Cherwell

Does Plate Choice Change Sampling Accuracy?

Full Report

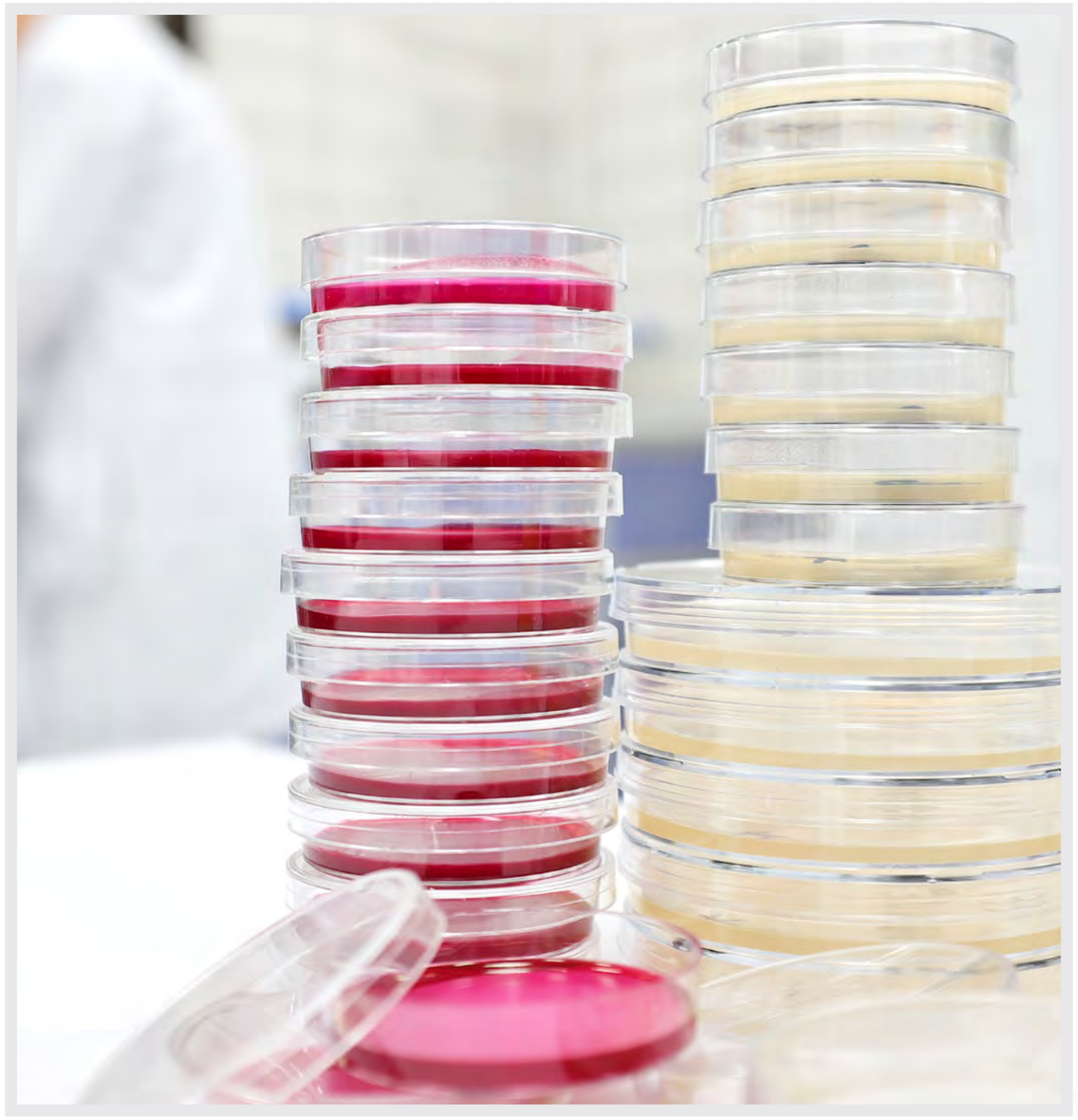
Table of Contents

About This Report

This report presents the results of our investigations into the performance of prepared media with our air samplers.

It is important to note that this report is not peer reviewed and is not intended to be a definitive white paper on the topic. Rather, we wanted to share our findings with the broader community in the hopes of promoting further investigation and understanding.

Our investigations yielded some interesting results, which we have presented in this report. We believe that our findings are worth considering, but they should be treated with caution and should not be considered definitive. We encourage others to conduct their own investigations and to share their findings with the broader community. Only by working together and sharing our knowledge can we truly advance our understanding of this topic.



