Active Air Sampler for continuous viable air monitoring with minimal plate changes

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Abstract

The EU Manufacture of Sterile Medicinal Products Annex 1 version 12 Revision is, as of 20 February 2020, undergoing a targeted three month consultation process. The proposed changes include the requirement for "continuous viable air monitoring in the Grade A zone to be undertaken for the full duration of critical processing, including equipment (aseptic set-up) assembly and filling operations."

In addition to this it is also a requirement to investigate any microbial counts and for any organisms isolated to be investigated and identified down to species level. Furthermore their potential impact on product quality for each batch affected and the overall state of control during sterile manufacturing should be investigated, as part of a documented system.

We consider now a new design of active air sampler that is capable of a very high, industry leading, Biological Efficiency, which can be used for an interval sample of 1 cubic metre of air. More importantly this new air sampler can be employed for up to 4 hours on a single plate of TSA (Trypticase Soy Agar) irradiated agar, at critically assessed risk locations in Grade A environments, with minimum human interventions.

This method of active air sampling can supplement or replace settle plates for more accurate microbiological sampling over an entire production run, providing a greater understanding of the level of microbiological control in the Grade A zone.

Introduction

Clean environments are used to control and limit microbial contamination where there is a risk to product quality, patient or consumer.

In Grade A clean zones, like in cleanrooms, isolators or RABS (Restricted Access Barrier Systems), the need to establish and maintain microbial control requires an understanding of the sources of contamination.

Selecting the most appropriate methods for assessing the presence of microbial bioburden with associated risks should be studied in depth for individual processes and facilities. Then the carefully selected best option should become part of a documented and validated Environmental Monitoring (EM) programme.

As part of this EM programme, recent regulatory perspectives need to be considered, especially around microbial monitoring during an entire sterile production process. From a microbial risk perspective, the Pharmaceutical Industry traditionally performs "snap-shots" when looking for presence/absence of bioburden. However, shouldn't we carry out microbial monitoring over an entire production run to gain a more reliable understanding of our critical areas?

Continuous microbial monitoring with an accurate, validated method is far more meaningful and can provide you with a far clearer understanding of microbial presence and potential risk to your product during sterile manufacturing.

Let's look at certain areas in a little more detail:

- Current Standards concerning the choice of the most appropriate AAS (Active Air Sampler) as part of an EM programme.
- Different AAS designs/methods.
- Pros and cons to consider when designing an AAS for Grade A and the plated media used.
- Introducing a new slit-to-agar design with initial validation results.

Current standards concerning the choice of the most appropriate AAS as part of an EM programme.

There are 3 main Standards/Regulations most relevant to consider:

ISO 14698-1:2003[1]

This Standard is in the process of being superseded by CEN 17141. However, a central part regarding AAS's is included. This ISO standard describes the need for a sampler to capture viable particles efficiently on an appropriate culture medium, from both biological and physical efficiency perspectives. A validation method for each is described.

Biological efficiency – is the ability of an AAS to collect microbe-carrying particles efficiently, with minimal drying out or shearing of microbes by the velocity of the air coming though the collection slit or sieve. Also, the drying effect on the chosen culture media needs to be considered. Is no growth present because you are in control, or because the medium has dried out?

Physical efficiency – is the ability of an AAS to collect microbe-carrying particles of different sizes efficiently. Multiple factors affect the physical efficiency including: the geometry of the head, the length and width of the slit or the diameter and number of holes in the impactor sampling head, depending on the method. Another important factor to consider is the velocity of the impacted air and the accuracy of the gap between the sampling head and the surface of the agar plate.

Also, within ISO 14698-1 are some clues (but no specific designs) on what an AAS should include into its specification when considering how to make a suitable AAS for Grade A, some key pointers being:

- Should be able to sample sufficient air in a reasonable time (interpreted as a minimum of 1 cubic metre of air sampled in several minutes at the fastest AAS level and up to 4 hours at the slowest)
- Should have the ability to sample efficiently down to particles of 1 μm. This can be expressed as the need for the Grade A AAS to have a d_{50}[2] value of 1μm (or smaller). Note that a d_{50} value of 1μm is the cut-off value at which 50% of 1μm particles are collected in the sampler and 50% are not collected.
- The exhaust should not disrupt the unidirectional airflow of the room, i.e. the exhaust air should be piped away from the vicinity or dissipated gently.

