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R. Meier and H. Zingre

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Qualification of air sampler systems: The MAS-100

*R. Meier and H. Zingre**

Key words: Air sampler, qualification

Summary
In the context of the newly developed MAS-100 air sampler system, the qualification areas
– Design qualification
– Installation qualification
– Operational qualification
– Performance qualification
illustrate the qualification procedure for this type of device.

Comparative studies of eight different methods yielding 1022 individual results at Novartis Pharma AG undertaken in Basel/Switzerland between 1981 and 1998 were evaluated. They show that, if meaningful statistical analyses are performed, adherence to important testing parameters such as sampling periods, culture media used, and placement of the devices, six of the eight methods examined yielded comparable cfu levels.

Introduction
Monitoring the number of microorganisms in the air is becoming an ever more important task due to increasingly stringent regulations, in the medical device industry as well as in the cosmetic or food industries, but especially in the pharmaceutical industry.
Reliable and accurate monitoring results, however, depend on a number of different factors:
- Composition and concentration of airborne microbiological contaminants and contaminant-carrying air particles
- Requirements of the tested area (clean room class)
- Correct operation of the device
- Device functionality
- Method of cfu counts and their evaluation
- Incubation times and temperatures
- Culture media

This article intends to demonstrate the qualification of air sampler systems using the MAS-100 developed by the authors as a practical example in order to assist interested readers in performing their own evaluations.

Materials and Methods

1. MAS-100: principle of operation
The MAS-100 operates on the impact principle. The contaminant-carrying particles are propelled onto a solid medium. The sampled air is aspirated either horizontally or vertically through a perforated plate with 400 holes 0.7 mm in diameter and propelled (blown) onto media plate with an impact speed of 10.8 m/s. The air then passes through an air flow meter that adjusts the sampling volume to a constant 100 litres per minute. This continuous adaptation of the air volume is a novelty in air sampler systems and greatly contributes to correct and reproducible results.

2. Qualification focus
Qualification of air samplers is primarily effected using the following parameters currently accepted primarily in English-speaking areas:
DQ Design qualification
IQ Installation qualification
OQ Operational qualification
PQ Performance qualification

2.1 Design qualification (DQ)
For manufacturers, *design qualification* means determining a profile of requirements for a given device. This profile may contain a number of different criteria.
The following criteria applied for the MAS-100:
- Easy to use
- Universal application
- Suitable for clean rooms
- High precipitation rate for bacteria and fungal spores
- Can be calibrated and adjusted

For customers, DQ may mean, for instance, the scope of delivery of the device.

2.2 Installation qualification (IQ)
Installation qualification means that the requirement profile as described in the DQ is met, or that the device and its accessories are delivered as ordered.

* *R. Meier, Novartis Pharma AG, CH-4002 Basel (retired 1. 9. 99),*
H. Zingre, MBV AG, CH-8712 Stäfa

Device No.: _____

Test No.	Description of test	Expected event	Actual event	P /NP *	Initials
1	Activate electronics	Display is lit	Display:		
2	Start-up delay	Start-up after 5 minutes	Start after:		
3	Sampling indicator	RUN indicator lit	Lit:		
4	End-of-sampling indicator	STOP indicator lit	Lit:		
5	Sampling period	2½ minutes for 250 litres	Minutes:		
6	Aspiration volume with plate	100 ± 2 litres			
7	Aspiration volume without plate	100 ± 2 litres			
8	Automatic shut-down	After 5 minutes	Display darkens		

* P: Test passed
NP: Test not passed

Date: _____

Technician: _____

Initials: _____

Fig. 1: Operational qualification – MAS-100.

