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REVIEW

The index of microbial air contamination

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Summary: The standard index of microbial air contamination (IMA) for the measurement of microbial air contamination in environments at risk is described. The method quantifies the microbial flow directly related to the contamination of surfaces coming from microbes that reach critical points by falling on to them. The index of microbial air contamination is based on the count of the microbial fallout on to Petri dishes left open to the air according to the 1/1/1 scheme (for 1 h, 1 m from the floor, at least 1 m away from walls or any obstacle). Classes of contamination and maximum acceptable levels have been established. The index of microbial air contamination has been tested in many different places: in hospitals, in food industries, in art galleries, aboard the MIR space station and also in the open air. It has proved to be a reliable and useful tool for monitoring the microbial surface contamination settling from the air in any environment.

Keywords: Air sampling; colony units; microbiology.

Introduction

In many human activities micro-organisms in the environment represent a hidden but dangerous risk factor. Concern has increased with the introduction of advanced technologies in hospitals, industry and agriculture.

In recent years, many studies have been carried out on this topic, and nowadays the evaluation of the level of air microbial contamination in places at risk is considered to be a basic step toward prevention.[1–8] However, there are still problems to be solved relating to methodology, monitoring, data interpretation and maximum acceptable levels of contamination.

At the Department of Hygiene at the University of Perugia, monitoraggio ambientale microbiologico – MAM (microbial environmental monitoring) has been devised. It is a system for microbial monitoring in any closed workplace at biorisk. A fundamental part of this system is microbial air monitoring, for which the index of the microbial air contamination (IMA) has been established.[9]

Counting microbes in the air is not an easy task. Many different methods are in use, which can be divided into four groups; the count of colony forming units per cubic meter of air (cfu/m^3); the count of cfu on settle plates; measurement of a chemical component of the microbial cells/m^3 of air; the count under the microscope.

The measurement of chemical components of microbial cells (ATP, DNA, enzymes) has not yet produced practical and reliable methods for the study of airborne micro-organisms because such methods are not sensitive enough. Counts under the microscope, or by automatic counters in fluorescence (flow-cytometry or fluorescent in situ hybridization) have limited applications and are still under study.

At the moment, the only effective means of quantifying airborne microbes is limited to the count of cfu. The cfu count is the most important parameter, as it measures the live micro-organisms which can multiply. Air samples can be collected in two ways: by active air samplers or by passive air sampling (the settle plates). Both methods are widely used.

In this paper we will summarize the advantages and disadvantages of active and passive samplings,

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analyse the method used to determine the risk of contamination on critical surfaces and describe the IMA standard, including classes and maximum acceptable levels.

Active air sampling

The microbial air contamination can be measured by counting the number of cfu per cubic metre (cfu/m^3) of air. For this purpose active air samplers are used, which collect a known volume of air, blown on to a nutrient medium by different techniques. There are many different types of active samplers on the market, each based on a different design (Table I): they are in use everywhere. Official standards for air control are based primarily on the measurement of cfu/m^3.

Unfortunately, there are many drawbacks that make it difficult to interpret correctly the results obtained by these devices (Table II).

Table I *Commercially available active air samplers*

Impingers
All-Glass impinger 30 and pre-impinger
Midget impinger with Personal Air sampler
May 3-stage Glass impinger
Folin Bubbler
Cyclone Sampler method
Impactors (slit-type)
Casella single slit and four slit sampler
Mattson-Garvin air sampler
New Brunswick STA air sampler
Bourdillon sampler
BIAP Slit Sampler
Reyniers slit sampler
Impactors (sieve type)
Andersen 6-stage, and 2-stage samplers
Andersen 8-stage sampler
Ross-Microban sieve air sampler
Personal particulate, dust, aerosol collector
Surface Air System sampler (SAS)
Joubert 3-stage biocollector
Filtration samplers
Millipore membrane filterfield monitor
Gelman membrane filter air sampler
MSF 37 monitor
Sartorius MD8 Air sampler
Centrifugal samplers
RCS Centrifugal sampler
Wells sampler
Electrostatic precipitation samplers
LVS sampler
General Electric Electrostatic Air sampler
Thermal precipitation samplers
Thermal precipitator, hot wire

