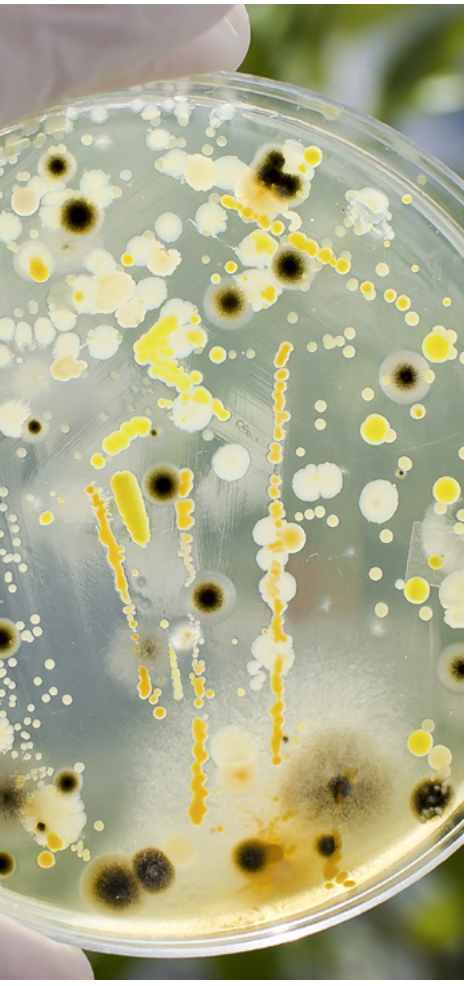
iVAS Physical and Biological Efficiency Testing

PHARMAGRAPH
Pharmaceutical Monitoring Systems

PG-WP-001(1)

Executive Summary

The release of the new European standard EN 17141:2020[1] in August of 2020, has reinforced the importance of considering the Physical and Biological Efficiency of microbial air samplers when selecting an air sampler.



This new standard supersedes EN ISO 14698-1:2003[2] and EN ISO 14698-2:2003[3] in Europe and introduces the recommendation for Physical Efficiency that "a d_{50} value smaller than 2 µm is considered appropriate". Recent testing conducted by Public Health England's Biosafety Investigation Unit has shown that Pharmagraph's next generation air sampler, the iVAS, is in full compliance with this recommendation when running at both 50 lpm and 100 lpm.

The Physical Efficiency testing showed that at 100 lpm the iVAS has a d_{50} of approximately 1.1 µm and at 50 lpm the iVAS has a d_{50} of around 1.4 µm. This represents an improved performance of >45% over the previous generation Pharmagraph AH8x90-10x Air Sampler, which when run at 100 lpm has calculated d_{50} of around 2 µm. The Biological Efficiency testing showed that at both flow rates the Biological Efficiency of the iVAS was greater than that of the reference sampler. These results indicate that the iVAS is effective at sampling bacteria-laden particles at both flow rates without an undue loss of viability.

Tom McCollin

TECHNICAL DIRECTOR - ENGINEERING

Introduction

Both EN 17141:2020[1] and ISO 14698-1[2] outline the requirements for Biocontamination Control within Cleanroom Environments. Both standards mention the importance of considering the Physical and Biological Efficiency of microbial air samplers like the iVAS, when selecting an air sampler.

The Physical Efficiency of the air sampler gives an indication of the proportion of particles of a certain size that will land on the agar within the petri-dish and an indication of the smallest particle size that can be collected by the air sampler.

The Biological Efficiency is a measure of how effectively a sampler collects micro-organisms on an agar plate in a manner that means the micro-organism will subsequently form a colony when incubated.

In order to demonstrate the Physical and Biological Efficiency of the iVAS, it was sent to an independent test facility run by Public Health England's (PHE) Biosafety Investigation Unit.

In order to demonstrate the Physical and Biological Efficiency of the iVAS, it was sent to an independent test facility run by Public Health England's (PHE) Biosafety Investigation Unit. The Biological and Physical Efficiency tests were conducted by PHE at both 100 lpm and 50 lpm flow rates.