2.2.1 User-friendliness
Modern air samplers should meet the following minimum requirements:
- Easy to carry
- Operation on rechargeable batteries
- Constant performance
- Low cost of consumables (culture medium)
- Calibration
- Covered air intake

Some additional parameters apply to the MAS-100:
- Programmable delay before air collection (1–60 min)
- Date and time
- Optical status indicators

2.2.2 Operation area
Depending on what class of clean room an air sampler system is to be used in, the system will have to meet the applicable requirements.
For sterile areas where contaminant counts are required to be less than 1 cfu/m³, the sampling volume must be at least 1 m³ to acquire quantities of less than 1 cfu/m³. This means that impact devices must have a relatively high air throughput to test 1 m³ of air within a reasonable amount of time (the throughput for the MAS-100 is 100 litres per minute). For areas that are subject to less stringent requirements, it is possible to extrapolate from the results of smaller sampling volumes to obtain cfu/m³ values.

2.2.3 Suitability for use in clean rooms
Suitability for use in clean rooms does not only mean that the device is suitable in terms of cfu counts, but also in terms of particle counts. Depending on the designated clean room class, a number of factors have to be evaluated in advance:
- Device decontamination
  Each device should be suitable at least for external disinfection. The MAS-100 is additionally suitable for ethylene oxide sterilisation. When microbiological air quality is monitored in sterile Class 100 clean rooms it is recommended to keep the air sampler permanently deployed in this zone following decontamination. This reduces the risk of secondary contamination.
- Particle emissions
  In all mechanical devices with moving parts, abrasion can generate smaller or larger amounts of particles. For this reason, any device that is to be used in areas with stringent air particle count requirements must be tested for particle emissions.
  One method for doing this is to turn on the air sampler inside the laminar flow of a sterile bench and to determine the particle count in the exhaust air. (The number of particles > 0.3 µm found in the MAS-100 exhaust air is less than 5 per cubic foot of air.)
- Suitability for isokinetic sampling
  Sterile rooms in the pharmaceutical industry are Class 100 clean rooms (Zone A according to EU classifications) and are usually equipped with laminar flow systems from 0.3 to 0.5 m/s. To avoid turbulence, all air sampling should be performed isokinetically. (This is why the MAS-100 airflow speed is 0.45 m/s.)

2.2.4 High precipitation rates
The quality of an air sampler is primarily determined by its precipitation rate, which in turn is influenced by the following factors:
- Air sampler design
- Physical properties of the contaminant-carrying particles
- Type of contaminants
- Rate of impact on the culture medium

However, opinions are strongly divided as to what constitutes the proper method for determining precipitation rates and what would be the proper reference method.
Attempts are currently under way to determine precipitation rates using artificial

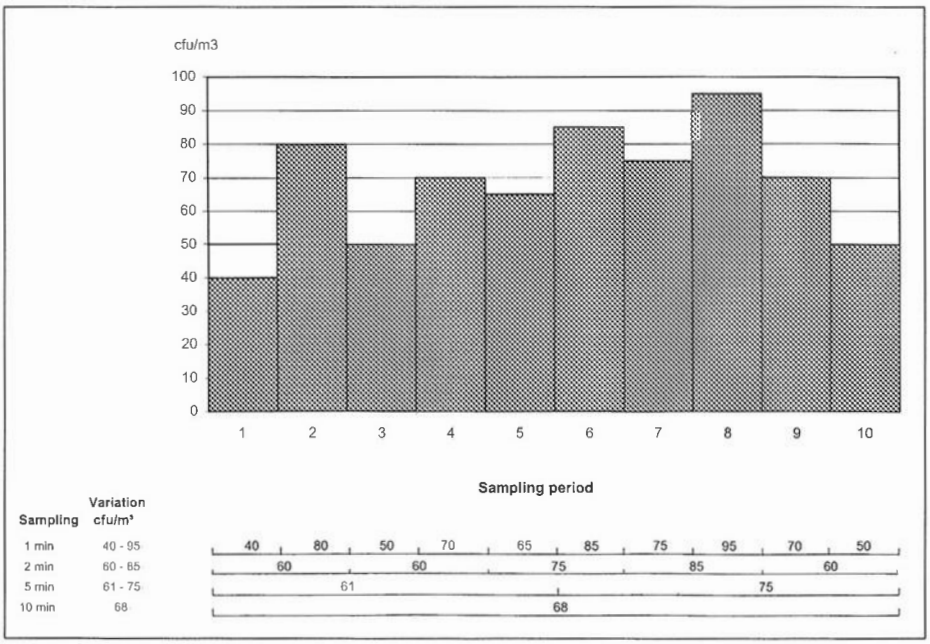


Fig. 2: Influence of the sampling volume and the sampling procedure on the resulting airborne microbe count using a fictitious variation in the microbial count as an example.