Introduction

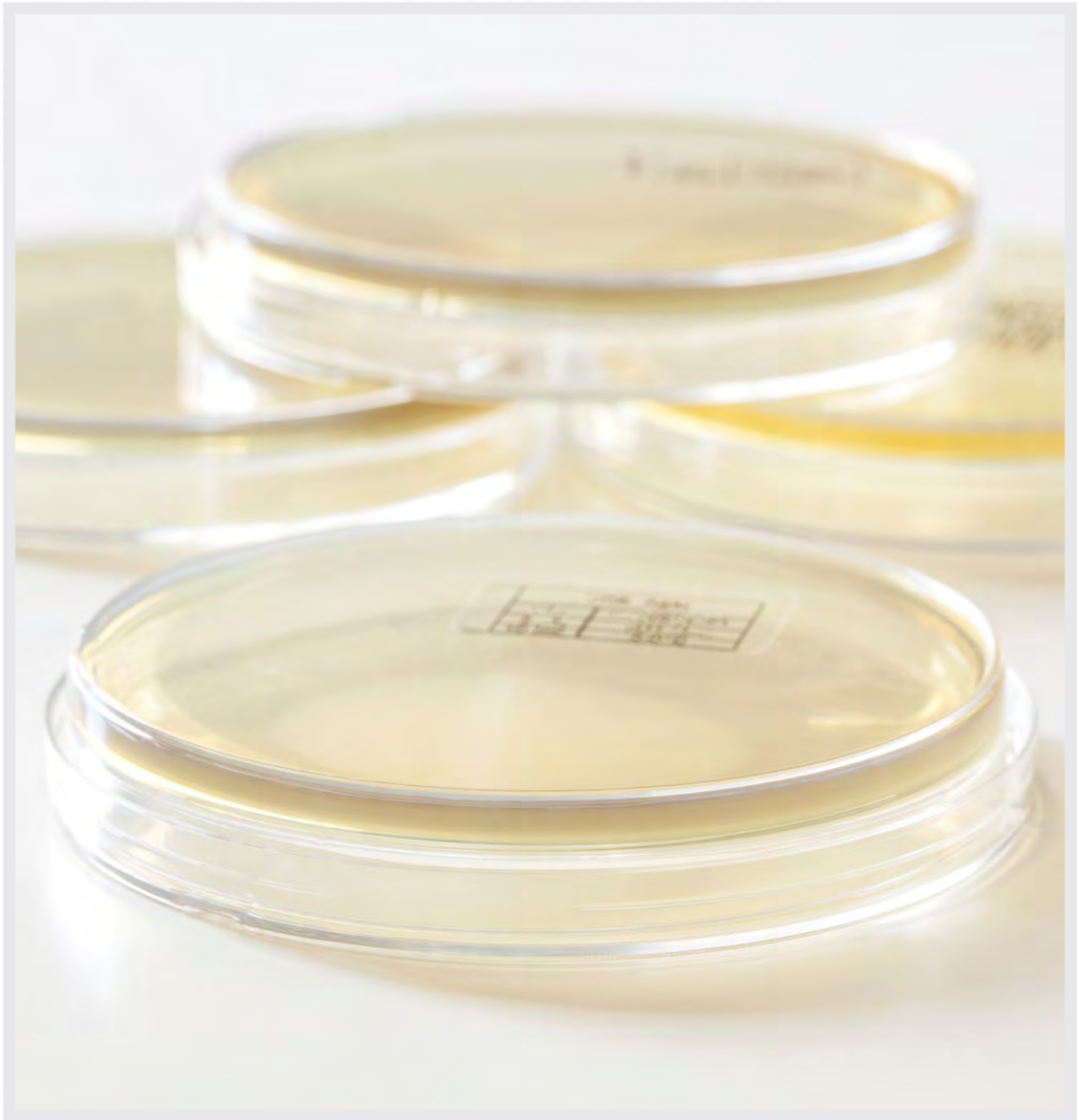
If you’re responsible for environmental monitoring in your organisation, you know the importance of accurate air sampling.

You’ll also know that the changes demanded by the latest revision of Annex 1 will require organisations to monitor cleanroom environments more closely and be able to demonstrate that the monitoring is to the required standard.

At Cherwell we have supplied, serviced, and calibrated air sampling devices for over 35 years. During that time, we’ve seen air sampling devices used with many types of plated prepared media. The question that has intrigued us is “does the choice of plate affect the sampling results achieved?”

In the autumn of 2021, this question became more urgent. At Cherwell, we have a highly skilled and experienced team of calibration engineers.

> *“Does the choice of plate affect the sampling results achieved?”*



We perform calibration of air sampling or air monitoring devices against a standard plate, and against other plates provided by our clients to reflect their own set-up.