- The AAS should not contaminate the surrounding area, i.e. the exhaust air should be piped away or passed through an appropriate HEPA filter.

EN 17141: 2020[3]
When published in the very near future, this European standard will replace ISO 14698 in Europe. It takes into account modern developments and practices. In particular, regarding AAS's, it emphasises the importance of biological efficiency and the need to have an appropriate collection efficiency for the area being tested. An important point to consider is the significance of this when read in connection with Annex 1. In principal this can be interpreted as meaning that to recognise "no growths" accurately, an AAS will need to have a d_{50} cut-off value of better than 1 (i.e. smaller than 1 μm) in conjunction with a high Biological Efficiency, otherwise there is a risk of only having at best a 50% chance of collecting any 1 μm particles.

EU GMP Annex 1: Manufacture of Sterile Medicinal Products (Revision 12 under final consultation as of 20th February 2020) [4]
Sections 9.24 to 9.33 entitled "Environmental and personnel monitoring – viable particles" are relevant to EM and the choice of appropriate methods.
Of specific interest are Sections 9.27, 9.29 and the Table 7 in 9.30 which are quoted here in full:
9.27 Continuous viable air monitoring in the Grade A zone (e.g. air sampling or settle plates) should be undertaken for the full duration of critical processing, including equipment (aseptic set-up) assembly and filling operations. A similar approach should be considered for Grade B cleanrooms 1977 based on the risk of impact on the aseptic processing. The monitoring should be performed in such a way that all interventions, transient events and any system deterioration would be captured and any risk caused by interventions of the monitoring operations is avoided.
9.29 Sampling methods and equipment used should be fully understood and procedures should be in place for the correct operation and interpretation of results obtained. The recovery efficiency of the sampling methods chosen should be qualified.
9.30 Action limits for viable particle contamination are shown in Table 7 [of Annex 1].

Table 7: Maximum action limits for viable particle contamination

Grade	Air sample cfu/m³	Settle plates (diam. 90 mm) cfu/4 hours (a)	Contact plates (diam. 55mm), cfu/plate (c)	Glove print, Including 5 fingers on both hands cfu/glove
A	No growth (b)			
B	10	5	5	5
C	100	50	25	–
D	200	100	50	–

a. *Settle plates should be exposed for the duration of operations and changed as required after 4 hours (exposure time should be based on validation including recovery studies and it should not have any negative effect on the suitability of the media used). Individual settle plates may be exposed for less than 4 hours.*
b. *It should be noted that for Grade A, any growth should result in an investigation.*
Note (a) above, provides the opportunity to consider replacing settle plates with an AAS that can sample for 4 hours on a single plate, giving a sample time equivalent to a normal exposure on a settle plate, but with a considerably better Collection Efficiency (consistently more than 10 fold) than a settle plate (see later).

Different Active Air Sampler (AAS) designs/methods

There are a number of different AAS methodologies which have evolved. The most commonly seen ones are:
a. Centrifugal – employs strips of agar. This sampler causes excessive turbulence and the strips require manipulation from the instrument for incubation. Not relevant to Grade A.

b. Filtration – employs a gelatine filter membrane to capture particles from the environment. After sampling, the gelatine membrane needs to be aseptically removed onto a petri dish of Trypticase Soy Agar (TSA) and allowed to dissolve releasing trapped particles onto the medium for incubation. This manipulation means this method is not suited for Grade A.

c. Sieve Sampler – employs a fixed sampling head positioned (typically) 2.5mm over the surface of a TSA plate. May be a sieve plate with usually 300 small holes or a number of radial slits, the orifice dimensions being designed to give sufficient velocity to give a d_{50} value down to about 1 μm or slightly over. There are a number of airflow rates available by different sieve samplers, ranging from 1 cubic foot per minute (equivalent to 28.3 litres per minute), which takes 35 minutes 20 seconds to sample 1 cubic metre, to 100 litres per minute which takes 10 minutes to sample a cubic metre. Even faster AAS's are available that will sample a cubic metre in just a few minutes. Beware though, as some sieve sampler designs are a lot less efficient with significantly higher d_{50} values (e.g. >10 μm) and these should only be employed for trending where significantly higher counts are anticipated.
A sieve sampler is an "interval sampler" and the media plate needs to be changed after each and every cubic metre of air.
As the air impacts onto the same fixed positions on the agar surface, there is a natural drying out of the media at those points and particles impacted can be desiccated, reducing the Biological Efficiency.
As a technique, it needs careful qualification if the intent is to use a sieve sampler in Grade A. However, the sample is only a snap-shot of the air at the time of the sample being taken. A positive result for growth is significant, but a negative result can be misleading, as there may well be long intervals where no samples are taken. For areas where slightly higher numbers of organisms are anticipated, like C, D or unclassified areas, it is a good method for trending.
Sieve samplers can be battery or mains operated or can be built into a