aerosols of contaminant suspensions. Since real-life contaminants suspended in air, however, are attached to dust, lint, or skin particles, this type of qualification makes little sense; this would be a comparison of the proverbial apples and oranges.

Since it is well known [1, 2, 3] that the size of contaminant-carrying particles exceeds 2 µm, it is important to know what size particles an air sampler is able to precipitate. Based on the physical data known for each impact sampler, the precipitation efficacy can be calculated by determining the cut-off size (d_{50}), as follows [4]:

$$d_{50} = \sqrt{\frac{9\,\eta\,WSTk\,50}{p\,U\,C}}$$

were

- η = Viscosity of the air ($1{,}81\times10^{-5}$ Pas)
- Stk_{50} = Stokes constant (¼ for round and ½ for rectangular air intake apertures)
- ρ = Particle density (1.03 g cm^{-3})
- U = Impact speed (m/s)
- C = Cunningham correction for particles < 1 µm
- W = Diameter of air intake aperture (mm)

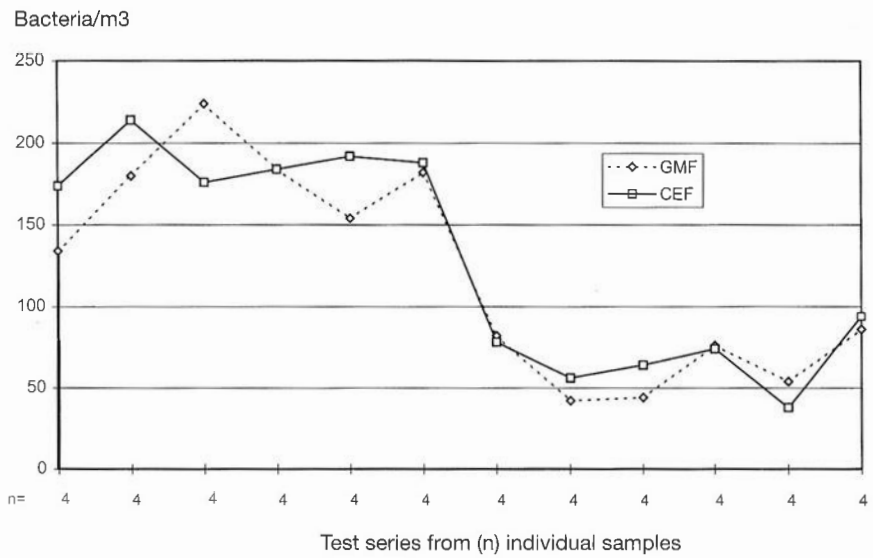
This formula can be simplified by predetermining the constant factors (air viscosity, particle density, correction factor):

$$d_{50} = \sqrt{\frac{40.725 \times W}{U}}$$ round air intake apertures

$$d_{50} = \sqrt{\frac{81.45 \times W}{U}}$$ rectangular air intake apertures

At an aspiration volume of 100 litres per minute and with 400 holes with a diameter of 0.7 mm each for the lid, the MAS-100 will attain a collision speed of 10.8 m/s. This results in a d_{50} value of

$$d_{50} = \sqrt{\frac{40.725 \times 0.7}{10.8}} = 1.62\ \mu m$$



Average cfu counts using GMF 120
Average cfu counts using CEF 128

t test *t* for 2p = 0.05 2.012
t for GMF-CEF 1.206

Result: No significant differences between the two filter types. At the same time, testing under these conditions refuted the claim that the contaminants on the CEF would be damaged [9].

