmaceutical cleanrooms should efficiently collect and count microorganisms. Methods are described in this paper that allow collection efficiencies to be determined and maximised, and comparisons to be made between sampling methods.

Key words: microorganisms, collection efficiency, surface sampling, air sampling, monitoring, cleanrooms

Introduction

Pharmaceutical cleanrooms are regularly monitored to ensure that the required limits of microbial contamination are not exceeded. Limits are laid down in documents such as the European Union GGMP[1] and the FDA Guidelines for Aseptic Processing[2] that expect microbial sampling to be carried out on:

- air, by the use of air samplers, to ascertain the number of microorganisms in a given volume,
- air, by the use of settle plates, to ascertain the number of microorganisms that will deposit onto a known surface area in a given time,
- hard surfaces, by surface sampling methods,
- clothing and gloves of personnel, by surface sampling methods.

The microbial sampling methods used in cleanrooms can therefore be categorised into:

1. air sampling,
2. settle plate sampling,
3. surface sampling.

Microbial sampling of clean environments started about 60 years ago in hospitals, and many of the methods are used today. Unfortunately, the collection efficiency and precision of most of these old methods were not properly investigated. Many relative new methods have also not been adequately validated to ascertain their collection efficiency. Therefore, a cleanroom can appear to have different microbial concentrations depending on the sampling methods used. Indeed, low efficiency methods can be chosen to ensure that an unsatisfactory cleanroom achieves the correct cleanliness standard. The collection efficiency of sampling methods should therefore be known, and preferably be high, and the variability of the results should be low i.e. the precision should be high. It is also necessary to ensure that the microorganisms sampled are efficiently counted, by demonstrating that the bacteriological growth medium is both sterile and fertile.

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Collection efficiency of surface sampling methods

The most common methods used to sample surfaces in pharmaceutical manufacturing rooms are as follows:

- Contact surface sampling, where an agar surface is pressed onto a surface. RODAC (Replicating Organism Detection And Counting) dishes were designed by Hall[3], and are circular with a surface area of 25 cm^2, although rectangular shapes and other sizes are now commonly available. They are used on flat hard surfaces and clothing fabrics. The method of application varies. They can simply be pressed onto a surface, or pressed down so that trapped air bubbles are dispersed. They can also be rolled over the surface from one edge of the plate to the other to avoid trapping air bubbles. Different pressures and application times can be used. The effect of these various application methods on the collection efficiency and precision of the results has not been reported.
- Swabbing is mainly used on surfaces where the contact plate cannot be used, such as uneven or inaccessible surfaces. At its simplest, a dry bud swab is wiped over a surface to remove microbes and then wiped on the surface of an agar plate. A variety of methods can be used that are likely to increase the collection efficiency. The bud swab can be moistened by either dipping it into aqueous liquid, or dipping and squeezing out excess liquid, and then wiping it over a given area of surface. Various wiping techniques can be used that require the swab to be turned, as well as using overlapping passes, or not, and the number and type of passes can be varied. The microbes removed from a surface can then be extracted from the swab by a variety of methods. The swab can be simply wiped over an agar plate surface, either randomly, or using a variety of methods. The microorganisms in the swab can also be extracted by washing (this is known as the rinse-swab technique), or dissolving. The efficiency and precision of these various collection and extraction methods are relatively unknown.

- Finger sampling. The finger tips of gloved hands are either touched onto, or wiped over the surface of an agar plate. The collection efficiency and precision of the methods, and hence the best method to use, has not been reported.

To ascertain the collection efficiency of surface sampling methods, two methods can be used. These are:

- surface seeding, where test organisms are artificially seeded onto a surface and sampled by the method under test;
- sequential testing, where naturally-occurring micro-organisms on a surface are sampled consecutively.

These test methods are now described and discussed.

Surface seeding method

In this method, a known number of test microorganisms are seeded onto a surface, the surface sampled, the number of microorganisms removed ascertained, and the collection efficiency and precision of the method determined. A number of sampling methods, mainly contact and swab methods, have been validated using this method, and these methods reported to have a collection efficiency of around 40% to 60% [4,5,6].