facility. They can also be positioned with the head at the chosen sample point, with the controls and vacuum source located away at a safe position.

a. Slit to Agar sampler – Optimal method for the most critical, risk assessed areas in Grade A. These samplers have a fixed radial slit in the sampling head, positioned over an agar plate which rotates up to 360 degrees over a user selected time. Air is impacted on to a fresh part of the agar surface continually and so a d_{50} value of better (smaller) than 1 can be maintained throughout. These AAS's come with the added advantage of an excellent Biological Efficiency as fresh agar is continually being presented to the impacted air. This method also allows for the attachment of an isokinetic probe over the slit assembly, so in certain situations the sampling head can be moved up to 8 feet away from the critical sampling location. The latest instruments can be battery/ mains operated, or by Power over Ethernet (PoE), or can be built into a customer bespoke software system or a Facility Managed System (FMS), with the controls and vacuum source based remotely.

Design requirements for an AAS for Grade A environments

1. If a sampler has a d_{50} value of 1μm, it has only a 50% chance of impacting an organism of 1 μm (ref). The latest Annex 1 revision gives a target of zero growth. Therefore the design target for a Grade A sampler d_{50} value should be lower than 1μm.

   As a guideline to achieve this, the air velocity between the slit or orifice in the sampling head to the impaction site on the agar surface should not fall below 30 metres/second, but can be considerably higher, providing that the biological efficiency does not drop off.

2. Biological Efficiency should be high. Maintaining an accurate and consistent slit-to-agar surface gap (typically 2.5mm) is important, otherwise impaction rates vary significantly. The type of media, fill volume and moisture content of the agar, all come into play. Fresh media needs to be continually presented to the stream of impacted air to achieve this.

3. The collection efficiency of an AAS should be appropriate to the Grade of the area being tested.

4. An ability to sample for longer periods of time on a single plate before a plate needs to be changed is desirable. A settle plate is typically exposed for 4 hours maximum, so that should be the target for an AAS. This will allow for more meaningful monitoring over an entire batch run, rather than taking a snap-shot interval sample, the latter providing little meaningful information about the air quality through a manufacturing campaign.

5. Minimum human intervention is important. Regular plate changing on an AAS is a sterility risk and should be minimised.

6. Media used can vary widely. This means there is a need for careful validation and regular GMP compliance audits of the manufacturer and their internal methods, controls and SOPs.

   The following considerations apply to media:

   a. Typically, gamma irradiated Trypticase Soy Agar (TSA) is used routinely and, additionally, Sabouraud Dextrose Agar (SDA) is used for moulds if suspected. Both are poured into 9cm petri dishes.

   b. Different agar plate fill volumes are available (typically 18ml, 25ml and 32ml), but exact volumes may vary from one supplier to another.

   c. Moisture content of plates can vary. Room temperature storage packs, triply wrapped and hermetically sealed, retain moisture in the agar better than the original breathable packaged plates. It is important to use fresh plates with the maximum level of retained moisture to optimise performance.

   d. Different manufacturers use different levels of gamma irradiation (anywhere from 12 to > 25 kilo Grays (kGy) are seen) to terminally sterilise their plates. This has significant effects on the fertility, gel strength and moisture retention of the media. Higher gel strength retains more moisture, but reduces the fertility of a plate. The chosen plate from an audited manufacturer needs to show a consistent fertility under challenge testing within each individual batch and also maintain acceptable batch-to-batch variation.

   e. Can a new design of AAS sample for the same length of time on a single plate, as a settle plate? Post impaction, a media plate is incubated for a total of 5 days: either 2 days at 30 to 35°C followed by 3 days at 20 to 25°C; or some incubate at just 30 to 35°C for 5 days, to allow for growth from environmental and human originated (operator) organisms. The PQ validation of an AAS has to prove that a plate has sufficient moisture and fertility remaining to grow organisms impacted over the whole of the selected sample time.