Fig. 4: Comparative airborne contaminant counts using GMF and CEF (1981) n = 48 pairs.

Air samplers with a d_{50} of less than 2 µm should theoretically be able to precipitate practically any airborne microbiological contaminant-carrying particles.

2.2.5 Calibration and adjustment

To obtain constant and reproducible results, the air sampler must be calibrated and, if necessary, adjusted at least once every year. Calibration can be effected using a certified anemometer or – as in the case of the MAS-100 – using a positive displacement gas meter.

2.3 Operational qualification (OQ)

Operational qualification demonstrates that the device under inspection is fit for use. Like performance qualification, OQ is not infrequently required by regulatory authority representatives. In OQ, certain functions that are important to the individual user – such as sampling periods, start-up delays, or automatic cycle termination – are tested using e. g. a stopwatch *(Fig. 1)*.

2.4 Performance qualification

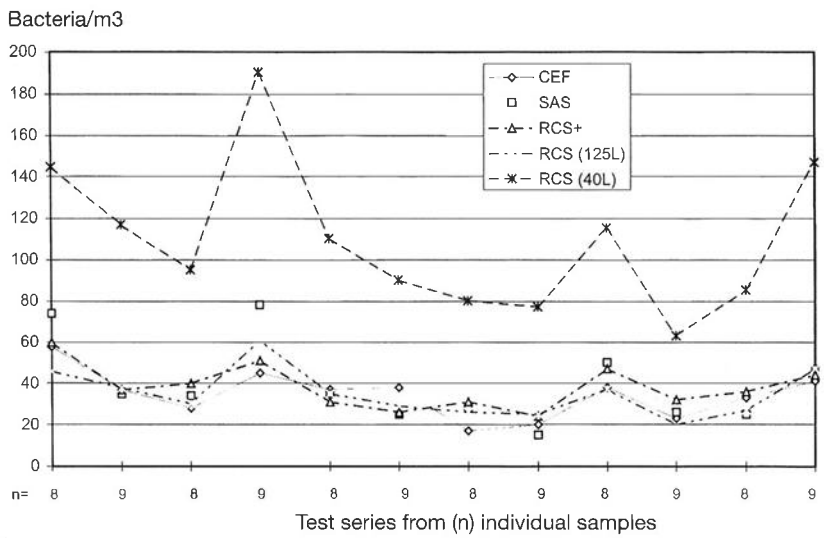
As mentioned before, correct determination of airborne contaminant counts depends on a number of different factors. The nature of the airborne contaminants and the contaminant-carrying particles, as well as environmental and testing conditions, will vary from one air sampler system application to another. Users will therefore have to obtain performance qualification for *their* specific applications and *their* specific testing conditions in order to demonstrate the biological efficacy of their samplers. Incidentally, the regulatory authorities and their representatives specifically require this.

Company-internal or regulatory requirements regarding airborne contaminant counts exist for most areas in which microbiological air quality is monitored. Once a new monitoring method is introduced, it must be determined whether the new device yields the same results as those obtained by the device used previously.

As a result of technical advances, Novartis Pharma AG repeatedly changed its testing methods, from the initial GMF (1975) through CEF (1982), RCS (1982), SAS-Compact (1988) to the MAS-100 (1996), and validated these methods accordingly

	Colony count per sampling volume			
Trial No.	Device 1 500 litres	Device 2 500 litres	Devices 1 + 2 1000 litres	Device 3 1000 litres
1	24	34	58	72
2	28	28	56	52
3	12	19	31	37
4	39	18	57	50
5	9	41	50	30
Average 1–5	–	–	50	48