A surface seeding method that is likely to give the most applicable results is one that mimics the way microorganisms occur naturally on cleanroom surfaces. This is difficult, as many microbes deposit gently from the air, whereas others are rubbed onto the surface by gloved hand or clothing. It is best to avoid applying to a test surface a microbial suspension containing dissolved material that, when dry, will form an adhesion bridge between the surface and the microbes; pure water is therefore best. The hard surface on which the test organisms are seeded should also be standardised; stainless steel has been used but its finish varies and rougher surfaces will reduce access to the microbes and hence the removal efficiency; glass is a useful available material. Both vegetative bacteria and bacterial spores have been used to seed a test surface but as bacterial spores survive with practically no loss in viability and should not influence the collection efficiency, they are the best option. A method developed by the author is as follows.

Washed spores of *Bacillus atrophaeus* (NCTC 10073, equivalent to DSM 2277), previously known as *Bacillus subtilis var. niger*, and prior to that as *Bacillus globigii*, suspended in pure water, are used. A sterile rectangular glass dish, of the 'casserole' type used in the kitchen, and approximately 30 cm x 20 cm in surface area, is also used. Sufficient spore suspension should be added to cover the surface of the dish: a good starting point is about 60 ml of a heat treated suspension of 100 spores/ml, and an uncovered dish placed into an incubator, levelled, and left for about two hours at about 60°C to 65°C until the water is driven off. A ten or hundred-fold concentrated suspension can be prepared in sufficient volume for many experiments, as the concentration of spores will not change through time.

The base of the dish should be divided up into equal areas of sufficient size e.g. 8 for the sampling dish described; drawing lines with a waterproof marker on the outside of its glass surface will aid this. Random sampling of the squares should be employed to remove any bias caused by an uneven spore concentration over the surface.

Surface sampling is now carried out on the test surface. If required, two or more methods can be compared, a fresh and randomly selected test area being used for each new sample. The samples should be processed and incubated for between 30°C to 35°C for 36 to 48 hours to determine the number of spores that have been removed from the surface.

The original number of spores on the test surface should now be found. This can be calculated from the count of the aqueous suspension of spores that has been added to the surface. This may be satisfactory, but the use of a direct surface agar overlay technique will also assist. This technique has been shown 7 to have very high removal efficiency for spores of 94% with high precision, having 95% confidence limits of 91.2% and 109.7%. 200 ml of cool molten agar (at about 65°C) is added to the dish after sampling is complete, and the dish placed in a unidirectional cabinet. When the agar has set and the water of condensation has evaporated, the dish is covered, inverted, and incubate at 30°C to 35°C for 48 hours. After incubation, the numbers of spores that have grown within the agar, at the interface of agar and dish, and on top of the agar, are counted. This count is added to the total of the counts from all the test samples, to obtain the original concentration on the test surface. The sampling efficiency can now be calculated from knowledge of the count obtained by sampling and the original number on the area sampled.

The seeding method can also be used to make a simple comparison between two sampling methods to see which is best. In that case, it is not necessary to determine the number of spores on the sampling surface; a simple comparison between counts taken randomly on the surface by the two methods will suffice.

Sufficient samples should be taken to be confident of the collection efficiency result. The use of a 'cumulative average efficiency' method is helpful, and this is described in the last two paragraphs of the next section. As well as the collection efficiency, the precision of the method should be ascertained.

The exact number of spores on the test surface may be calculated by the following equation:

$$\text{Number of bacteria on surface/cm}^2 = \frac{\text{number of bacteria/ml x number of ml added to the test surface}}{\text{area of test surface (cm}^2\text{)}}$$

For the dimensions and numbers given above the following numbers will apply:

$$\text{Number of bacteria on surface/cm}^2 = \frac{100 \times 60}{30 \times 20} = 10 \text{ bacteria/cm}^2$$

Sequential sampling method

The seeding method described in the previous section assumes the test microorganisms on a test surface mimic the natural microbial flora on cleanroom surfaces. This is unlikely. Also, it can only be used to determine the collection efficiency of hard surface methods and is not suitable in determining the collection efficiency of sampling methods used on clothing or gloves. Another method has been devised by Whyte *et al*[8] to establish the collection efficiency of sampling methods using microorganisms in their natural state, and its use reported [8,9,10].