Introducing the ImpactAir ISO-90, a new Slit-to-Agar design

Main design features

The main design features of the new unit are:

a. Slit to agar AAS employing a 9cm agar plate;

b. Factory interchangeable slit assemblies. All slits are 22mm long, but the width can be selected from 0.1mm to 0.8mm;

c. Variable flow rates, in litres per minute: 5, 10, 15, 28.3 (1cfm), 50 and 70;

d. Achieved d_{50} values from 0.46μm to 0.95μm;

e. Can sample 1 cubic metre of air, or can sample continuously for 4 hours on a single plate, sampling typically 3 or 4 cubic metres of air, if set-up selected appropriately;

f. Biological Efficiency is greatly improved, especially important when a set-up with a d_{50} of lower than 1μm is employed. Allows for longer sampling periods;

g. Design options include a stand-alone unit, powered by mains, battery/ mains or Power over Ethernet (POE).

Also, a remote unit, powered by an external vacuum source and control, positioned away from the clean zone, which can optionally be integrated into a client's Laboratory Information Management System (LIMS), a bespoke local system or a Facility Managed System (FMS).

Table 1 shows the effect of varying the slit dimensions and altering the air flow rates through the sampling head on the volume of air sampled over prolonged sampling periods of up to 4 hours, on a single plate. This is achieved whilst maintaining high impaction velocities delivering d_{50} performance of well below 1 μm.

Initial validation tests

The initial validation tests were run by a client in a controlled laboratory area and reported on at a recent conference.[5] Four different AAS's were used in the tests, of which one was a client validated reference sampler used in their Grade A areas:

- ImpactAir-140 (14cm TSA plate), slit-to-agar sampler – reference sampler.
- ImpactAir ISO-90 Head (9cm plate) with ISO-CON remote vacuum source and operational touch screen – the new design of slit-to-agar sampler.
- Sieve sampler A (9cm plate) – targeted for Grade A Isolators
- Sieve sampler B (9cm plate) – targeted for Grade A Isolators

The reference sampler had been independently tested against its own

Figure 1: Two alternative ISO-90 Head options: a) mounted on pod with sanitary flange connector for quick release (left); and b) freestanding (right)

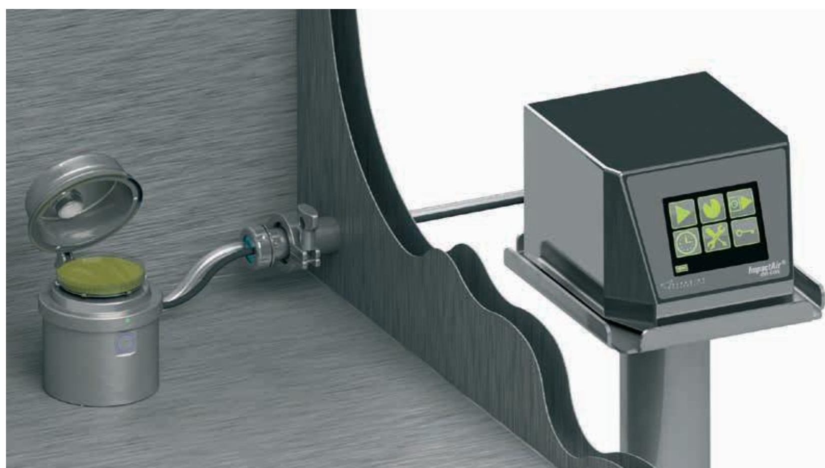

Figure 2: Free standing ISO-90 Sampling Head situated in a Grade A sampling location (left), connecting via a vacuum tube and electrical connection through an easy access gland to an ISO-CON control unit safely situated away from the Grade A area (right). A range of glands and stainless steel stands are available for a variety of installations.

Table 1: The effect of different slit dimensions and different air flow rates on d_{50} values and volume of air sampled.