Our calibration engineering team began to see evidence that seemed to indicate that the choice of plate was affecting the calibration results in some circumstances. The logical inference was that the choice of plate was affecting air flow through the device. If true, this in turn would render any reading from the device inaccurate.

> *"We promise to be independent in our work and findings"*

So we decided to investigate.

In this report, we present the results of that investigation so far. We've taken plates from the leading manufacturers and tested them against each other, via their performance in a standard set-up of an SAS air sampler. As manufacturers of the Redipor® range of plated media, we declare an interest. However, for this exercise, we promise to be independent in our work and findings.

Findings

We tested six leading contact plates available in the market today. The testing equipment, methodology, and data obtained are discussed in detail in the appendix.

The results show that the flow rate is affected by the type of plate chosen. This in turn may well cause under-sampling, which will lower the consistency of EM reporting. In all cases, the fill volume was 17ml in a 55mm diameter well. This produced the typical convex agar required in a contact plate.

We also found:

- In some cases, additional plastic moulding around the agar elevates the agar on the housing platform (sometimes this moulding was part of the lid locking mechanism).

- The contact plate from one brand was larger in diameter at 69 mm than the other plates at 65-66 mm. This meant that it was unable to align properly, which offset the plate and influences not only the flow rate, but the position under the sieve. This in turn means that some particles may not impact the agar.

- The petri dish results also showed varying degrees of sample volume. Two brands are marginally and one brand notably under-sampling and two are significantly over-sampling.

Petri Dish Flow Rates

The petri dish flow rate variation is possibly due to a difference in fill volume. The standard fill in the Cherwell dish is 18ml and any variation on this would change the height of the agar and consequently affect the flow rate.

> *"The risk in over- or under-sampling is an unreliable understanding of the environment"*

Over and Under-Sampling Risks

Under-sampling carries the risk of failing to detect bioburden.

Over-sampling will have an impact when trying to determine the levels of background flora in lower graded areas of the facility. Over-sampling increases the risk of a false positive result. This may result in poor microbial action and alert limits, which in turn results in unreliable data, and so false information on the facilities environment.

The risk in over- or under-sampling is an unreliable understanding of the environment, which has implications for capture of a possible excursion, microbial contamination of product, and consequential repercussions.

Implication For Annex 1

As a result of over or under-sampling, the sampling process may fail to comply with the latest revision of GMP Annex 1, released 25th August 2022.

Annex 1 now provides clarity on the required CFU values for microbial contamination during qualification of cleanrooms. It also clarifies action limits from viable particle contamination in all graded areas. However, to achieve the required standard, the correct air volume must be sampled, which requires the correct air flow.

> *"The sampling process may fail to comply with the latest revision of GMP Annex 1"*

There is an associated risk that viable organisms within the environment will not be captured. Annex 1 demands that the sampling taking place should demonstrate accuracy, which in turn provides confidence that the clean room is under control.