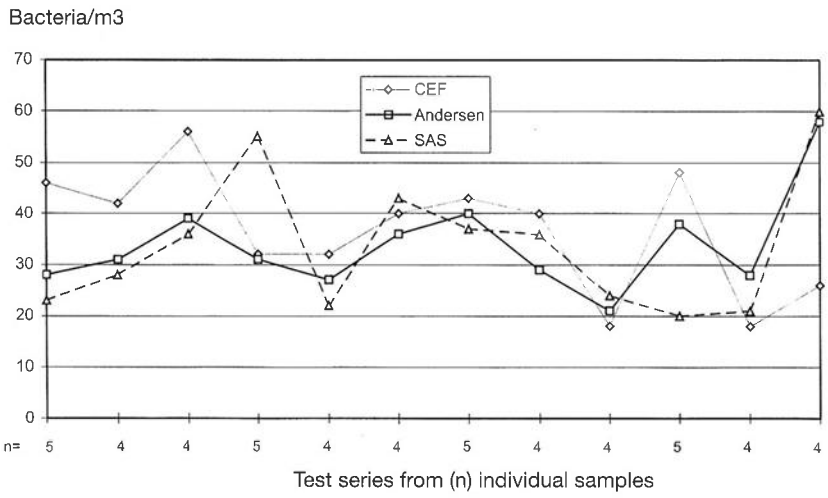
Fig. 3: Comparison of colony counts using sampling volumes of 500 and 1000 litres of air.



Average cfu counts using CEF		35
Average cfu counts using SAS		39
Average cfu counts using RCS (40 l/min)		109
Average cfu counts using RCS (125 l/min)		35
Average cfu counts using RCS-Plus		38
t test:	*t* for 2p = 0,05	1.984
	t for CEF-SAS	1.631
	t for CEF-RCS-Plus	1.575
	t for CEF-RCS (125 l/min)	0.316
	t for CEF-RCS (40 l/min)	15.009

Result: No significant differences between CEF-SAS, CEF-RCS-Plus, and CEF-RCS (assumed air volume flow: 125 l/min). RCS, by contrast – 40 l/min according to manufacturer data – yielded about three times as many contaminants as any other method, an observation confirmed by other authors [11].

Fig. 5: Comparative airborne contaminant counts using CEF, SAS, RCS and RCS-Plus (1990) n=102 pairs.



Average cfu counts using CEF		37
Average cfu counts using SAS		34
Average cfu counts using Andersen		34
t test	*t* for 2p = 0.05:	2.008
	t for CEF-SAS:	0.699
	t for CEF-Andersen:	0.854

Result: No significant differences between methods.

Fig. 6: Comparative airborne contaminant counts using CEF, SAS and the Andersen sampler (1991) n = 52 pairs.

[5, 6]. Care was taken to ensure that identical testing parameters were used over the years to the full extent this was feasible:

2.4.1 Mode of operation

Airborne contaminant counts are dependent on the person operating the measuring device. The more stringent the air purity requirements, the greater the influence of air sampling techniques. Given the fact that humans are the most important disseminators of germs in clean rooms, operator influence on air sampler results is considerable.

When several air samples are taken in the same area, it is therefore possible that the first sample shows a higher contaminant count than subsequent samples because the operator may be a source of additional microorganisms entering the environment while the air sampler is installed. It is therefore necessary to wait 1–2 minutes after installation before starting the device. Being able to program a start-up delay is therefore an important advantage.