Physical Efficiency Testing

The physical efficiency of the iVAS at both 50 l/min and 100 l/min was compared against a 0.8 µm membrane filter sampler using the techniques described in EN 17141:2020[1]. This testing was carried out by an approved external body (Public Health England's Biosafety Investigation Unit at their Porton Down facility).

The testing was performed in 20m^3 room with a turbulent air flow. A spinning top aerosol generator was used to produce an aerosol of bacterial spores (*Bacillus atrophaeus*) at controlled particle sizes. The aerosol generator was then placed in the centre of the test room and samplers were arranged around the aerosol generator in a semi-circle with a diameter of 0.8 m.

Once the aerosol generator had been run for long enough to create an effective distribution of the aerosol throughout the test chamber (20 seconds), the samplers were then operated together for a fixed period of time. During each test the iVAS sampled onto a Beckton Dickinson BB IC-XT Petri-Dish filled with Typticase Soy Agar. On completion of the sample the comparative membrane filters were removed and carefully placed upon a Typticase Soy Agar growth media and incubated alongside the Petri-Dishes within the iVAS.

After incubation the plate's counts obtained were standardised in to colony forming units per cubic meter. The results for the iVAS plate were then compared to the membrane sampler in order to calculate its physical efficiency at each particle size

as a percentage of the colonies found on the membrane filter sampler.

The testing described above was completed a minimum of 10 times for each of the following particle sizes:

- 0.8 µm
- 1.4 µm
- 2.5 µm
- 6.8 µm
- 13.1 µm

The physical efficiency for the iVAS is then determined to be the average physical efficiency observed across all of the testing at each of the particle sizes listed above. The results of this at both 100 lpm and 50 lpm can be seen in Figure 1.