Conclusion

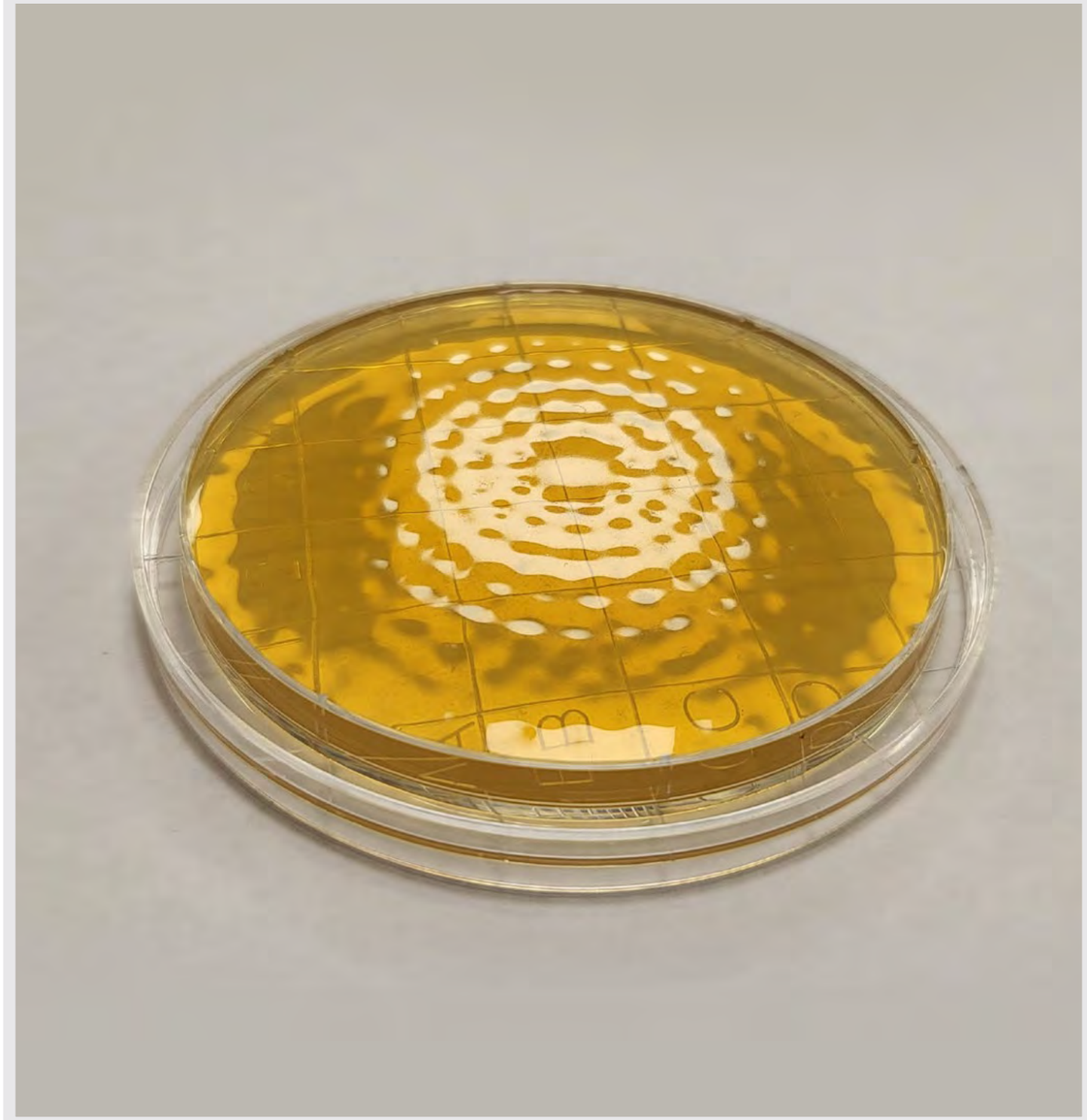
The use of plates or dishes which deviate from the recommended standard to which the air sampler was calibrated will have an impact on the flow rate of air.

This leads to a risk of under- or over-sampling and subsequent unreliable data.

Compliance with the latest revision of Annex 1, published in August 2022, will require organisations to ensure that their chosen plates, in combination with their chosen sampler, gives an accurate result.

If the flow rate of air is affected, this will impact the volume taken onto the plate, which in turn may make the sample non-compliant with respect to Annex 1.

Annex 1 demands better risk assessment and risk management. Consistency and accuracy in air sampling will be part of this. We conclude that plate selection does make a difference, and that selection of the right plate, providing consistency and accuracy in sampling, is an important part of Annex 1 compliance.



Recommendations

1. Increased understanding that cleanroom environmental monitoring is an ecosystem rather than a set of product choices and processes. The media/ sampler combination is an important part of this.

2. Calibration of samplers against establish standards should include testing in conjunction with the prepared media with which they are normally used.

3. Annual (at least) calibration of sampling equipment to reduce the risk factor associated with incorrect sampling.

> *"Selection of the right plate, providing consistency and accuracy in sampling, is an important part of Annex 1 compliance"*

Appendix

Appendix

Equipment Used:

All equipment used is calibrated to a recognised standard:

- SAS 180 isolator head
- Power supply with maintained voltage to produce a constant flow rate of 0.62m/s.
- This ensures the air sampler runs at 180 litres per minute and samples 1000L (1m³) .
- Anemometer (Schmidt probe)

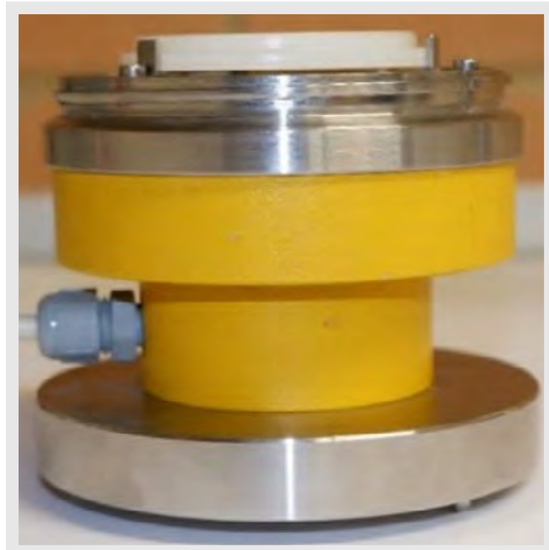


Figure 1 - Resin Plate Calibration

Methodology and Data:

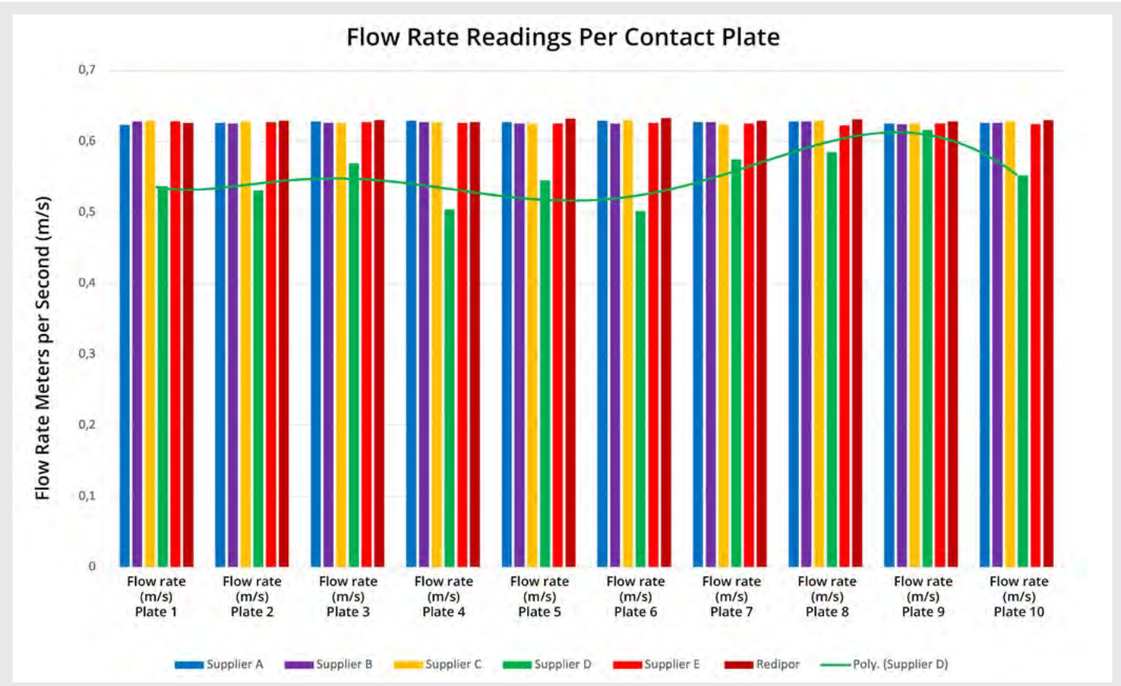
An SAS 180 isolator head was calibrated according to the Cherwell in-house method. This is covered by our ISO 9001:2015 certification.
This method requires the use of a standard resin contact plate and a standard petri dish to perform the calibration. Resin plates are used as these are constants within the process.

Following calibration, we ran these tests:

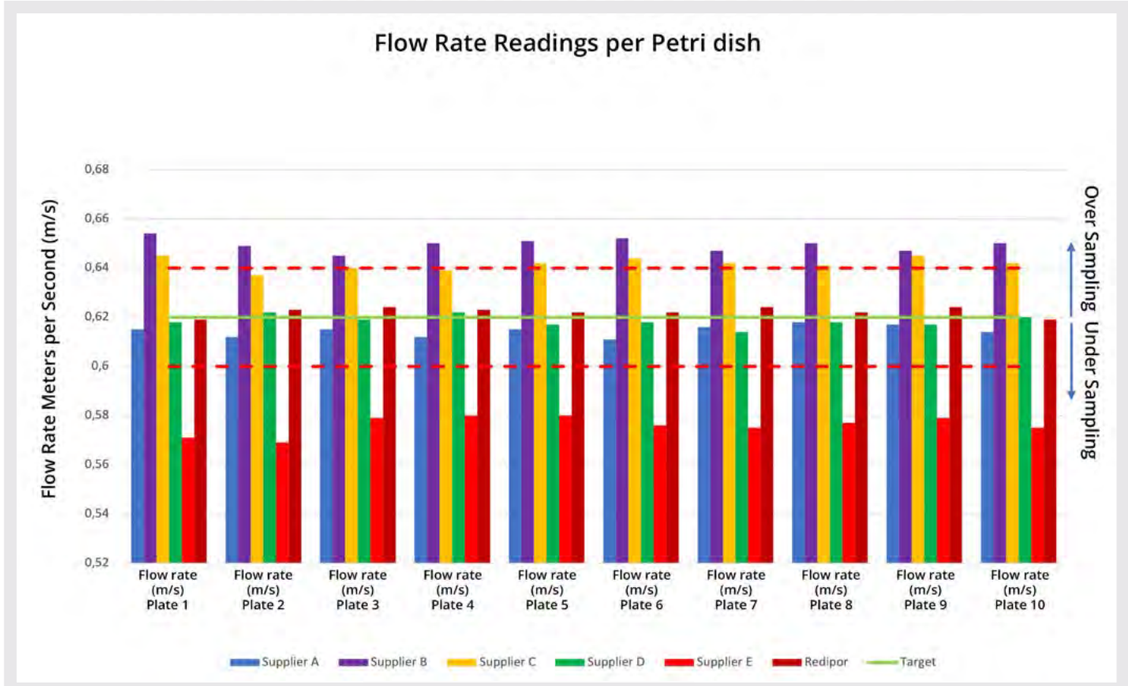
- For each supplier in turn, we placed a contact plate in the unit and then ran the air sampler for five minutes and thirty-three seconds to achieve a sample volume of 1000l, based on a flow rate of 180l per minute. This is in accordance with standard operation during the calibration process.
- We repeated this exercise ten times for each type of plate, in order to get more reliable data.
- The flow rate data was captured in a spreadsheet.
- We repeated the tests with the petri dishes (90mm).

SAS Super 100/180 Isolator

Findings



Bar Chart 1 - Flow Rate Readings per Contact Plate



Bar Chart 2 - Flow Rate Readings per Petri Dish

The results from the reading on the contact plates show some varying consistency in maintaining a 0.62 m/s flow rate. However, supplier D has markedly reduced flow rate and demonstrates an impact on the flow rate of air.

The trendline is polynomial showing the fluctuation in data demonstrating no consistency.

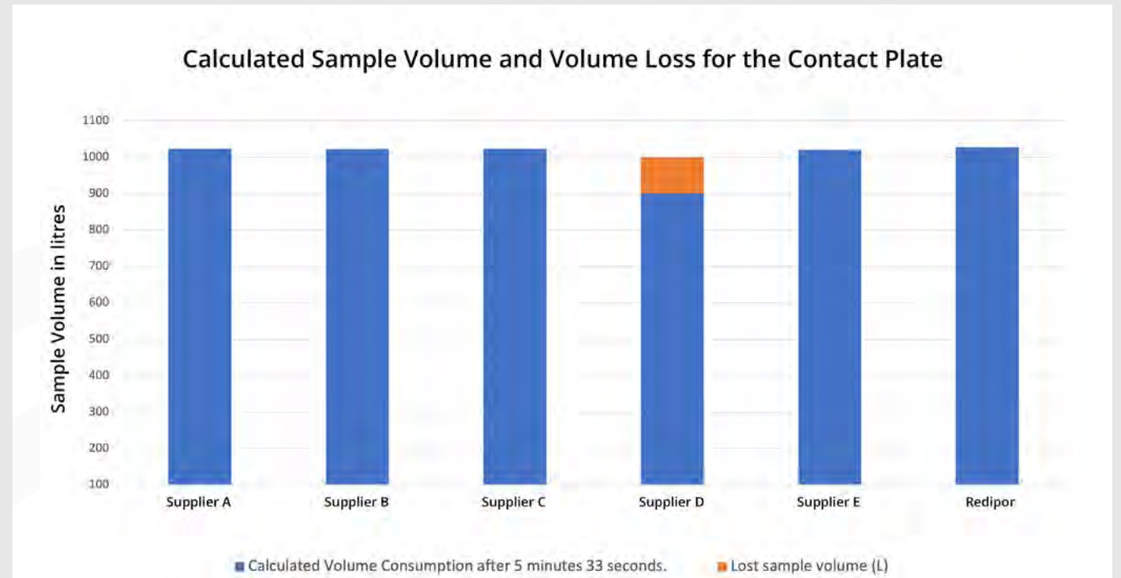
The results of the petri dish (90mm) show varying results:

- Supplier A and D presented a reduction in flow rate
- Supplier B and C demonstrating greater flow rate
- Supplier E Showed significant decrease in flow rate

As there are minor fluctuations in the Cherwell plate readings; the tolerance allowed for calibration in accordance with procedure is ±3%. The data from both the contact plates and petri dish were averaged to produce a mean result on flow rate, using the following calculation to convert meters per second to litres.

