d_{50}-Value: A convenient way to compare the physical collection efficiency of microbiological air samplers and a practical approach for the validation.

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Abstract

The air in pharmaceutical production areas has to be monitored for microorganisms. International Standard ISO 14698-1/2[1] describes how to check for microbiological contamination and explains the principles and methods regarding these measurements.
In this paper the important parameters for the physical collection efficiency are introduced. With an easy mathematical formula they can be summarized into a single d_{50} value which can be used to compare different models of impaction based air samplers.
As most of the companies will not have access to a test chamber as required in the ISO 14698 a simplified approach to test the performance of any air sampler is described.

Key words: d_{50}-value, impaction method, validation, microbiological air samplers, Isolators, clean rooms,

1. Introduction

The air in pharmaceutical production areas has to be monitored for the presence of microorganisms. Also other production areas where perishable products like cosmetics, eye drops, food additives etc. are produced should be monitored about the presence of microorganisms. Microorganisms mostly present on dust particles may develop quickly when they come into contact with water and some nutrient media.
On the market a substantial number of different air samplers are offered. For the end user it is very difficult to choose amongst those proposals.
This paper shows some theoretical and practical methods and approaches which everyone may perform in his laboratory without investing in expensive test materials.

This paper will concentrate on the comparison of impaction type sampler because most used samplers are based on this principle.

2. Impaction principle

Air is drawn through a lid which has a defined number and a defined diameter of holes or a narrow slit. The speed of the air drawn through these holes will increase. A Petri dish filled with a nutrient medium (see Figure 1) is put into the instrument to collect the microorganisms. Microorganisms which are in the air stream will impact to the surface of the medium in the Petri dish due to the sudden change of the air stream from vertical to the horizontal direction. After collecting a known volume of air the Petri dish is removed from the instrument and incubated. After incubation the colony forming units (CFU) can be counted and expressed directly as colony forming units per cubic meter of air [CFU/m^3].

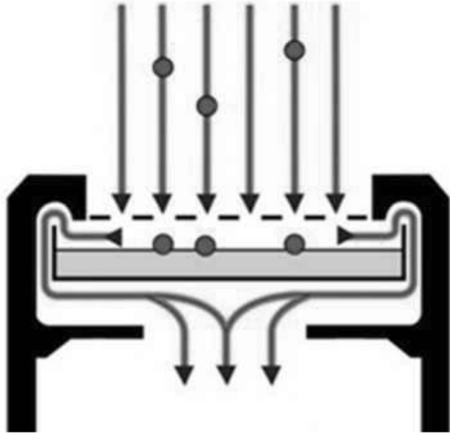

Figure 1: Cut through a sampling head
yellow: agar in the Petri dish, blue: air stream,
red: micro-organism carrying particles

3. Alternative sampling methods and instruments

3.1 Anderson[2] based sampler

The Anderson 6-stage sampler was developed in the late 50ties and was used to collect microorganisms and enumerate them according to size. For this reason it has different stages with different impaction speeds separating the particles according to their size. Most of today's microbiological air samplers are based on the Anderson sampler and work according to the principle of impaction.

3.2 Slit Sampler

The slit sampler works with slit type of air accelerator instead of a hole based type in the Andersen based instrument. The Petri dish will move 360° under the slit and collect the microorganisms as described with the impaction method. The instrument due to the mechanics for rotating the Petri dish is rather heavy and seldom used as a portable instrument.

3.3 Impinger

Impinger is drawing air through bottles which are filled with a nutrient bouillon to collect microorganisms which are in the air. The advantage of this method is that there is no drying out of the media possible and the microorganisms are concentrated in the liquid. Since the handling of liquids in the pharmaceutical environment is not desired this method is not very much used in the pharmaceutical production places.

3.4 Direct detection laser instruments

New on the market are laser based particle detection counters. They will excite the microbial particles with an UV laser beam and measure the size of them. The microorganisms containing metabolites such as NADH, Falvins or Triptophane will change the wave length of the laser and emit a different wave length of light. This wave length will indicate the presence of microorganisms. The big draw back so far is that also dead microorganisms behave as living ones since the metabolites are still present in the dead microorganisms.

4. Volume versus mass-flow or Standard liters

The market offers both volume and mass flow (Standard liter) based instruments.