Table II *Advantages and disadvantages of active air sampling*

Advantages:
Most official guidelines refer to cfu/m^3
Sample collection is rapid
Disadvantages:
Device difficult to sterilize
Expensive
Noisy
Different samples give different results
The same sampler gives different results
Fallout of microorganisms is not evaluated
The sampler must be frequently calibrated
The air exhaust must be removed
The airflow is disturbed
A certain number of microbes are inactivated by the impact on the nutrient

Each active sampler gives different results in the same place at the same time, showing a high variability.[10] Different active samplers give different results. Therefore it is difficult, if not impossible, to compare data collected using different samplers. Many papers have been published, in which the efficiency of different samplers is evaluated and compared.[11–28] The results are always the same: the final counts differ from one device to the next. Thus 'there is often no obvious choice of the correct sampler to use'.[5]

Some studies demonstrate that the Andersen sampler recovers a significantly higher number of micro-organisms,[8,26] but the Andersen eight-stage sampler is better than the Andersen two-stage impactor.[14] Lembke, on the other hand, complains of a high degree of variability in results using the Andersen six-stage impactor.[17] At over 1000 cfu/m^3 the AG-30 impinger yielded counts up to six times higher than the gelatin membrane filtration (GMF) method,[24] while the Reuter Centrifugal sampler (RCS) was found to be more efficient than a slit sampler or a liquid impinger.[15,22,23] The same result was obtained by comparing the RCS with the Surface Air System sampler (SAS): the RCS sampler gave counts three or four times higher.[21] The SAS Super 90 and RCS measurements were significantly lower than those obtained with the Andersen two-stage or Burkard samplers.[19] Verhoeff *et al.* documented different results when different air samplings were used for the enumeration and identification of viable moulds. A comparison was made between the results obtained with five commercially available air sampling devices (slit-to-agar sampler, N6-Anderson sampler, SAS sampler, RCS, Gelatine Filter sampler) in combination with

four culture media. The coefficients of variation were high for all combinations. Statistical analysis showed that the slit sampler and the N6-Andersen sampler in combination with DG18 (dichloran 18% glycerol agar) and MEA (malt extract agar) gave the highest yield in terms of cfu/m^3.[28]

Active samplers are expensive, heavy, noisy and difficult to sterilize. They must be continuously calibrated, otherwise the volume of processed air does not correspond to expectations.

One of the major limitations of mechanical air sampling is the limitation in sample size of air being sampled. Typically, slit-to-agar samplers have an 80 L/min sampling capacity. If 1 m^3 of air is tested, then it would require an exposure time of 15 min. It may be necessary to use sampling times in excess of 15 min to obtain a representative environmental sample. Although there are samplers reported to be capable of very high sampling volume rates, consideration in these situations should be given to the potential for disruption of the airflow patterns in any critical area, or to the creation of a turbulence that could increase the probability of contamination.[29]

The air being sucked in or pushed out by volumetric air samplers can disturb the surrounding area, because it remains in the area being checked, producing an artificial turbulence and thus altering the counts.[30]

Any laminar airflow is either interrupted or accelerated. Large amounts of living particles are inactivated during the sampling on impact with the device and on the nutrient medium.[31,32]

Nevertheless, all the official regulations on the control of airborne micro-organisms are primarily based on the count of cfu/m^3, without specifying the kind of active sampler to be used. The only exception is the National Health Service (NHS) regulation for bacteriological sampling in UK.[33] This is a serious problem because the active air samplers on the market vary in efficiency.