Sample Volume Calculations

The results are demonstrated in the bar chart below with the difference in sample volume to the required 1000L indicated as volume loss in a different colour.



Bar Chart 3 - Calculated Sample Volume and Volume Loss on Contact Plates

The mean air flow rate from each supplier plate is multiplied by 294 to expressed litres per minute (L/ m).

Example: 0.62 x 294 = 182 L/min

Multiply this by the time that a calibrated air sampler will sample 1000L (5 minutes and 33 seconds).

Example : 182L/min x 5.55 = 1,010 Litres. (Note 5'33" = 5.55 minutes.)

$$\frac{\text{Speed} \times \pi r^2 \times 60 \times 100}{1000} = \text{litres/minute}$$

Cross Sectional Area of Tube = πr^2 (where r is in cm)

Air Speed = m/sec (x 60 to convert to minutes and x 100 to convert to cm)

Divide by 1000 to convert cm3/minute into litres/minute

In removing the air speed, which is the variable being tested, the conversion formula is adjusted as shown below:

$$\frac{(\pi r^2) \times 6000}{1000} = \text{(conversion factor rounded to whole number)}$$

Simplifying the calculation by performing the division of the 1000 reads:

$\pi r^2 \times 6 = a$ (conversion factor rounded to whole number)

The 'r' is the radius of the tubing which is 3.95cm, the final calculation reads:

$\pi \times 3.952 \times 6 = 294$

Potential Causes

The results show supplier D has a volume loss of just over 100 litres (10%).

The potential cause can be linked to the height of the plate base as shown in Table 1, Figure 2 and Figure 3.

Plate	Plate base diameter (mm)	Plate height (mm)
Redipor®	65	9
Supplier A	69	11
Supplier B	65	11
Supplier C	65	10
Supplier D	66	13
Supplier E	65	10

Table 1 - Contact plate base and height measurement

Figure 2 - Left: Supplier D plate during sampling Right: Redipor® plate

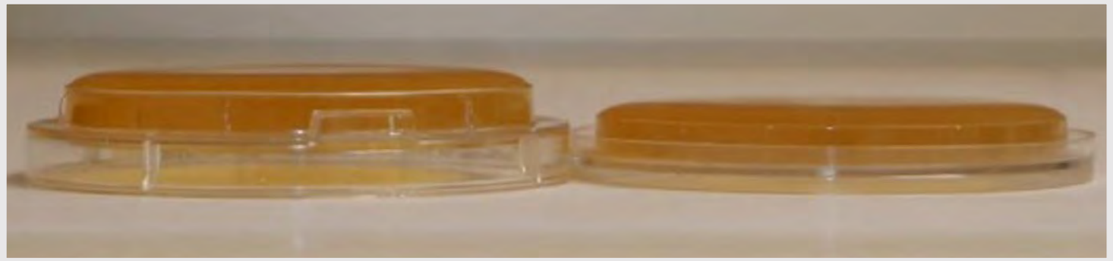


Figure 3 - Supplier D (left) plastic plate height compared to Redipor® plate (right)

The impact due to change in flow rate can be physically seen in the impressions left on the surface of the plate. On the right (figure 5) the lower flow rate delivers a reduced sample volume. The air in the middle of the plate is not impacting the agar at a high enough speed to make the indents in the centre of the plate because the flow is restricted.