This method requires a surface to be consecutively sampled at exactly the same place, and the collection efficiency of the sampling method calculated by the equation:

$$\text{Collection efficiency of sampling (\%)} = 1 - \frac{\text{Count from second sample}}{\text{count from first sample}} \times 100$$

The derivation of this equation is given in a separate paper[8], where further information is given on the method. That paper also describes a multiple sequential sampling method, but it has been found by the author that the simpler two-stage consecutive method is best.

If the sequential method is used on cleanroom surfaces it will not give accurate results, as the counts will be too low. Therefore, it is best to sample surfaces, such as laboratory benches or used clothing, where high microbial counts are obtained. If a comparison is carried out between two or more sampling methods it is best to divide the test area into a box grid, and randomly sample in the boxes. The collection efficiency of some sampling methods will be more consistent than others, and the precision should be reported using a statistical method such as the standard deviation, or 95% confidence limits.

Shown in the first two lines of **Table 1** is a series of consecutive samples previously reported by Whyte *et al*[8] and taken on a laboratory bench to establish the collection efficiency of the RODAC contact plate method. The overall mean collection efficiency was found to be 53%, and the standard deviation, 13.

Microbial counts are more variable that physical counts and to ensure an accurate estimate of collection efficiency, sufficient tests should be taken and averaged; ten is a good start. A simple method of recognising if sufficient samples have been gathered is to use a cumulative average efficiency. This is done by adding each new result to a running cumulative sum of the efficiencies (line 4 of table 1) and dividing this sum by the number of results to obtain the cumulative average efficiency (line 5 of table 1). The cumulative average collection efficiency as a percentage of the final overall cumulative collection efficiency can then be calculated (line 6 of table 1) and when this result is shown to be within a few percent of 100%, the final result may be considered to have been reached. This approach is shown in **Table 1** and **Figure 1**.

Table 1. Rodac sampling on benches

Sample test number	1	2	3	4	5	6	7	8	9
Count from 1st sample	3	16	6	40	19	40	49	38	25
Count from 2nd sample	1	5	2	23	8	23	26	20	17
Collection efficiency (%)	67	69	67	43	58	43	47	47	32
Cumulative sum of efficiencies	67	136	203	246	304	347	394	441	473
Cumulative efficiency (%)	67	68	67.7	61.5	60.8	57.8	56.3	55.1	52.6*
Cumulative efficiency as a % of overall collection efficiency	127	129	129	117	116	110	107	105	-

* overall mean collection efficiency

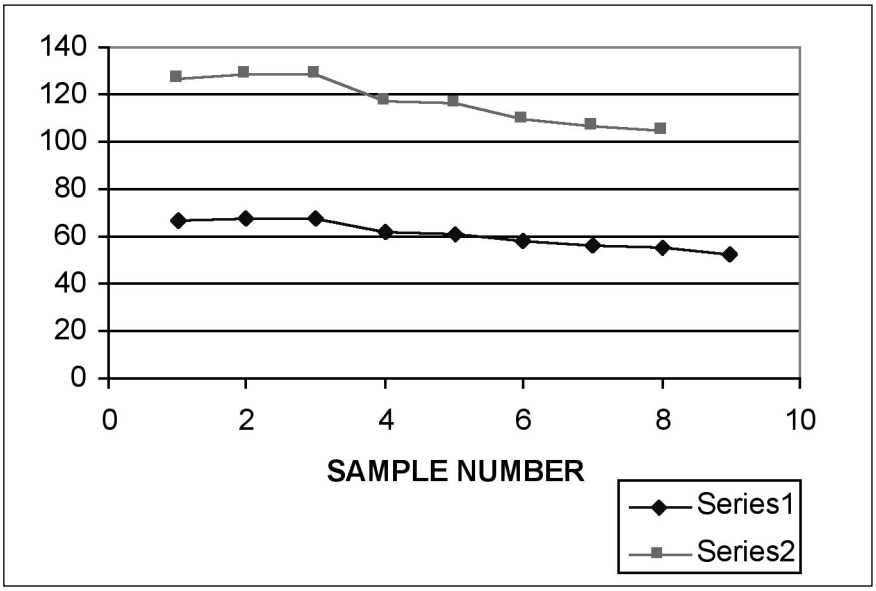

Figure 1. Cumulative average efficiencies (series 1), and cumulative average efficiencies as a percentage of the overall mean (series 2).