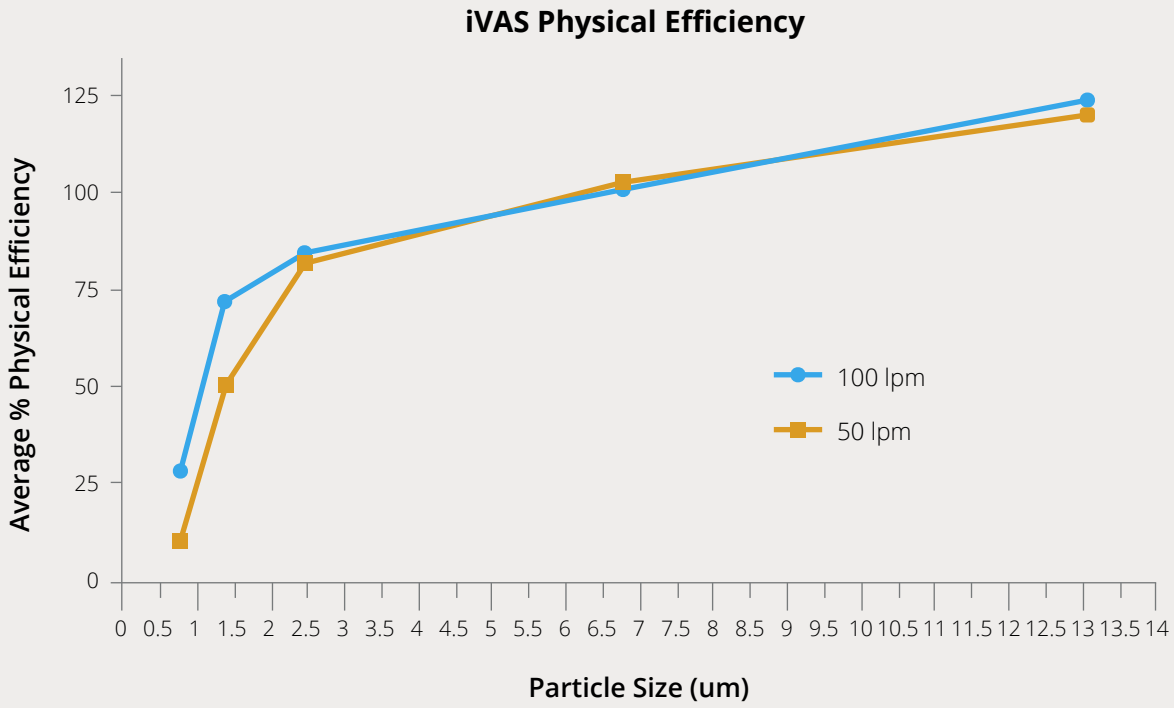


Figure 1 – Physical Efficiency Results

Due to the nature of a 0.8 µm membrane filter sampler it can be assumed that it is 100 % efficient at all of the above particle sizes therefore, the d_{50} value for the iVAS is the particle size at which the Physical Efficiency is 50 %. As can be seen from Figure 1 above this is around 1.1 µm when sampling at 100 lpm and at around 1.4 µm when sampling at 50 lpm. These values correlate well with the calculated d_{50} values for both flow rates of 1.1 µm for 100 lpm and 1.6 µm for 50 lpm, and in fact at 50 lpm the iVAS appears to perform slightly better than expected.

The d_{50} value for the iVAS is around 1.1 µm when sampling at 100 lpm and at around 1.4 µm when sampling at 50 lpm.

Further information on the physical efficiency test method and test results can be found in Porton Down's test reports[4,5] which can be made available if required.

Biological Efficiency Testing

According to the recommendations of ISO 14698-1[2] the human-derived environmental contaminant *Staphylococcus epidermidis* should be used as an indicator of the biological efficiency of an air sampler.

However, when *Staphylococcus epidermidis* is aerosolised in a laboratory, it forms a fine aerosol with particles that will be smaller than 1 µm, for this reason it is not used alone to determine the Biological Efficiency as the results can be skewed by the sampler's physical efficiency. The Biological Efficiency is therefore determined by comparing the recovery ratios of a hard-bacterial spore, *Bacillus atrophaeus* (BA) and the more fragile *Staphylococcus epidermidis* (SE).

As per the Physical Efficiency, the Biological Efficiency is a comparative value therefore, the results obtained by iVAS at both 100 lpm and 50 lpm are compared against a known standard sampler, in this case the Casella Slit Sampler. The Biological Efficiency was calculated using the formula shown in Eq.1 below:

$$\textit{Biological Efficiency} = \frac{\textit{Ratio of } \frac{SE}{BA} \textit{ sampled by the iVAS}}{\textit{Ratio of } \frac{SE}{BA} \textit{ sampled by the Casella sampler}} * 100$$

Eq.1

Again, the Biological Efficiency testing was performed by an approved external body (Public Health England's Biosafety Investigation Unit at their Porton Down facility) with both of the air samplers using the Beckton Dickinson BB IC-XT Petri-Dish filled with Typticase Soy Agar.

The Biological Efficiency reported by PHE has been derived from an average over a minimum of 20 repeat measurements as seen in Figure 2.

Further information on the Biological Efficiency test method and test results can be found in Porton Down's test reports[6,7] which can be made available if required.