In the USA, the standard for the measuring of particulate air contamination is defined by the Federal Standard 209E.[34] This concerns airborne particles in general, including inert and viable particles. The first Federal Standard was written in 1957, and has been used as a basis reference for all analogous documents approved thereafter in other countries. Except for size classification, FS 209E is not intended to characterize the physical, chemical, radiological or viable nature of airborne particulate contamination (FS 209E, point 1.2). The methods and equipment suitable for measuring airborne particle concentrations, for class verification and monitoring of air cleanliness are described in great detail, but no commercially available sampler is suggested. It is stated that 'Even recently calibrated instruments of like design may show significant differences. Caution should be used when comparing measurements from different instruments.' (FS 209E, point 5.3.4.)

The guidelines for the measurement of airborne viable particles in the USA have been established by different institutions, mainly professional associations. To protect outer space from terrestrial microbial contamination, NASA published a standard based on the count of cfu by active sampler and settle plates, in 1967.[35] Other guidelines, based on cfu/m^3 came from hospital and industrial associations to protect different activities at bio-risk.

Various active samplers are suggested by different institutions.[5] Brachman recommended the AGI-30 sampler.[36] The American Conference of Governmental Industrial Hygienists Committee on Bioaerosols used the Andersen multi-stage air sampler.[37] In the pharmaceutical industry a slit sampler is the most widely used.[29,38]

In the UK, the Health Technical Memorandum 2025 (Ventilation in Healthcare Premises) defines the standards of air microbial contamination for clean rooms and ultra clean rooms.[33] The tests should be performed by a microbiologist using the technique described by Whyte *et al*.[39] In this paper it is stated that: 'The large volume Casella slit sampler, without any extension head or inlet connector, working at 700 L/min, should be regarded as the standard instrument for measurement and any other sampler should be calibrated in relation to this. Any extension tube, including those designed to be used with the Casella sampler, must be tested and shown to cause particle losses not exceeding 20%. The sampling time should be limited to avoid drying of the medium. With the large volume Casella sampler the limit when using untreated agar plates is about 10–15 min (7–10 m^3 of air sampled) … During each operation at least 20 m^3 of air should be taken'. Thirteen years later, Whyte published a note entitled: 'In support of settle plates'.[40]

In France, the standards and guidelines for the control of operating theatres (clean rooms) are set out in the document NF S 90-351, December 1987. The classes and maximum acceptable limits of microbial contamination are expressed as cfu/m^3. The sampler to be used is not specified.[41]

The guidelines for ventilating systems in Swiss hospitals distinguish between five classes of tolerable airborne micro-organisms.[42] The corresponding German guidelines, DIN 1946/4 includes requirements for the absence of micro-organisms without giving specific values.[43]

It appears that although active air samplers are the most common method for the measurement of the cfu/m^3, in reality the indications for their practical use remain open to criticism.

According to the CEN/TC 243 document, the selection of a sampling apparatus shall take the following criteria into account: (a) the ability to reliably detect low levels of bio-contamination; (b) a suction flow rate suitable for (a); (c) an appropriate impact/air flow velocity; (d) the specific volume of air to be sampled; (e) an appropriate culture medium; (f) an appropriate size/weight of the device to allow easy handling; (g) ease of operation; (h) ease of cleaning, disinfection and sterilization; (i) the apparatus shall not intrinsically add to the biocontamination being measured. Proper validation of the apparatus chosen may be performed.[44]

Passive air sampling: settle plates

Passive air sampling is performed using settle plates. Petri dishes containing a solid nutrient medium are left open to air for a given period of time. Microbes carried by inert particles fall onto the surface of the nutrient, with an average deposition rate of 0.46 cm/s being reported.[45] After incubation at 36 ± 1°C they grow colonies in a number proportional to the level of microbial contamination of the air.