Slit Width (mm)	Flow Rate (LPM)	d_{50} (μm)	Impact Velocity (m/s)	1m³ Time (Mins)	1 Hour Vol (m³)	2 Hour Vol (m³)	3 Hour Vol (m³)	4 Hour Vol (m³)
0.1	5	0.46	38	200	0.3	0.6	0.9	1.2
0.2	5	0.92	19	200	0.3	0.6	0.9	1.2
0.2	10	0.65	39	100	0.6	1.2	1.8	2.4
0.2	15	0.53	57	66.7	0.9	1.8	2.7	3.6
0.3	15	0.80	38	66.7	0.9	1.8	2.7	3.6
0.4	29	0.76	55	34.5	1.74	3.48	5.22	6.96
0.6	50	0.87	63	20	3	6	9	12
0.8	75	0.95	71	13.3	4.5	9.0	13.5	18

Notes:

1. The set-up shown in the olive green row was used in the initial validation tests.
2. The yellow box is not recommended as a set-up, the information is just for illustration purposes.

Gold Standard test sampler at Public Health England, Porton Down, an ISO 14698 test-house, and shown to be 25% more efficient. It is also used routinely in the client's Grade A critical areas.

The new design sampler comprises an ISO-90 Sampling Head, which works in conjunction with an ISO-CON control unit, the latter comprising a vacuum source, operational touch screen and a HEPA filtered exhaust (essential only if the ISO-CON needs to be positioned in Grade A).

The ISO-CON controls the flow rate, time and other user functions (such as operator details, location and run data) by the touch screen and holds the Run Data Memory. The flow rate ranges from 5 LPM to 100 LPM. Lower rates of 5 LPM or 10 LPM will maintain a d_{50} of about 0.5µm by minimising the drying out of the culture medium, thus allowing longer sampling on a single plate (up to 4 hours). A higher flow rate of 100 LPM would take a 1 m^3 sample in 10 minutes, if a more rapid sample needed to be taken, but the plate would need to be changed after the 1 m^3 sample, due to the media drying out more quickly.

Figures 1 and 2 show illustrations of the new sampler comprising the ImpactAir ISO-90 Sampling Head and the ISO-CON control unit.

The test procedure consisted of the reference sampler and the three samplers under evaluation, being tested simultaneously for 20 minutes at each of four sampling locations, several metres apart.

It can be seen in Table 2 that the average count per location, which is the average from all four samplers, had negligible variation of 5% between the maximum and minimum counts, indicating that the testing environment remained constant throughout the test period. Plates were counted for cfus per m^3 of air sampled, after 5 days incubation at 30 to 35°C. Results were normalised to compare counts per cubic metre of air sampled. A control plate, a 9cm settle plate, was also exposed at each location for 20 minutes, in parallel with the air sampling.

Table 2: Average counts per sample location and normalised counts in cfus per m^3 by air sampler type

Air Sampler	Sampler location and plate (cfu) count				Average plate (cfu) count	Normalised average plate (cfu) count per m^3
	1	2	3	4		
Reference sampler	44	83	71	37	59	105
New sampler	41	34	39	41	39	130
Sieve Sampler A	70	71	66	103	78	78
Sieve Sampler B	71	47	63	47	57	57
Control	5	3	3	0	3	-
Average plate (cfu) count per location	57	59	60	57	-	-

Table 3: Comparison of Relative Recovery Scores and Collection Efficiencies of the four samplers

Air Sampler	Description	Air Flow (lpm)	Air Velocity at slit/ sieve (m/s)	Time for 1 m^3 sample (mins)	d_{50} value (µm)	Biological Efficiency (% v ISO 14698 Test Lab Sampler)	Relative Recovery Score	Calculated Collection Efficiency
Reference sampler	Slit to agar Single slit 0.152 x 44mm 14cm TSA plate	28.3	72	35.3	0.42	125	1.00	1.25
New sampler	Slit to agar Single slit 0.2 x 22mm 9cm plate	15	56.8	66.7	0.53	To be determined by Independent Test House	1.30	1.63
Sieve Sampler A	Sieve sampler 179 holes Radius 0.375mm 9cm plate	50	10.5	20.0	1.6	To be determined by Independent Test House	0.74	0.93
Sieve Sampler B	Sieve sampler 300 holes Radius 0.300m 9cm plate	50	19.65	20.0	1.11	To be determined by Independent Test House	0.54	0.68

The Biological Efficiency, Relative Recovery Score and Collection Efficiency of the 3 samplers on test were compared to the reference sampler. If the counts recovered per m^3 of air by the reference sampler are assumed to be an absolute score of 1, then the Relative Recovery Scores associated with the 3 samplers under test are shown in Table 3. The new Slit-to-Agar sampler had a Relative Recovery Score that was 1.3 times better than the Reference Sampler and 1.8 times and 2.4 times better than Sieve Samplers A and B respectively.