The Boyle-Mariotte law defines the law for a closed gas system:

$p * V = \text{const.}$ (1)

and the definition of a standard liter at 20°C is defined as:

The volume in liters at 20°C at a atmospheric pressure of 1013.25 mbar (2)

4.1 Calculation of Standard Volume $Vn_{20°C}$

Norm or standard volume will be calculated from the following formula:

$$V_{n20°C} = \frac{V * p * T_{n20°C}}{p_n * T_{amb.}} \quad (3)$$

$Vn_{20°C}$ = Standard volume in liters at 20°C
V = Volume
p = Atmospheric pressure
p_n = Norm or standard pressure (1013.25 mbar)
T_n = Gas Temperatur in °K = 20°C + 273.15°K
$T_{amb.}$ = Gas Temperatur in °K = °C + 273.15°K

The following example shows the difference between two volume based samples at different temperatures and pressures.

A volume based sample of 1000 liters is taken at 950 mbar air pressure and 28°C temperature and another sample is taken at 22°C and 1005 mbar.

Conversion from Volume to Standard Litres		
Litres (=Volume)	1000.0	1000.0
Temperature in °C	28.0	22.0
Pressure in mbar	950.0	1005.0
Standard-Litres	**912.7**	**985.1**

Table 1: Example to show the difference between mass and volume

Note: If two or more air samplers are compared with volume and mass flow control they have to be corrected to the same base.

Its recommend using mass flow based air sampler because they automatically compensate for temperature and pressure changes and therefore always deliver comparable results one to each other.

5. ISO Norm 14698-1/2 validation

The Annex B in the ISO 14698 Standard describes how to evaluate air samplers according to their physical and biological efficiency. Unfortunately the methods described are not easy to perform from an individual customer. Only a few companies worldwide have the necessary equipment and are specialized to do the performance tests. Also the methods seem to be very old; some equipment for testing is no longer available. The test chamber is very small and a homogenous spore distribution seems almost impossible. Further to this the aspiration speed of the control device is 5 l/min and the instrument to be tested runs at 100 l/min. This is a factor of 20 times quicker.

6. A simple laboratory approach for validation is suggested as follows:

General Remarks

This proposed method could be performed in any location with sufficient concentration (up to 300 CFU/m^3) of natural occurring microorganisms. The instruments which have to be tested may be compared to an existing method ore a qualified reference.

Test arrangement

The instruments to be tested (could be more than one) and the reference is put on a table within a distance of about 1 meter. Put the Petri dishes filled with an appropriate growth medium or a filter in the samplers and start them simultaneously. Wait until the programmed volume is aspirated. Remove the Petr dishes from the instruments. Change now the position of the instruments. Put the first instrument from position A to B and the reference from B to position A. If more than one instrument is compared with a reference move the instrument after each test to the next position. Perform about 30 test runs in order to have statistical relevant test results.
Put the Petri dishes into an incubator and incubate them according to the specification of the manufacturer. Remove the plates after the incubation time and count the colonies (CFU).

Interpretation of results

Use the yield from the test sampler and the membrane filter sampler or another reference sampler from the same volume of air to calculate the efficiency using the following equation:

Efficiency of sampler (%) =

$$\frac{\text{test sampler count * 100}}{\text{total count (from reference sampler)}} \quad (4)$$

7. The d_{50} value, a mathematical approach

Key variables:

To influence the efficiency of an air sampler the following variables may be influenced by the manufacturer.

1. The aspiration speed of the sampler: The aspiration speed of an air sampler influences the efficiency of the impaction speed. It also determines the duration of a sample. The lower the speed the longer the sampling time. See chapter 8. Alternative sampling techniques.

2. The number of holes in the sampling lid

3. The diameter of the holes

The annex in the Iso 14698 International Standard describes techniques for determining the collection efficiency of samplers used for evaluating airborne microbial levels. Manufacturers or third-party testing organizations will usually perform this evaluation.
The collection efficiency of microbial air samplers can be considered in two ways: physical efficiency and biological efficiency. Physical efficiency is the ability of the sampler to collect various sizes of particles. Physical efficiency is the same whether the particle is a microorganism, carries a microorganism or is a non-living particle. Biological efficiency is the effectiveness of the sampler in collecting colony forming units. Biological efficiency may be lower than physical efficiency for a number of reasons, such as the survival of the microorganisms during collection and the ability of the collection medium to support their growth.