The values shown in **Table 1** and **Figure 1** are not very typical, as it is more common to find the cumulative average efficiency moves up and down and then settles down to a more constant value. The results shown in **Table 1** had low counts at the start of the series and these probably gave unreliably high efficiencies. Efficiencies calculated from low counts are therefore best discarded. **Table 1** and **Figure 1** also shows that a few more counts are required to be added to the series to give more confidence that a consistent result has been obtained.

Air samplers

Most air samplers used in cleanrooms are impaction samplers. Impaction samplers accelerate microbe-carrying particles in the air to a high velocity by means of a hole, slit, or by a fan blade, and direct it onto an agar surface. As the air turns away from the agar surface the microbe-carrying particles, which cannot follow the air stream, are impacted onto the agar surface. Shown in **Figure 2** is a drawing of the principle of this method, as found with a slit or hole.

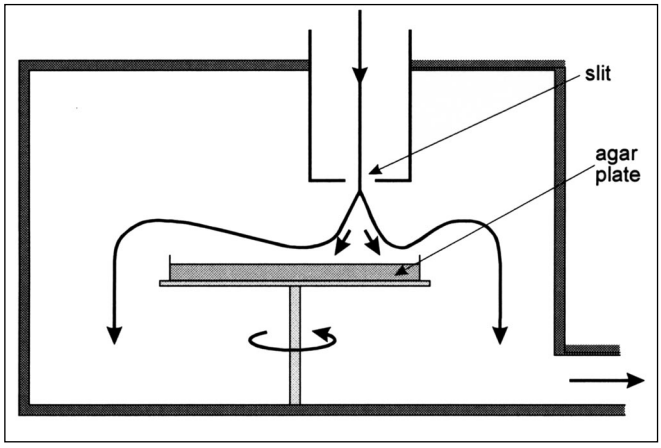

Figure 2. Airflow within a slit-to-agar sampler. Note that both the slit width and the distance of the slit to the agar will be very much less than shown.

It is not a simple task to design an air sampler to achieve high collection efficiency, as there are likely to be losses of microbe carrying particles in the sampler intake, within the sampler, and poor collection efficiency on the agar. The most common problem is poor collection efficiency on the agar surface; smaller particles with insufficient mass, and hence low inertia, are not thrown onto the agar and pass through the air sampler. ISO 14698-1[11] suggests that an air sampler should collect microbe carrying particles down to 1mm. To sample these smaller sizes of particles, a high velocity is required. The collection efficiency of impactors has been calculated by Ake Moller[12] who showed that some commonly used air samplers do not achieve a high enough impaction velocity to efficiently collect microbe containing particles.

The collection efficiency of air samplers have been studied for over 60 years and much information has been published. For example, Hemington[13] has summarised 94 articles on the collection efficiency of 101 different samplers. It is generally considered that the collection efficiency of air samplers should be ascertain either by:

(1) comparing the number of naturally-occurring microbes collected by different samplers in a place similar to where they are used, or,
(2) measure the collection efficiency of artificial test particles of sizes typical of the naturally-occurring microbe-carrying particles found in the place where the sampler is used.

Hemington reported that 46 studies compared air samplers using the prevailing microbial flora in a natural environment, and 57 used an artificial test aerosol. These two types of validation methods are now discussed.

Collection efficiency of microbes generated by people

This method compares the collection efficiency of two or more samplers when collecting naturally-occurring microbes in a place similar to where the samplers are normally used. It is important that a similar type of place should be studied, as the type of microbes and the size distribution of the microbe carrying particles may vary from situation-to-situation and this is likely to affect the collection efficiency. The concentration of microbe carrying particles can vary over time and simultaneous sampling with the samplers should be carried out. It is not realistic to compare samplers in a cleanroom because of the low concentration of microorganisms in the air. To obtain a suitable concentration of microbe-carrying particles, a room with a modest supply of air, or none, and where people exercise or move, should be used. No standard method exists, although the method suggested by Ljungqvist and Reinmuller[14] is the basis of such a method.

