Airborne germ counts and particles in round jet impactor

This article shows reasons and solutions to resolve the issue of deviations between the number of colony-forming units (CFU) and particle numbers for air sampling operations performed using air samplers in accordance with the round jet impaction method.

Round jet impactors have proved to be extremely effective as air samplers during microbiological air analyses. Most of the models available on the market allow the use of cost-effective culture media in standard petri dishes, and are robust, and user-friendly.

How round jet impactor work

To facilitate understanding once again of how round jet impactor work:

A defined volume of sample air, usually 50 to 400 litres per sample, is propelled using an air feed device (pump or fan) with a defined volume flow, depending on the type from 28.8 to 100 litres/min, through numerous round jets arranged side by side.

The number and cross-sectional area of the round jets varies based on the maker and type. This means, for example, that the “Andersen Sampler N6” has 400 round jets, the “LKS 30” air sampler has 324 round jets and the “LKS100” has over 500 round jets. The sample air is conveyed from top to bottom through the air sampling collection head. The sample air is conveyed from top to bottom through the air sampling head. The flow velocity is considerably higher in the jet area and the airborne particles to be collected are accelerated in the direction of the culture medium in the petri dish. Underneath the round jet plate, the flow velocity is reduced due to the enlarged cross-section to the extent that most particles, based on their inertia, collide with the culture medium in the petri dish and remain there. (Image 1).

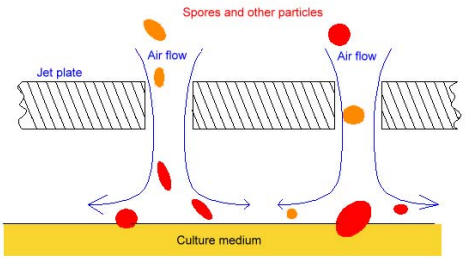

Image 1: Functional principle of a round jet impactor

Depending on the viscosity of the culture medium, the air flows leave visible dents on the agar surface (Image 2).

And it is at these very spots, the bacterial sites, that the microorganisms

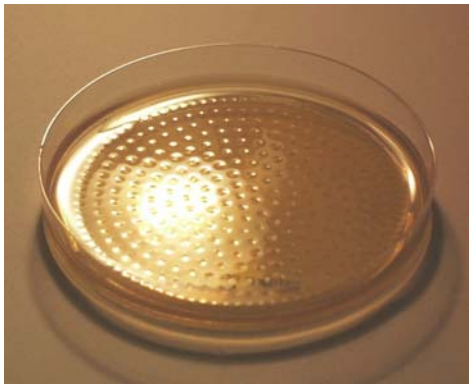

Image 2: Loaded culture medium

having grown after cultivation as colony-forming units (CFU) are visible (Image 3).

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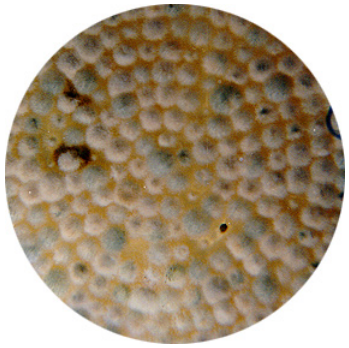

Image 3: Colony-forming units

The evaluation
The evaluation of the air sample depends on the respective task being performed. The total bacterial count can be determined by counting the CFU on the culture medium and subsequently standardising over 1000 l (1 m³) air.

$$\text{germ count}[\text{CFU}] = \frac{1000\,\text{l} * \text{counted CFU}}{\text{Sample volume}[\text{l}]}$$

For the purpose of qualitative evaluation, the genera of CFU are identified. For genera, which encompass potentially pathogenic varieties (e.g. aspergillus), the CFU in question are further identified in terms of the specific varieties of genera in question (e.g. A. fumigatus), or these varieties are excluded. The counted genera and varieties, standardised over a volume of 1000 litres, reflects the spectrum of genres.

The above-mentioned evaluation methods will precisely reflect the actual content of the sample air provided:

1. during the sampling, only one spore is respectively accelerated through a circular nozzle towards the culture medium,
2. This spore reaches the culture medium and
3. This spore also germinates and is thus visible as KBE.

Problem
In reality these conditions are not always met, because:
To 3: Whether a spore germinates, depends initially on the germination capacity of the spore itself. Reasons for the loss of this germination capacity may include e.g. the age of the spores or damage due to the use of fungicidal agents prior to sampling. However, the culture medium used must also be suitable for the germination of a spore in terms of water activity, substrate etc..
Last but not least, the germination of this spore may be hindered by the growth of other microorganisms in the vicinity or even prevented altogether (e.g. due to trichoderma).

To 2: Whether each spore actually "lands" on the culture medium, namely is separated from the sample air, depends on the separation rate of the air sampler.
It is easy to imagine: the smaller a spore and the lower the mass of this spore, the sooner the spore is carried away with sample air drawn away over the culture medium.
The **performance** of an air sampler thus depends primarily on its ability to separate as many of even the smallest spores (particles) in the sample air onto the culture medium. The separation rate, also known as the cut-off value, of an air sampler reveals the smallest aerodynamic diameter of spherical particles with unit density of 1000 kg/m³, which are separated with a likelihood of 50 % by the collector from the sample air [2]. Of course, the separation rate specified by the manufacturer only applies during operation with nominal operating data, e.g. volume flow.
The separation rate deteriorates with

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increasing volume flow. This means that when operating an air sampler with a volume flow of 5 l/min, instead of the 30 l/min specified on the data sheet, namely similar KBE figures for mainly larger spores (e.g. cladosporium) are collected. If the volume flow is too low, however, the risk remains that the small spores (e.g. aspergillus), which are often respirable given their small size and are hence of particular biological significance, can no longer be separated onto the agar.