Quantifying the physical cut-off size and collection efficiency

Since viable microorganisms or colony forming units (CFU) are generally larger than 1 Micron, a cut off size (d_{50}) of ≤2 µm is considered appropriate.

NOTE: Cut off size d_{50} describes the aerodynamic equivalent particle diameter removed by 50% from the air stream and impacted 3/4. The d_{50} value can be calculated for impaction samplers with multiple holes or rectangular slit. For other types of instruments (impingement, centrifugal samplers) ask the manufacturer or compare the sampler with the proposed “Alternative sampling methods and instruments” described in chapter 3. The effects of impact stress[5] (air velocity should not exceed 40 m/s) and the effect of the media drying during the sampling time has to be considered6.

7.1 Physical efficiency

The physical sampling efficiency of an aerosol sampler is influenced by inlet or extraction efficiency and by separation efficiency:

Inlet or extraction efficiency is a function of the inlet design of the sampler and its ability to collect particles from the air in a representative way and transport the particles to the impaction nozzle or the filter.

Separation efficiency is the ability of the sampling device to separate and collect particles of different sizes from the air stream by impaction onto the collection medium or into the filter medium.
The physical sampling efficiency is the same whether the particles consist of single microorganisms, carry microorganisms, or are nonviable (inanimate). The physical sampling efficiency is based on the physical characteristics of the sampling device such as airflow, orifice shape, and orifice size.

A simplified formula to calculate the d_{50}-value in µm is described below:

$$d_{50} = \sqrt{\frac{40 * Dh}{U}} \qquad (5)$$

where *40* = constant factor for air viscosity
D_h = Hydraulic diameter of the air inlet nozzle (mm)
U = Impact velocity (m/s)

NOTE: For a circular opening, the hydraulic diameter is the whole diameter. For a rectangular slit, the hydraulic diameter will be approximately twice the slit width.

Example:

The d_{50} value was calculated for two air samplers.

Air Sampler	**MAS-100 NT**	**Other**
number of holes/slit	**300**	**380**
aspiration speed l/min	**100**	**120**
diameter holes in mm	**0.6**	**1.0**
speed in km/h	**70.8**	**24.1**
d_{50} value in µm	**1.1**	**2.4**

Table 2: d_{50} value of two different air samplers.

7.2 Biological efficiency test:

The two air samplers in the above mentioned d_{50} value calculation have been tested according to the procedure described in chapter 6. “A simple laboratory approach for validation”. The tests were performed in a closed room. Five times 250 litres have been collected with both air samplers onto a Petri dishes filled with CASO-Agar (Heipha Dr. Müller GmbH) and incubated for 72 hours. The colonies have been counted and expressed as CFU/m^3. The results of the biological efficiency

tests are shown in the following graph. As expected the biological efficiency tests confirmed that the MAS-100 NT shows a better biological efficiency due to the superior d_{50} value of 1.1 µm.

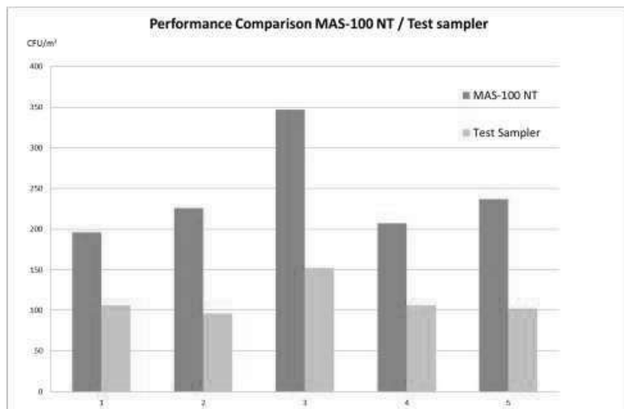

Figure 2: Biological efficiency graph of two air samplers

8. Alternative sampling techniques

8.1 Total volume sampling

In pharmaceutical environment the volume of air to be tested is 1000 liters or 1m^3 of air.
With an aspiration flow of 100 liters/minute the sampling time is 10 minutes.
Total volume sampling means that the defined volume is sampled at once. In a production process, this would mean, that the result found in this sample is a random sample representing the total duration of the manufacturing process which might be 8 hours.