Shown in **Figure 3** is Ljungqvist and Reinmuller's comparison of a selection of air samplers used in cleanrooms. Over the years, the 6-stage Anderson sampler, without the inlet cone, has been shown to be one of the most efficient collectors of airborne microorganisms and suggested as the standard sampler to which other samplers should be compared[13,15]; this is good advice. **Figure 3**, and other information given by Ljungqvist and Reinmuller, demonstrates that some samplers are very inefficient e.g. the Anderson 6-stage sampler is about 10 times more efficient than a SMA.

Collection efficiency against different sizes of artificially-generated microbe-carrying test-particles

The collection efficiency of microbe carrying test particles by an air sampler can be considered in two ways:

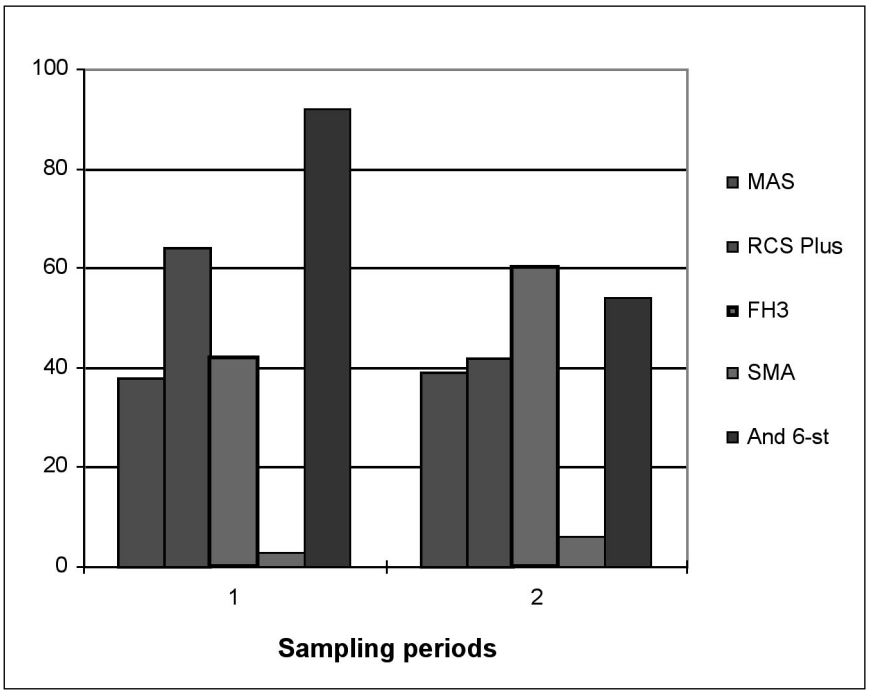

Figure 3. Comparative counts of five air samplers during two sampling sessions (information from Ljungqvist and Reinmuller).

- physical collection efficiency,
- biological collection efficiency.

The physical collection efficiency measures the collection efficiency of inert particles and takes account of the sampler's failure to capture particles; this failure occurs because of deposition on the sampler's intake, within the sampler, and inefficient collection onto the agar. The biological efficiency measures the collection efficiency of viable microbe-carrying particles and takes account of the losses caused by physical inefficiency together with the effect a sampler has on the viability of microorganisms. If microbes are excessively dried or stressed during collection they may die or fail to grow, and this will cause a reduction in the collection efficiency; this is shown by the biological efficiency.

Annexe B of ISO 14698-1 describes a method that can be used to measure the collection efficiency of air samplers. This method originates from a method described by Clark, Lach and Lidwell[16]. It should be noted that the ISO 14698-1 method requires apparatus unavailable in a routine microbiology laboratory and should be carried out in a test laboratory that can give an independent account of a sampler. It is expected that this information will be provided by the manufacturer of the air sampler.

The ISO method suggests the physical efficiency of samplers can be determined by the use of airborne particles containing spores of *Bacillus atrophaeus* (NCTC 10073, equivalent to DSM 2277), previously known as *Bacillus subtilis var. niger*, and prior to that as *Bacillus globigi*i. These spores are robust and unaffected during sampling by drying or stress; they can therefore be used to determine the physical collection efficiency of air samplers. Other test particles such as polystyrene latex spheres, or dye particles, can also be used[17, 13]. ISO 14698 recommends that the physical collection efficiency of the air sampler should be reported. Measuring the biological collection efficiency of non-spore bearing microorganisms is less reliable because drying and stress problem makes it difficult to be sure of the actual concentration in the test chamber. If biological efficiency is measured, a microbe should be used that is typical of those found in cleanrooms i.e. *Staphylococcus epidermidis* (NCTC 11047, equivalent to ATCC 14990). Shown in **Figure 4** is a drawing of apparatus similar to that used by the author to test the collection efficiency of air samplers to ISO 14644-1. The size is approximately 1.5m x 1m x 1m.