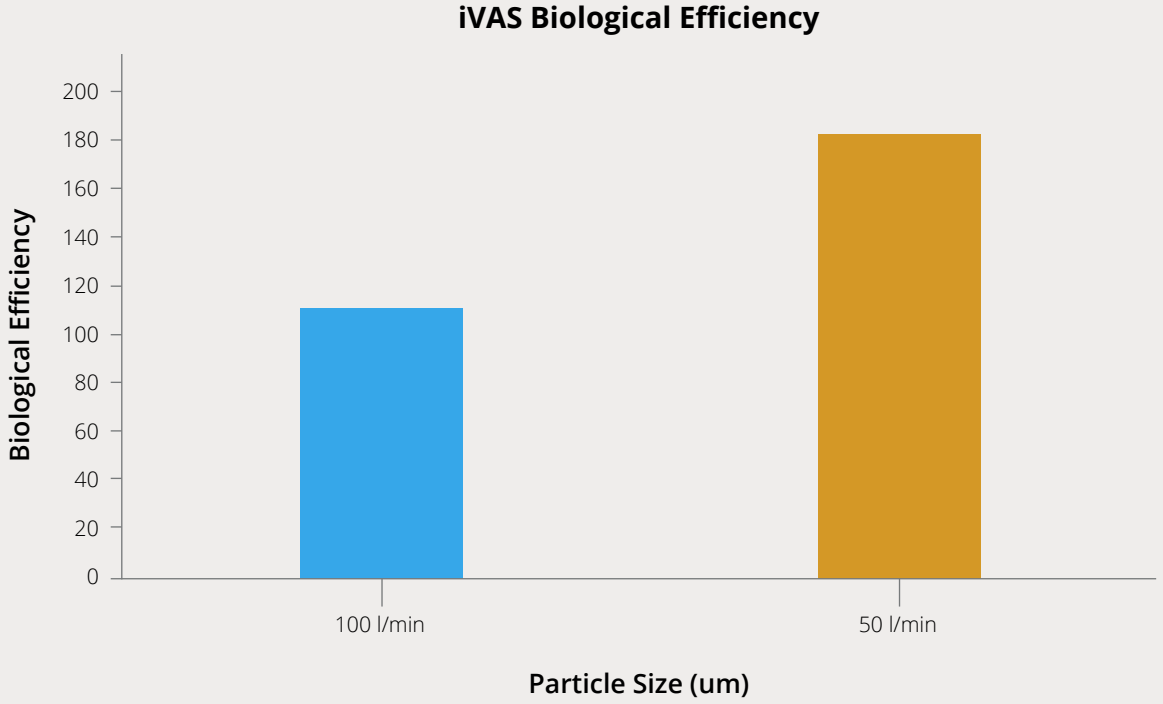


Figure 1 – Physical Efficiency Results

The Biological Efficiency testing showed that at both 100 lpm and 50 lpm the Biological Efficiency of the iVAS was greater than that of the Casella reference sampler.

Conclusion

Physical and Biological testing was conducted on the iVAS at both 100 lpm and 50 lpm by Public Health England's Biosafety Investigation Unit at their Porton Down facility. The Physical Efficiency testing was conducted according to techniques derived from EN17141:2020[1] and the Biological Efficiency using the techniques described in ISO146980-1[2].

The Physical Efficiency testing showed that at 100 lpm the iVAS has a d_{50} of approximately 1.1 µm and at 50 lpm the iVAS has a d_{50} of around 1.4 µm. EN17141:2020[1] states in Section E.5.2 that *"the equivalent diameters of the microbe-carrying particles (MCPs) that form the cfu are generally larger than 1 µm and a d_{50} value smaller than 2 µm is considered appropriate"*. Based on the results outlined above the iVAS meets this recommendation at both 100 lpm and 50 lpm. This represents an improvement over the Pharmagraph AH8x90-10x/VF8023 Air Sampler which when run at 100 lpm has calculated d_{50} of around 2 µm which is on the limit of the recommendation in EN17141:2020.

The Biological Efficiency testing showed that at both 100 lpm and 50 lpm the Biological Efficiency of the iVAS was greater than that of the Casella reference sampler (both numbers are greater than 100%). This indicates that the iVAS is effective at sampling bacteria-laden particles at both flow rates without an undue loss of viability.

References

1. EN17141 (2020): Cleanrooms and associated controlled environments – Biocontamination Control
2. EN ISO 14698-1 (2003): Cleanrooms and associated controlled environments – Biocontamination Control – Part 1: General Principles and Methods
3. EN ISO 14698-2 (2003): Cleanrooms and associated controlled environments — Biocontamination control — Part 2: Evaluation and interpretation of biocontamination data
4. Physical Efficiency Testing of the 100 l/min Pharmagraph iVAS Active Air Sampler Using Techniques derived from EN 17141:2020 (Report No 19-030 C)
5. Physical Efficiency Testing of the 50 l/min Pharmagraph iVAS Active Air Sampler Using Techniques derived from EN 17141:2020 (Report No 19-030 D)
6. Biological Efficiency Testing of the 100 l/min Pharmagraph iVAS Active Air Sampler Using Techniques Described in ISO14698-1 (Report No 19-030 A)
7. Biological Efficiency Testing of the 50 l/min Pharmagraph iVAS Active Air Sampler Using Techniques Described in ISO14698-1 (Report No 19-030 B)