To 1: During the sampling, depending on the spore concentration and the nozzle total, multiple spores in the sample air are accelerated into a single nozzle and collide with the bacterial site. This fact, referred to in the following section as multiple occupancy (of the bacterial site), was already published in 1950 as a discovery by Feller [1]. The greater the number of occupied bacterial sites, the lower the possibility for a spore to occupy a still-unoccupied (free) bacterial site. Hence with a coverage of 90 % of the possible bacterial sites, the chance of a spore occupying a free bacterial site is now only 1 in 10. Spores on a bacterial site that is subject to multiple occupancy form only one CFU after germination and are no longer visible as multiple individual colonies. The number of CFU after cultivation is thus lower than the actually “collected” (impacted) spores.

But how many?
The approach taken to determine the number of spores actually collected involves statistics. If we assume a uniform distribution of sample air over the jet plate, the following statistical method can be used to calculate the actual number of collected particles based on the number of CFU.

The calculation is performed in this instance via the distribution of the occupied holes with a given particle total (n) and known jets total (N). If we determine the distribution of the occupied holes by (n - 1) particles by the number of nozzles (N), the distribution of n particles can be calculated (conditional probability). Here, the distributions for 1, 2, ., n particles are recursively calculated respectively. The expected values of this distribution can be calculated in accordance with the following equation:

$$En = N\left(1 - \left[\frac{N-1}{N}\right]^{n}\right)$$

En reveals the CFU-total for *n* collected particles. The function of the statistically determined particle total over the CFU total is graphically illustrated in diagram 1 for various jets totals.

Correction of the CFU total, however, with the statistically determined particle value is only possible for the total bacterial count. Projections of the CFU figures for the individually identified CFU genera are not possible using this statistical method.

This method does not take into consideration the following:
Spore clusters (groups of connected spores) forming only a single CFU, are also considered to be a single ‘particle’ in statistical terms. The total of individual spores, however, may be many times greater. This fact has already been observed with particle collectors (e.g. PS 30) on multiple occasions.

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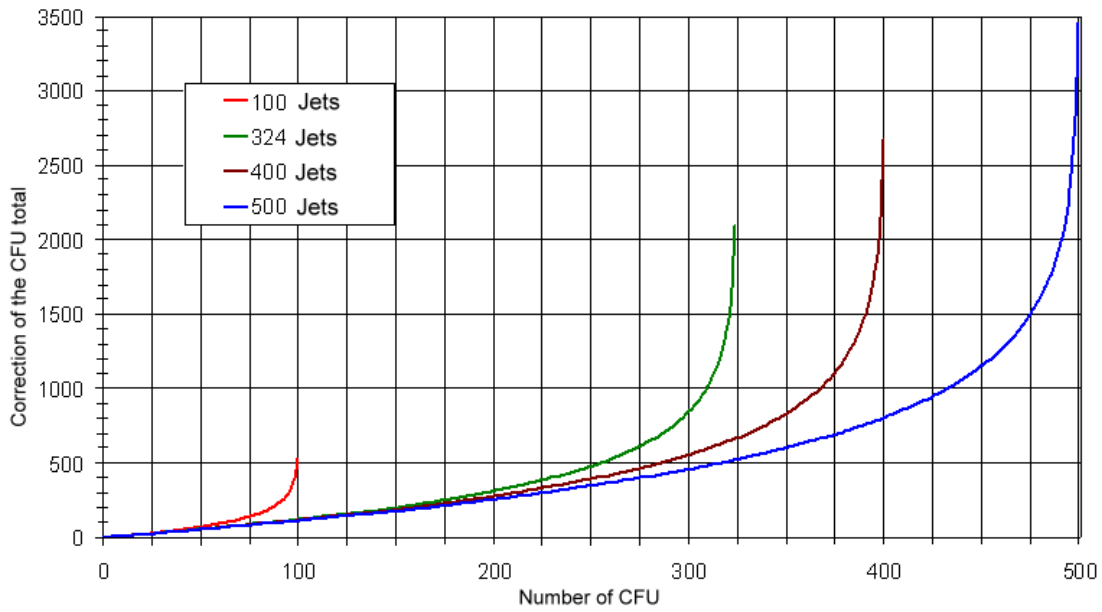

Diagram 1: Statistical correction values for round jet impactor

In addition to spores, other particles (dandruff, dust particles etc.) are also separated onto the culture medium. However, the ratio of spores and other particles in the sample air need not be the same.
The diagram shows the quickly increasing multiple occupancy for impacters with fewer than 300 jets. The greater the number of jets in an impactor, the higher the linear stage and the lower the expected multiple occupancy.
Despite the above-mentioned uncertainties, however, the use of statistical calculation can be helpful when interpreting sampling results.
Using the specially created program 'Correction', which runs on MS Windows, facilitates the calculation process. This program can be downloaded online at the URL: http://www.holbach.biz in the download section.

The total number of jets is freely adjustable in the program, meaning the calculation can be performed for all common forms of air sampler.

Sources:
Operating Manual for the single stage viable particle sizing sampler, ANDERSEN Sampler Incorporated

Expert survey of air samplers LKS 30, Fraunhofer Institut für Toxikologie und Aerosolforschung, Hannover