The air in the test chamber is stirred with a fan as the air movement should be similar to that found in a turbulently ventilated cleanroom. The microbe carrying particles are usually generated by a Spinning Top Aerosol Generator (STAG)[16] or spinning disc[18]. To obtain the concentration of spore bearing particles in the chamber air a membrane filter is used. The counts from the sampler being tested and membrane filter are compared over five sizes of particles spread between 0.6 mm and 15 µm. Shown in **Figure 5** is the physical collection efficiency of a sampler obtained by the author.

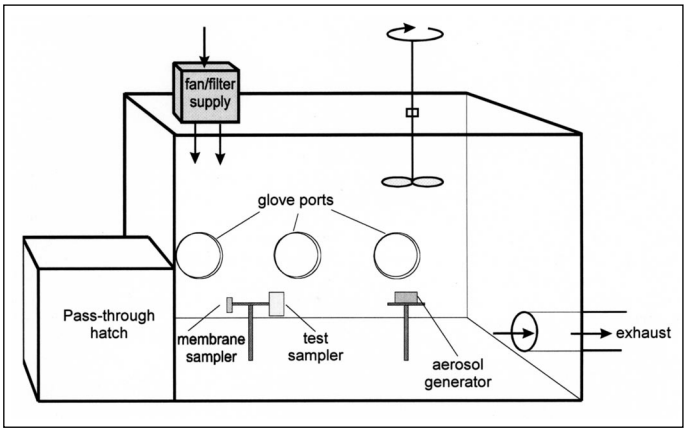

Figure 4. Microbial air sampler test chamber. Glove ports would have gauntlets attached.

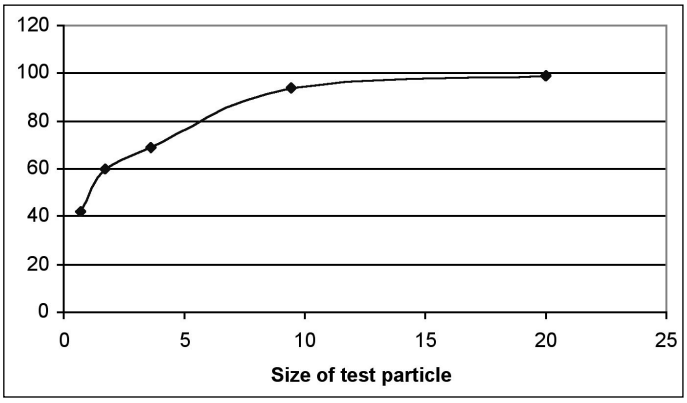

Figure 5. Physical collection efficiency of an air sampler against different sizes of airborne test particles.

Validation of any extension sample tubes

A further problem may occur during air sampling if extension tubes are used. When sampling during production it may not be possible to get an air sampler close to the critical area. Non sterility, bulk, and disturbance of the air flow[19], are problems. Sampling may therefore be carried out through a tube extension to the sampler, but there may be large losses within the tube, or the connection to the sampler.

The losses in sample tubes are normally associated with large microbe carrying particles, these being deposited by gravitational settling or impaction on the wall of the tube. The microbe-carrying particles in the air of a cleanroom have an average size usually between 8 mm and 20 mm[20, 21], and there can be substantial loss in a sampling tube; around 50% is not unusual. It is therefore necessary to test the sample tube to ensure that the losses are known. It may also be necessary to improve and optimise the size and shape of the tube, and its connection to the sampler, to minimise losses.

Extension tubes can be validated using the ISO 14698-1 method outlined above. They can also be checked by use of a comparative method using naturally-occurring microbe-carrying particles; the number of microbe carrying particles sampled without an extension tube can be simultaneously compared in the laboratory to counts from a sampler with a tube extension. A cumulative average collection efficiency can be used to assess when sufficient results have been obtained.