2.4.2 Sampling volumes and sampling periods

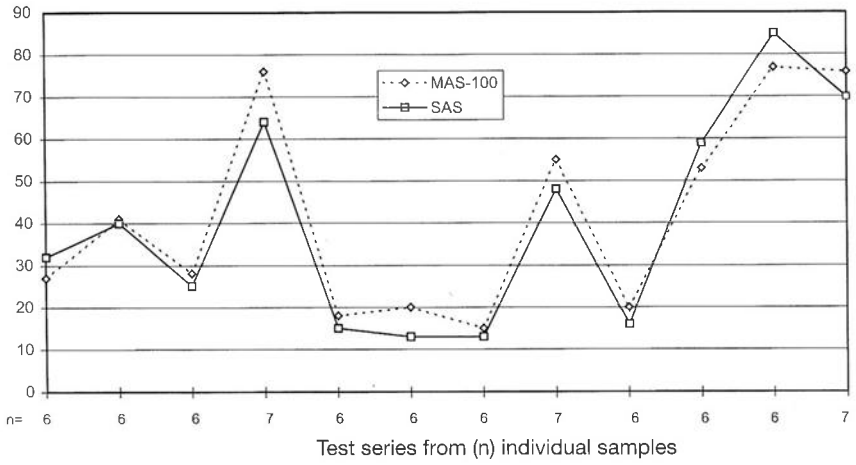
The experimental set-up needs to take device-specific properties and the expected microbiological air quality into account. If an air sampler with an aspiration volume of 100 l/min is compared to one with an aspiration volume of 50 l/min, results can vary considerably, depending on whether the same air volume or the same sampling period was used for both devices. If measurements are made in a conventionally ventilated room, airborne contaminant counts may also vary greatly if people are moving around or if work is being performed, sometimes from minute to minute *(Fig. 2)*. If, as shown in Figure 2, 100 litres of air are tested with these two devices, this corresponds to 1 minute of sampling for one device and 2 minutes of sampling for the other. The first device (100 l/min) will therefore yield a cfu count of 40/m³, while the second device (50 l/min) will exhibit 60/m³, (40 plus 80 divided by 2). For a sampling volume of e. g. 400 litres, the cfu counts obtained are 60 and 70/m³, respectively. If, however, the two devices are configured for the same sampling period, the microbial counts/m³ will always be identical. When comparing two devices with the same aspiration volume, their microbial counts will always be the same, independent of whether the same sampling period or the same sampling volume is selected. The example in Figure 2 shows:

1. When comparing two or more air samplers, sampling periods rather than sampling volumes should be identical wherever possible.
2. The longer the sampling period or the higher the sampling volume, the more accurate the measured cfu count.

3. For routine use: Several individual measurements averaged are more reliable than a single measurement.

2.4.3 Pathogen counts and sampling periods

When determining airborne microbiological contaminant counts in Class 100 clean rooms where the microbiological requirement is for less than 1 cfu per m^3 of air, it is indispensable to sample at least 1 m^3 of air. This of course invariably results in longer sampling periods and thus to potential cfu loss due to agar surface drying.

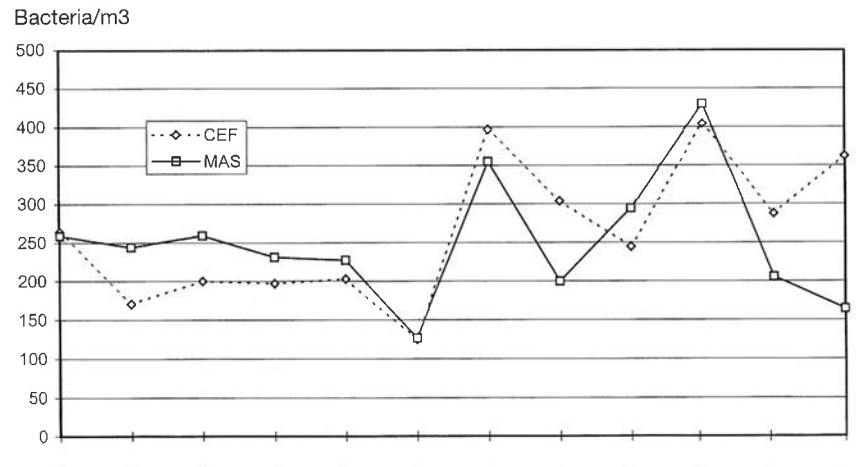


Average cfu counts using SAS 41
Average cfu counts using MAS 43

t test *t* for 2p = 0.05 1.993
t for SAS-MAS 1.552

Result: No significant differences between SAS-Compact and MAS-100.

Fig. 7: Comparative airborne contaminant counts using SAS-Compact and MAS-100 (1996) n = 75 pairs.



Average cfu counts using CEF 264
Average cfu counts using MAS 253

t rest: *t* for 2p = 0.05 2.030
t for CEF-MAS 0.621

Result: No significant differences between CEF and MAS-100.