The main criticism of settle plates is that the measured microbial fallout is not at all or is only weakly correlated with the counts determined by other quantitative methods and with a defined volume of the surrounding atmosphere.[24] Therefore gravity or depositional sampling is considered a non-quantitative collection method,[46] affected by the size and shape of particles and by the motion of the surrounding atmosphere.[47] The volume of air from which the particles originate is unknown. The results obtained by gravity sampling are not qualitatively or quantitatively accurate and do not compare favourably with those obtained by other sampling methods.[46,48–50] Another objection to the use of settle plates is the length of the time required to collect samples: from 15 min to 1 h or more.

According to the USP, the settle plate method is still widely used as a simple and inexpensive way to qualitatively assess the environments over prolonged exposure times. Settle plates are not to be used for quantitative estimations of the microbial contamination levels of critical environments.[29]

Humphreys affirms that in operating theatres agar settle plates, although inexpensive and convenient, are unsuitable because this method is not quantitative and selectively collects larger air particles.[51] Humphreys writes: 'Settle plates have no role in monitoring operating theatre counts'.[52] However, in a recent article, Humphreys cites a study by Friberg[53] where the results suggest that settle plates may have a role because they reflect the bacterial load nearest the operative site.[54]

Some authors have listed several advantages of passive air sampling (Table III).[1,40,53,55–59]

Settle plates are sterile, economical and readily available. The results obtained by settle plates are reproducible and reliable. Many places in an environment can be checked at the same time. Data collected on settle plates set in different places, by different operators, can be compared and understood.

The natural trend of the microbial population in the air is not disturbed during the sampling time nor are the laminar air flows interrupted in any way. Settle plates give the measurement of the harmful part of the airborne population which falls on to a critical surface in a given time. Settle plates allow the evaluation of surface contamination settling from the air.[44]

This property is their greatest advantage. Charnley wrote: 'The settle plate counts are considered more valid for comparing the different phases of air contamination because the settle plate reproduces

Table III *Advantages and disadvantages of settle plates for passive air sampling*

Advantages:
Cheap
Available everywhere
Sterile
Many samples can be taken in different places at the same time
Meaningful samples (for the contamination of critical surface)
Reliable results
Comparable and generally valid results
The airflow is not disturbed
Reproduce real conditions
Disadvantages:
Not always accepted by official guidelines

the circumstances of infection by dust particles sedimenting into the wound better than a slit-sampler'[1] and French wrote: 'Air sampling in the operating room should measure microbial fallout rather than air-suspended microbes. Types and numbers of bacteria falling into the wound and on instruments is of primary importance'.[57] In a recent paper[53] Friberg *et al*. propose that settle plates showing bacterial surface contamination are both a more practical and a more relevant indicator of actual wound contamination rate than air counts. They suggest, in addition to the current British bacteriological standard for ultraclean operating room air of $<10\,cfu/m^3$ a corresponding standard for surface contamination rate of $<350\,cfu/m^2/h$ measured by means of settle plates.[53]

It has been demonstrated that the bacterial counts obtained from the wound wash and the settle plates at the wound were closely correlated.[8] This clearly demonstrates that an exposed wound is essentially the equivalent of a settle plate.[58]

Referring to the pharmaceutical industry Whyte affirms: 'as air sampling is carried out to monitor the risk of microbial contamination to the product and settle plates do this best, a programme set up to monitor pharmaceutical production should be based on settle plates rather than volumetric sampling'.[40] We consider this statement is valid for any environments at risk.

The time span required to collect samples is also an advantage. The cfu collected on settle plates are like a photocopy, or a mirror of what was going on at a particular point, during a period of time. Long sampling periods may increase measurement significance and reproducibility.[60]

We have studied the reliability of data obtained by settle plates in comparison with two active air samplers: the SAS, Surface Air System sampler (Pool Bioanalysis Italiana) and the RCS, Reuter Centrifugal Sampler (Folex-Biotest). In measurements carried out in environments with different degrees of microbial contamination, collecting a number of air samples in the same place and at the same time using these three methods, it has been possible to show that the settle plates yielded data with the lowest standard deviation in comparison with the active air samplers (Figure 1).[21]