Settle plate sampling of air

The settle plate is the method of choice for evaluating the air quality in a cleanroom 22, being particularly useful in the critical area where it has advantages over air samplers by not disrupting the air flow, being sterile, and by minimising the risk from intervention of personnel who change plates. The settle plate also mimics the microbial deposition mechanism and is therefore a more direct means of measuring product contamination than an air sampler, and indeed, can be used to calculate microbial contamination[22]. The settle plate method of sampling air is a fundamental method of measuring the number of microbe-carrying particles that will deposit onto a given area in a given time; there is therefore no need to determine its collection efficiency. Having a constant collection efficiency therefore gives settle plates an additional advantage over air samplers

Validation of microbial growth media

Microbial growth media should be validated to ensure they are sterile and that microbes of interest are counted. It is therefore necessary to ensure that:

1. the growth medium is sterile,
2 the medium supports the growth of the types of microorganisms being sampled,
3. disinfectants on the sampled surfaces do not inhibit the growth of the microorganisms,
4. the medium used to sample airborne microorganisms is not over-dehydrated during sampling, and growth inhibited.

Sterility and fertility
Information on these two requirements i.e. ensuring the growth media is sterile and supports the growth of microbes considered to be important is not considered here but given in the European and United States pharmacopoeias[23, 24].

The method used to determine the fertility of agar medium by the use of test organisms is not fully explained in the pharmacopoeias. However, it is generally accepted that a small quantity of the test microbes suggested in the EP/USP for checking the fertility of sterility test media (0.1 ml of 10^3 microbes/ml is often used) is added to both the surface of the agar plate to be tested and medium of known fertility. The growth of the microbes is compared and if fifty percent of every test microbe grows, the medium is considered to be suitably fertile.

Disinfection inhibition
The third requirement is to ensure that the counts are not reduced by disinfection residue picked up from the surface. Microbial growth media should incorporate chemicals e.g. lecithin and Tween, to neutralise any surface disinfectants, and the effectiveness of these neutralisers should be demonstrated. This can be done as follows by, obtaining:

1. test plates used to sample experimental surfaces in the laboratory where the disinfection method is simulated, or,
2. • contact plates that have been used on disinfected surfaces within the cleanroom and, after incubation, found to be sterile,
   • plates used to streak-out swabs used to sample disinfected surfaces and, after incubation, found to be sterile.

To these test plates the EP/USP growth promotion test microbes are added. The counts from the test plates are compared with counts from fresh plates that have not come into contact with disinfectants.

Dehydration
The fourth requirement is to ensure that microbial growth media used in air sampling is not over dehydrated. In air samplers, air may be accelerated to a high velocity to ensure particles are efficiently impacted onto the surface. This high velocity, associated with a large air volume, may cause the agar medium to dry and hence fail to support the growth of some microbes. Also, settle plates laid out in a cleanroom, especially in unidirectional flow, can become dehydrated by the air flowing over them. In general, dehydration is not a problem in cleanroom sampling if the Petri dishes contain sufficient medium; Petri dishes used for air and settle plate sampling should always be generously filled and, generally speaking, they should be three-quarters full and not less than two-thirds. The dehydration phenomena has been investigated in slit samplers and settle plates using a variety of microorganisms of the type known to contaminate pharmaceutical products[25]. It was shown that the microbial count reduction on settle plates after 4 to 6 hours exposure to unidirectional flow, and in agar plates used for 60 min in a 30 l/s slit sampler was insignificant (<10%), especially when compared to count variability associated with air sampling, and considered acceptable. However, the effect of dehydration with respect to local sampling conditions should be checked.

The dehydrated medium is often checked by adding to the agar plates a suspension of the EP/USP standard growth promotion test microbes. However, this requires water to be added with the test organisms and rehydration of the medium is likely to affect the result. Also, the recommended EP/USP organisms are not typical of the type of microorganisms found in cleanrooms. It is therefore best to check possible dehydration using other methods. There are two possible test methods.