The Biological Collection Efficiency for the reference sampler was determined by an independent test house (PHE, Porton Down), comparing it to their Gold Standard Casella Slit sampler using the method outlined in ISO 14698-1 and was found to be 125%. Using the Relative Recovery Scores, reasonable estimates of the Collection Efficiencies for the 3 other units when operating in a natural environment were determined. The values are shown in Table 3.

Further considerations

The ability of an air sampler to recover airborne contamination can be determined from its Performance Rating (PR). The PR of an air sampler is the concentration of airborne contamination that the sampler is capable of recovering for a defined airborne concentration and can be calculated by the equation:

Performance Rating = n / (t *r *)

n = Minimum number of microbes needed to show the sampler will measure microbes at the airborne concentration under consideration

t = Sampling time (min)

r = Air sampling rate (m^3/min)

= Collection efficiency of sampler (as a proportion)

Table 4: Air sampler PRs

Air Sampler	Performance Rating (cfu/m^3)
Reference sampler	0.80
New sampler	0.61
Sieve Sampler A	1.08
Sieve Sampler B	1.47

For an EU Grade A zone, the action limit for airborne microbial contamination is 1 cfu per m^3. Using a value of 1 for n and using the calculated collection efficiencies, the PR for each sampler can be calculated as shown in Table 4. It can be seen that the reference sampler and the new sampler are capable of recovering airborne concentrations below 1 cfu/m^3 but the two commonly used sieve sampler units are not.

Test conclusion

A comparison of the number of airborne microbes simultaneously recovered by the four air samplers within the same environment determined that the new sampler has a recovery that is 1.8 and 2.4 times higher than Sieve Sampler A and Sieve Sampler B respectively and also 1.3 times higher than the recovery of the reference sampler). When the Collection Efficiencies are calculated from this information, the data can be used to determine the Performance Rating for each sampler for use within an environment with an action limit of 1 cfu per m^3 for airborne contamination. The Performance Ratings for both the reference sampler and the new sampler confirm each would be capable of detecting contamination below this limit. However, the Performance Ratings for the Sieve Samplers A and B indicate that both of these units would not be capable of detecting contamination below this limit. Consequently, it is concluded that the reference sampler and the new sampler would be suitable for monitoring in EU Grade A areas but not the Sieve Samplers A and B.

Overall conclusion

The new sampler, the ImpactAir ISO-90 is an innovative Active Air Sampler, which can sample at critical risk assessed locations within an EU Grade A area, whilst exceeding all the Guidelines as outlined in ISO 14698-1, the forthcoming EN 17141 and the latest Revision 12 of the EC GMP Annex 1. The new sampler design has an industry high Biological Efficiency and a d_{50} value in the region of 0.5µm (depending on the slit dimensions selected), enabling accurate sampling down to at least 1 µm particle size in an area where zero growth needs to be proven. Furthermore, the new sampler can run for up to 4 hours on a single 9cm TSA plate reducing human interventions and the potential introduction of microbial contamination into your critical areas.

Monitoring microbiologically throughout an entire production run with minimum human intervention for plate changes is now possible and worth considering for the enhancement of product quality and patient safety.

A final thought is that the settle plate, an inefficient passive air sampler employed for up to four hours, could be replaced by a monitoring AAS over the same time period.

References

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4. EU GMP Annex 1: *Manufacture of Sterile Medicinal Products*, Revision 12 for consultation, 6th March 2020
5. Eaton T. *Effective Risk Management of Microbial Contamination*. London, UK. SMi ; Pharmaceutical Microbiology Conference. 2020.

John Cobb is a GMP Microbiologist who works with PMT (GB) Ltd. and also consults for other clients. He has over 40 years' experience including original studies for irradiating culture medium, developing formulae, conducting gamma irradiation trials and sourcing packaging suitable for Grade A cleanrooms. He has consulted on product development with leading manufacturers of media. He has also been involved with the design of active air samplers since 1985 and currently advises clients on the most appropriate samplers for their Grade A facilities.