Fig. 8: Comparative airborne contaminant counts using CEF and MAS-100 (1997) n = 36 pairs.

Because the MAS-100 was designed specifically for determining low cfu counts, we were interested to see how doubling the sampling period to yield 1000 instead of 500 litres would affect the resulting cfu counts. To this end, 3 MAS-100 were loaded with TSA plates and placed adjacent to each other. Devices 1 and 2 were programmed for 500 litres, while device 3 was programmed for 1000 litres. Devices 1 and 3 were started concurrently, while device 2 was started after device 1 had completed its cycle. In theory, the cfu counts of device 3 should equal the sum of the cfu counts of devices 1 and 2.
The results (in no. of colonies per plate) are shown at Fig. 3.

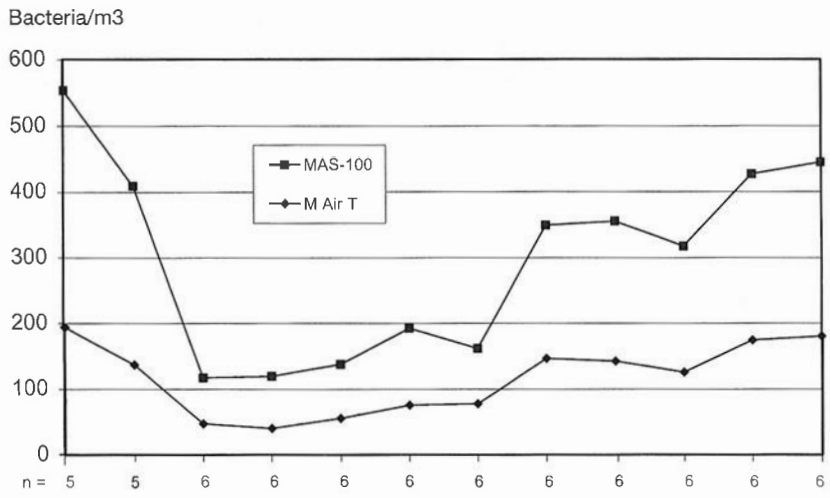
Result: Doubling the sampling period does not cause any significant contaminant loss.

2.4.4 Incubation periods and incubation temperatures

Both incubation periods and incubation temperatures will depend on the nature of the contaminants whose counts are to be determined. In hospitals, an incubation period of 2 days at 35–37°C may be sufficient to demonstrate the presence of human contaminants. In industrial environments, it is usual to determine the total bacterial and mould counts from one and the same air sample. In terms of incubation conditions, this means a compromise 5–7 days at 25–30°C. In an unpublished company-internal study, it was shown that the mould counts is considerably higher at 20–25°C than at 30°C. The exact opposite is true, by contrast for bacterial counts. Practical experience has shown that taking a first cfu count after 2–3 days is beneficial in that mould colonies appearing later could overgrow the already counted bacterial colonies. If bacterial counts were first taken after 5–7 days, this would have a negative influence on the accuracy of the bacterial counts.

2.4.5 Types of cfu counts and methods of evaluation

The cfu counts of all filter-type samplers has to be corrected using a conversion table (Feller 1950 [7]). This is the case because with increasing airborne microbiological contaminant concentrations, the probability will increase that more than one contaminant is propelled through the same air intake aperture onto the plate and is counted as a single colony-forming unit.
The higher the contaminant count, the greater the counting error. This error in turn will be the greater, the smaller the sampling volume. If the airborne contaminant count is, say, 300 cfu/m^3, a counting error of ± 2 micro-organisms in a 1-minute sample (= 100 litres), when converted to microbial count/m^3, will have a variation of ± 20 micro-organisms. The same counting error of ± 2 colonies in a 5-minute sample (= 500