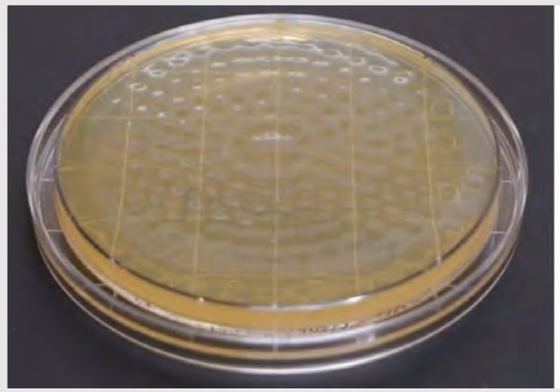


Figure 4 - Redipor® plate after sampling

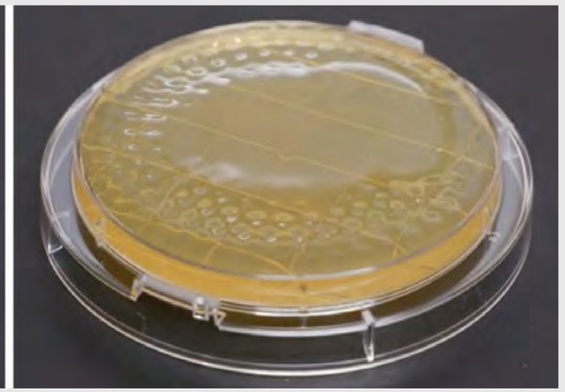


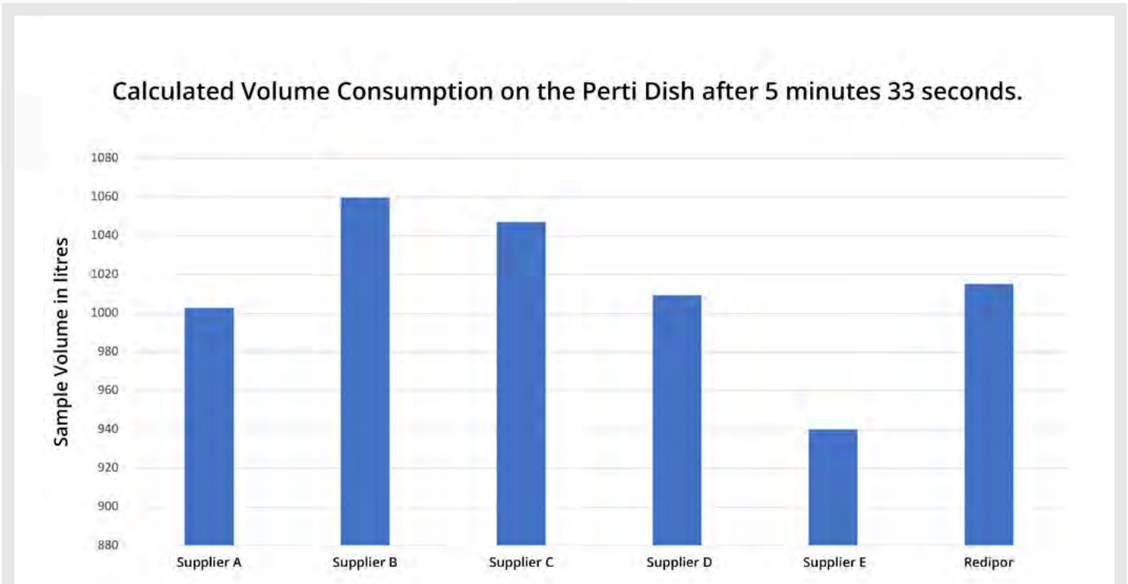
Figure 5 - Supplier D plate after sampling

In addition to the impact on flow rate the diameter of the plate showed some misalignment. Supplier A had a larger base (69mm compared to all other plates sampled which are 65mm) and therefore didn't align to the sampler, Figure 6 and Figure 7. This required adjustment to enable effective sampling.

Figure 6 & 7 - Supplier misalignment in samplers

The petri dishes from each supplier show a large variability in sample volumes that were achieved by the air sampler in Bar Chart 4. Supplier B and C supplied petri dishes have exceeded the required volume of 1m³ as B takes close to a 1060L sample. Petri dish supplier E shows a total volume of 940L which puts into question the accuracy of the sample.

Petri dish suppliers A, D and the Redipor® control are all providing samples within the required 1000L.



Bar Chart 4 - Calculated Volume Consumption of the Petri Dish

About Cherwell Laboratories

Cherwell Laboratories, located in Bicester, Oxfordshire in the UK, is a family run manufacturer of prepared microbiological media and supplier of environmental monitoring equipment. We supply to the UK, Ireland and a number of western and central European countries, primarily to aseptic manufacturing sectors, such as pharmaceuticals and medical devices.

We are unique to many of our larger competitors in that we are able to offer tailored solutions to match customer needs. This not only applies to our range of prepared media, but also for the air sampling equipment and EM accessories we specialise in.

We never dreamt when we founded Cherwell in 1971, as a veterinary diagnostic laboratory, that we would transition to be the company we are today. It was in the late 80s that the veterinary lab closed, but we retained the microbiology facility and turned our focus to the marketing of Redipor® prepared media and developing sales of EM samplers into the pharmaceutical sector.

Since the early 80s, we have been the UK distributor for the SAS range of air samplers. Recognised across the industry, many of the leading pharmaceutical companies across the world use the distinctive yellow SAS as the cornerstone of their EM programs.

During the 35 plus years of selling SAS we have created bespoke solutions for individual clients. More recently Cherwell has added to its capabilities with the addition of the ImpactAir slit to agar sampler to further meet the monitoring needs in critical environments.

With many, many years of insight and experience with environmental monitoring applications, we have intricate understanding and expertise that ensures we continually deliver high calibre products and services to our many clients.

If you would like to explore our product range in greater detail, you are welcome to do so here.

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