1. Use fresh plates to sample air in a sterile environment e.g. a unidirectional flow cabinet, using the same sampling method and time as when sampling air within the cleanroom. An alternative source of plates is from those used to sample in the cleanroom and found to be sterile. The dehydrated plates should now be used to sample air in a room with a high concentration of airborne bacteria dispersed from people e.g. a laboratory. Simultaneous sampling should be carried out using two identical samplers, one using the dehydrated plates and the other fresh

plates. Sufficient comparisons should be carried out in a balanced experiment to ascertain the effect of dehydration (cumulative average counts can be used) and no significant reduction (< 20%) in the count demonstrated.

2. A microbial suspension of an EP/USP growth promotion test organism is added to the agar surface of a fresh plate, spread over the agar, and dried in a unidirectional flow cabinet. The plates are removed from the cabinet when the last of the water just dries. Air sampling should then be carried out in a unidirectional cabinet, or empty cleanroom, as appropriate, using either an air sampler, or settle plates, and for the normal sampling time. These plates should then be incubated and the counts obtained compared to counts from identically prepared plates that have not been used for air sampling. This should be carried out for all EP/USP test organisms and over sufficient comparisons there should be no significant loss in the average microbial viability (< 20%).

Test 1 is likely to be a more stringent test that test 2, as more dehydration than normal is expected. However, Test 2 does not test the types of microbes generally found in cleanrooms, although this can be partially remedied by the addition of typical microbes isolated from the cleanroom.

Discussion and Conclusions

Cleanrooms must be regularly sampled to ensure that the concentrations of microbial contamination on surfaces and air are within the standards set for the room. The methods used should be valid ones that accurately reflect the concentration present. Many airborne and surface sampling methods used at present have unknown collection efficiencies, and their efficiency varies between sampling methods. This may allow cleanrooms to pass or fail cleanliness standards according to the method used. Sampling methods should have high collection efficiency with good precision, and the growth media and growth conditions should be optimum for the microbes being investigated.

The collection efficiency of surface sampling methods can be determined by two methods. One uses an artificial seeding method and the other sequential sampling. Both methods have some advantages and disadvantages but the sequential sampling method can be used to test all methods and surfaces, and employs the flora existing in cleanrooms. The collection efficiency and precision of the surface sampling methods should be ascertained but need not be retested unless there are changes in the sampling conditions that might affect the results.

Two methods are suggested for checking the collection efficiency of microbiological air samplers, and their tube extensions. One method is published in ISO 14698-1[11] and requires the collection efficiency to be measured using artificial test particles of a size range found in cleanrooms. The collection efficiency of air samplers according to ISO 14698-1 should be carried out by the manufacturers who should provide data from an independent test house. The second method compares the collection efficiency of a sampler being tested with another of known high collection efficiency e.g. an Andersen 6-stage sampler; this comparison should be carried out in an environment where microbes are dispersed by people. Settle plates use a fundamental method of sampling and the collection efficiency need not be ascertained.

The sterility and fertility of media used with sampling methods should be tested to ensure a high recovery of microbes of the type being sampled in cleanrooms. The choice of growth medium and incubation temperatures is largely governed by information given in pharmacopoeias and standards, and not considered in this paper. The effectiveness of neutralisers against disinfectants in surface sampling methods needs to be checked, but this need not be repeated unless there is any change in the disinfection type, concentration, or method of application, that might change the concentration of residue left. The effect of air dehydration of sampling media will also have to be ascertained using a method that does not re-hydrate the media, but this need not be repeated except if the sampling time used with an air sampler or, in the case of settle plates, sampling time and air turbulence, changes. However, the physical characteristics of the air sampler e.g. air volume through the sampler, should be routinely checked.

Some of the sampling methods presently used in cleanrooms need to be more efficient and their collection efficiency and precision known. It may surprise some readers that the efficiencies of sampling are so low, with some surface sampling methods substantially less than 50%, and some air sampling methods lower than 10%. However, collection efficiencies in the region of 50% are perfectly acceptable if the efficiency is known and the variation in the results is low i.e. the precision is good. A sampling method that gives a very low efficiency and poor precision is not acceptable. In addition to the problems associated with the collection efficiency there are further problems associated with dehydration, disinfectant residue and fertility of the media. It is clear that sampling methods used in cleanroom need further investigation and the information shared with others through publication. Based on these publications, standard sampling methods with good collection efficiencies and precision should then be published in an official document, such as an ISO standard or pharmacopoeia.

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