Louis Pasteur was the first to use a nutrient medium exposed to air to collect living microorganisms. Some years later Robert Koch was the first to use settle plates to measure microbial air contamination indoors.[61]

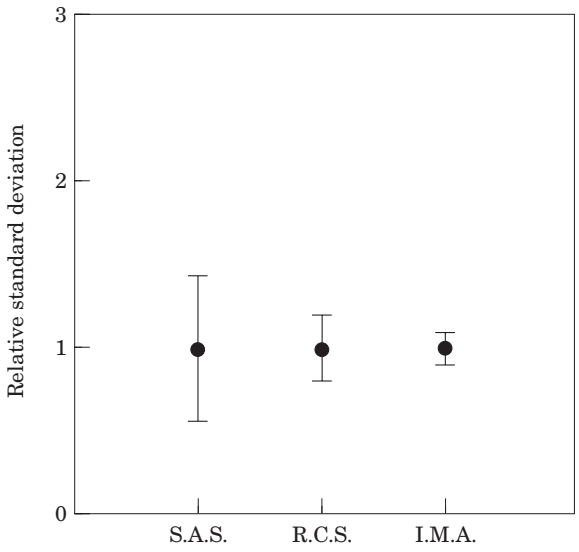

Figure 1 Relative accuracy (relative average values and relative standard deviations) of the microbial air contamination measurement using three different methods (SAS, RCS, IMA) at the animal facility of the Hygiene Department of the University of Perugia.
SAS: surface Air System sampler; RCS: Reuter Centrifugal sampler; IMA: index of microbial air contamination.

Since then, settle plates have continued to be used in many different environments for evaluating microbial air contamination. The UK survey of 438 operating theatres in 147 hospitals showed the extent of the use of settle plates for the control of microbial air contamination. The air was monitored in almost every operating theatre: 72 (49%) hospitals used settle plates, a slit sampler was used in 58 (39%), other methods (e.g., surface air system) were used in 62 (42%). Eighty-five percent of hospitals measured only the total bacterial count.[52]

Attempts to standardize passive air sampling

Unfortunately, settle plates are used in very different ways. Dishes of different diameters, different exposure times, different nutrient media and different incubation temperatures and times, make it difficult to compare data obtained by different operators.[62–67] Moreover, a rating of the results obtained by settle plates has never been established.

The first attempt to standardize the use of settle plates was made in the 1970s by Fisher. His study was aimed at defining the best sampling parameters and methods.[56,68–70] Using Petri dishes 9 cm in diameter with blood agar, after 24 h incubation at 37°C and normalizing the results to cfu/dm^2 he was able to show that air turbulence does not affect the

cfu counts on Petri dishes open to air.[68] Leaving the Petri dish open to air for 1 h and positioning it 80–100 cm above the floor and at 100–150 cm from the wall he obtained an average and useful value for the microbial fallout from the air in the environment.[68,69] The result was expressed as total microbial count ('Gesamtkeimzahl').

Hence the schedule 1/1/1 was devised as a standard for measuring the microbial air contamination in hospital environments at bio-risk: the Petri dish must be left open to the air for 1 h, 1 m above the floor, 1 m from the wall.[56]

As a second step Fisher studied the 'Gesamtkeimzahl' in different places in the hospital and was able to demonstrate how this changed in relation to the structure and the management of the environment. He did not face the problem of defining microbial contamination classes generally valid in any environment at bio-risk. He tentatively set safe, acceptable and unacceptable air contamination levels in different hospital environments at different degrees of bio-risk (Table IV).[70]

In 1984 Fisher's suggestions were vindicated. Russell found that the standard 9 cm plate is a good indicator of the number of viable particles falling from the atmosphere. The results obtained after 1 h of exposure implied an increase in efficiency in comparison with different exposure lengths. The water loss of the nutrient medium did not reduce the cfu counts significantly.[71]

The measurement of microbial air contamination by settle plates appears in some official standards, without a rational definition of how to use the Petri dishes or how to interpret the results. The evaluation of airborne living particles by settle plates has not attained full acceptance.