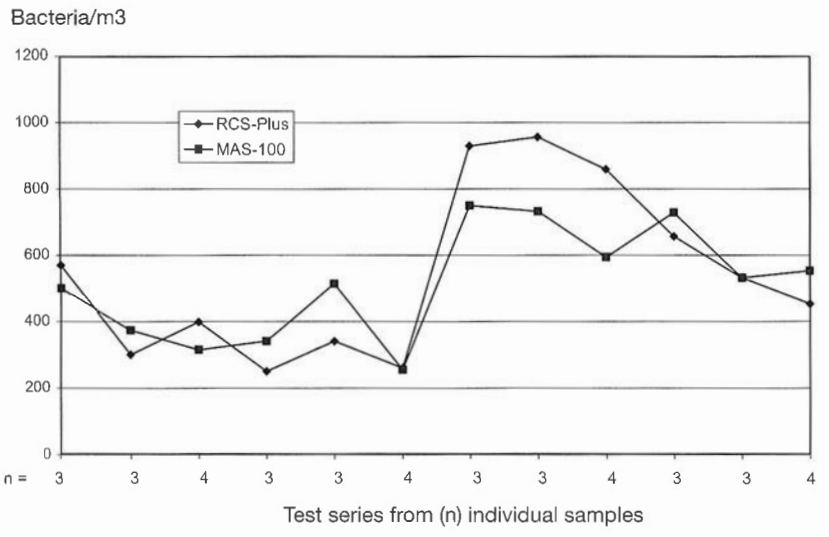


In 1998, at the request of Millipore, the newly developed M Air T air sampler was compared to the MAS-100, and the uncorrected values were evaluated statistically.

Average contaminant count M Air T		46
Average contaminant count MAS-100		69
t test	*t* for 2p = 0.05	1.995
	t (not corrected)	4.223

Result: MAS-100 contaminant counts were significantly higher than M Air T contaminant counts.

Fig. 9: Comparative airborne contaminant counts using M Air T and MAS-100 (1998) n = 70 pairs.



An independent study commissioned at the Centre for Applied Microbiology and Research at Salisbury (UK) in 1997 was also evaluated statistically:

Average cfu counts using MAS		507
Average cfu counts using RCS-Plus		537
t test	*t* for 2p = 0.05	2.023
	t for MAS-RCS-Plus	1.103

Result: No significant differences between MAS-100 and RCS-Plus.

Fig. 10: Comparative airborne contaminant counts using RCS-Plus and MAS-100 (1997) n = 40 pairs.

litres) will therefore yield results of between 296 and 304 micro-organisms/m³, which is a variation of only ± 4 micro-organisms. The rule therefore continues to hold: the longer the sampling period, the more accurate the results.

Results
Comparative studies, 1981–1997

Testing conditions

The devices to be compared were each placed on a table at 1.2–1.5 m above the floor, in a row with distances of 1 m each between them. The air intakes of the devices were at the same level and facing upward and so that the exhaust air of one device did not affect the aspired air of another device.

Total bacterial and mould counts were determined following 5–7 days of incubation at 29–31°C.

Devices and materials

- Sartorius Collectron with 3-µm gelatine membrane filters (GMF), loaded with TSA (tryptic soy agar) plates
- Sartorius Collectron with cellulose ester filters (CEF) 0.8 µm, loaded with TSA plates
- Biotest RCS with TSA foil
- Biotest RCS Plus with TSA foil
- PBI SAS-Compact with OMIKO plates [8] with TSA
- Andersen with 6 TSA plates
- Merck MAS-100 with TSA plates
- Millipore M Air T with special TSA plates

Resulting contaminant counts (cfu/m³)

All data were evaluated in pairs using the Student t test ($p > 0.05$). (To enhance the clarity of the presentation, individual values were converted to 12 mean values for the purposes of this publication).

Discussion

When examining comparative studies of different methods for determining airborne microbiological contaminant counts it becomes apparent that the claims made relative to the number of contaminants retrieved are often contradictory. This may confuse potential buyers with regard to their choice of method and existing users of a specific device with regard to its performance.

This article intended to show users of air samplers how their physical performance is calculated and how their biological performance can be demonstrated based on strict adherence to the most important testing criteria.

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