Example:

If the sample is taken at 09:00 and a microbiological incident would happen around 10:00 o'clock this method would miss the microorganisms (red dots).

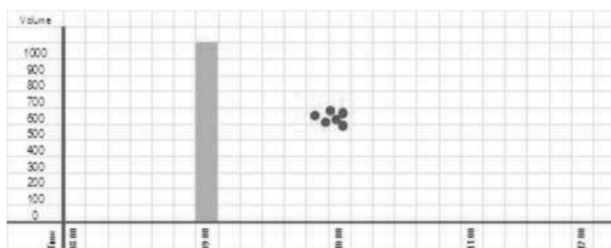

Figure 3: Standard sampling

8.2 SQS[7] sampling

The SQS-Cycle (sequential sampling) will split the total volume of 1000 Liters in equal fractions of volume for example 10 times 100 liters over a total sampling time of 4 hours.

Example:

If the sampling start at 08:00 and a microbiological incident would happen around 10:00 o'clock this method would probably find some of the microorganisms (red dots).

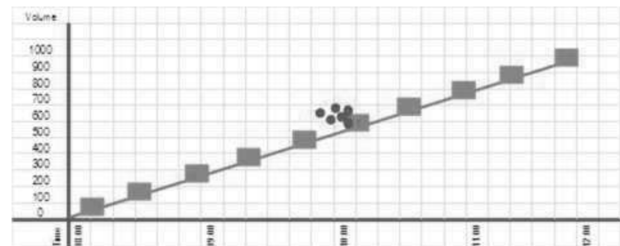

Figure 4: Sequential sampling

8.3 Continuous sampling

The continuous monitoring will sample during up to 4 hours on the same Petri dish with the same sampling efficiency or d_{50} value as above mentioned methods. The big advantage is that if a microbiological incident happens at any time during the sampling time it will detect the microorganisms.

Example:

If the continuous sampling starts at 08:00 and a microbiological incident would happen around 10:00 o'clock this method would find the microorganisms (red dots).

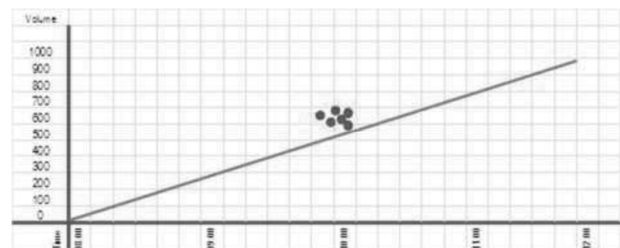

Figure 5: Continuous sampling over 4 hours

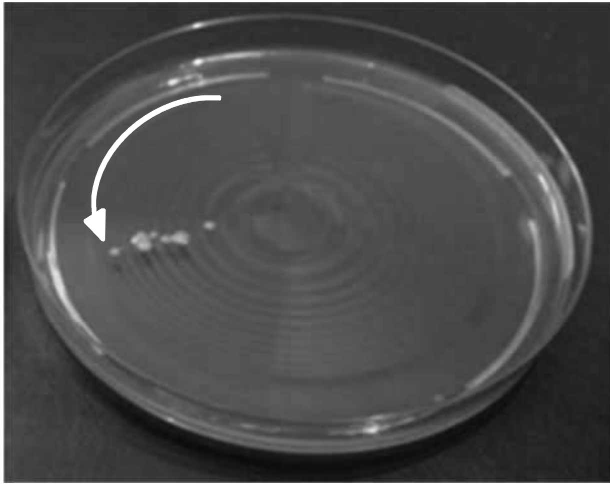

Figure 3: Continuous sampling test 1000 liter in 4 hours. The microbial incident happened after 1 hour and detected the microorganisms as expected.

8. Conclusions

The d_{50}-value is a very practical easy to calculate method to compare the physical collection efficiency of impaction type air samplers. It gives reliable indication on the efficiency which could be confirmed by practical laboratory tests.
The practical validation is easy to perform and gives excellent and comparable results to the d_{50} value calculation.

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