Table IV *Air total microbial count ('Gesamtkeimzahl') according to Fisher in different hospital environments (cfu on Petri dishes 9 cm in diameter, with blood-agar, left open to air according to the scheme 1/1/1)*[70]

Place	Total microbial count (cfu/dm^2/h) ('Gesamtkeimzahl')		
	Optimal	Acceptable	Not acceptable
Medical wards	0–450	451–750	>751
Surgery	0–250	251–450	>451
Pharmacy	0–100	101–180	>181
Aseptic room	0–50	51–90	>91
Operating theatre (at rest)	0–4	5–8	>9
Operating theatre (in activity)	0–60	61–90	>91

The FS 209E says that, 'For monitoring purposes only, determining the extent to which particles are contaminating surfaces may be accomplished by allowing airborne particles to deposit on test surfaces and then counting them by appropriate methods. …' (FS 209E, note 3 to point 5.2). This statement may be equally applied to the count of cfu fallout.[34]

In the NASA standards for clean rooms and work stations for microbially controlled environment, the counts of cfu on settle plates are listed in parallel with the cfu/m^3.[35] The sampling is done on Petri dishes 73.5 cm^2 wide after 1 and 2 h, and on 1 m^2 for one week. This is clear evidence of the lack of a defined standard for the use of settle plates.

Settle plates are also included in other standards, again without any clear indication about the method of sampling and the interpretation of the data. In the EURACHEM Guide, for European cooperation for Accreditation of Laboratories (EAL-G18) the use of settle plates for the measurement of airborne living particles is accepted.[72]

The Joint Commission on Accreditation of Hospitals recommends the use of settle plates for the microbiological monitoring of the laminar airflow systems.[73]

In the USA the 15th edition of the Standard Methods for the Examination of Dairy Products classifies settle plates as a class D method and recommends 15 min exposure of Petri plates 9 cm in diameter containing general or selective media.[5,74]

As of January 1, 1997, the Guide to the Manufacture of Sterile Medicinal Products delivered by the European Working Party on 'Control of Medicines and Inspections' (revision of Annex I to the EU Guide to Good Manufacturing Practice) came into effect.[75] For technical procedures, the document refers to the CEN/ISO standards.[76] Air, surfaces and hands are taken into consideration. Four levels of increasing environment cleanliness are stated, each one defined by maximum acceptable inert particles/m^3 of air; cfu/m^3 of air; cfu/settle plate 9 cm in diameter exposed to air for 4 h; cfu/RODAC plate; cfu/gloved hand (Table V).

Mathematical description of fall-out

The aim of microbiological sampling is mainly to assess the contamination of a critical surface (wound, medicament, food) produced by the fallout of micro-organisms coming from the air. For this

Table V *Recommended limits for microbial contamination according to the European Union Good Manufacturing Practice*[75]

Grade*	cfu/m^3	cfu/plate†	cfu/RODAC‡	cfu/glove
A	<1	<1	<1	<1
B	10	5	5	5
C	100	50	25	–
D	200	100	50	–

* According to the EU GMP.
† Settle plates (diameter 90 mm) exposed to air during 4 h.
‡ On surfaces, RODAC contact plates, 55 mm in diameter.
§ cfu on hands wearing sterile gloves.

purpose the most reliable method is passive sampling since it gives a direct indication of the microbial contamination of the surface.

An aerosol can be defined as a suspension of microscopic solid or liquid particles in air for an appreciable period of time. Biological aerosols include bacteria, yeasts, moulds, spores of bacteria and moulds, viruses. The dynamic behaviour of an aerosol is influenced by several factors: physical (i.e., Brownian motion, electrical gradient, electromagnetic radiation, gravitational field, particle density, thermal gradients, humidity, ventilation) and biological (e.g., presence of nutrients, presence of antimicrobial compounds).[77–79]

Brownian motion plays a role when particles have dimensions comparable or inferior to the middle free path of the molecules in the atmosphere. Convective effects occur in the presence of a thermic gradient. With charged particles, atmospheric humidity and electrostatic fields must be taken into account. Air friction influences the motion of particles with different dimensions in different ways. However, we consider spherical uncharged particles whose dimension and density is such that their deposition is influenced mainly by the gravitational field and environments with a uniform temperature and no perturbation. Under these conditions the particles in the air sediment with a constant velocity according to the following formula:

$$v_c = \frac{2}{9} r^2 g \frac{\rho - \rho_a}{\eta}, \quad (1)$$

where v_c is the contamination velocity, i.e., the settling velocity of cfu; r is the particle radius; g is the acceleration due to gravity; ρ is the particle density; ρ_a is air density and η is air viscosity. Equation (1) shows that the velocity of sedimentation depends mostly on the radius and mass of the particle.

Table VI *Velocity of sedimentation of particles of different diameters dispersed in the air (supposedly spherical with density = 1 and temperature = 25°C)*

Diameter (μm)	Velocity of sedimentation	
	cm/s	m/h
1	0.003	0.108
2	0.012	0.43
3	0.027	0.97
5	0.075	2.7
10	0.3	10.8
20	1.17	42
30	2.7	97
40	5.5	200

Examples of the velocity of sedimentation for particles >1 μm are given in Table VI.[80]

In order to apply Table VI to non-spherical particles, the correction suggested by Whitlaw-Gray and Patterson must be made.[81]

The rate of micro-organisms (biological aerosols) falling on to a critical surface can be calculated by the following formula:

$$\phi_c = v_c \cdot \rho_c, \quad (2)$$

where ϕ_c is the contamination flow, i.e., the count of settling cfu per unit surface and per unit time; ρ_c is the contamination density, i.e., the count of cfu per unit volume; v_c is the contamination velocity, i.e., the settling velocity of cfu. In equation (2) fallout is expressed according to the microbial density of the air. From the measurement of microbial fallout and contamination velocity, it is possible to obtain the microbial density of the air:

$$\rho_c = \frac{\phi_c}{v_c}, \quad (3)$$

and from the measurement of microbial fallout and microbial density, it is possible to obtain the contamination velocity:

$$v_c = \frac{\phi_c}{\rho_c}, \quad (4)$$

However, equations (2), (3) and (4) are valid only in optimal conditions, i.e., with uniform spatial particle distribution; particles of the same shape, same dimension, same density; regular airflow; high cfu/m^3 values; no static charging of particles; no temperature gradients.

In practice, optimal conditions never exist because:

(1) the spatial particle distribution is not uniform: the closer the contamination source (mainly the operating staff), the higher the number of cfu/m^3;
(2) the particles vary greatly in shape, dimension and density;
(3) the operating staff cause air turbulence;
(4) for low values of cfu/m^3 – the norm for environments submitted to regular microbiological monitoring – sampling shows a broad statistical distribution, increasing the discrepancy between data obtained by active and passive methods because of differences in sampling times and spatial location.

For all these reasons, a generally valid mathematical formula cannot be established.

On the other hand, in any environment, the number of micro-organisms falling is related to the number of micro-organisms present in the air: the greater the air contamination, the higher the number of micro-organisms sedimenting due to gravity.

Some studies, from the classic NASA study[35] (Table VII), to the leading studies of Charnley[55] (Table VIII) and more recent notes, indicate that a relation between cfu/m^3 and counts on settle plates does exist. Over time this statement has been supported by important evidence and the relation between fallout and cfu/m^3 has been studied by comparing the data collected by use of settle plates and active air samplers at the same time and in the same place. In these studies, the counts of cfu/m^3 have been made by different active samplers and in different experimental conditions. It is therefore difficult to find good correlation between the results.[35,40,53,60,75,82–84]

Table VII *NASA NHB 5340.2. Guidelines on microbial air contamination, in comparison with the FS 209*[35]

FS 209 classes	cfu counts			
	cfu/m^3	cfu/m^2/week*	cfu†	
			1 h	2 h
100	3.5	12 900	0.6	1.2
10 000	17.6	64 600	3.0	6.0
100 000	88.4	323 000	15.0	30.0

* Microbial fall out;
† on settle plates 73.5 cm^2 wide.

Table VIII *Air cleanliness over the four phases of Charnley's study from 1959 to 1967*[55]

Phase	Air changes/h	Settle plates* cfu/h	Slit sampler cfu/m^3
I	0	70	18.0†
II	10	10	2.5†
III	130	1.8	0.2‡
IV	300	0.2	0.1‡

Phase I: 1959–61; phase II: 1962; phase III: 1962–66; phase IV: 1966–67.
* Blood agar plates (3 $\frac{1}{4}$ inch plate/h) on the operating table;
† estimated; ‡ observed.

Through a series of parallel counts by settle plates used according to the 1/1/1 scheme (IMA) and the bacteriological air pollution detector (BAPD) active impact air sampler (PBI), Pitzurra found a regression line with an angular coefficient of 2.47 and a correlation coefficient (r) of 0.63.[83] The design of the BAPD sampler is the same as the SAS. Orpianesi also found a meaningful correlation between cfu/m^3 and IMA ($P<0.001$) values, in the ratio of 2 to 1. He used the SAS active air sampler (PBI).[82]

An indirect, relevant confirmation of this grading recently came from the Guide to Good Manufacture Practice of Sterile Medicinal Products provided by the European Working Party (Table V). This document gives the values of measurements made using settle plates 9 cm in diameter, exposed to the air for 4 h, and measurements performed by active samplers. From these data, it is possible to estimate a ratio of 2 to 1.

In a recent paper, Friberg gives various angular coefficients measured during strictly standardized sham operations.[53]

From these data it appears that a correlation between the counts of the microbial fallout and cfu/m^3 exists, but the regression coefficients differ from one to another.

Differences occurred because active air samplings were performed by different operators with different instruments and in different experimental conditions.

Measurement of the contamination of a surface

Choice of method

To describe the microbial contamination, it is essential to have a reliable method of measurement

which must:

(a) perturb the parameter under investigation as little as possible;
(b) have good reproducibility;
(c) be sustained by clear evidence that what needs to be measured is really measured;
(d) be expressed in units containing all quantities necessary to characterize it.

When applied to microbial surface contamination, the standard and recommended measurements, based mainly on cfu/m^3, do not meet the above requirements, but measurements performed with settle plates do. Therefore, it may be possible to develop a science, based on measurements with settle plates, which correlates the measurement of microbial contamination of the air with the risk of the microbial contamination of surfaces.

Active Sampling

Perturbance of the parameter under investigation. Active sampling produces airflows which would normally not exist.

Manufacturers ensure that there is a laminar flow inside the sampler, but collection creates external turbulence of the air and interruption or acceleration of laminar flows.

Reproducibility. Only the control of the parameters of interest will secure high reproducibility, but many questions remain:

(i) What is the size of the solid angle of aspiration?
(ii) At what distance is the air collected?
(iii) What pressure gradient exists outside the instruments?
(iv) What is the velocity distribution for the aspired particles?
(v) Particles of different shape and dimensions are collected diversely, in which way?
(vi) Where does the collected air go?
(vii) How many micro-organisms escape when the air is expelled from the sampler?
(viii) How many of the collected microorganisms land on the nutrient?
(ix) How many micro-organisms die due to the impact on the nutrient?

The answers to these questions vary from one sampler to another and from one measurement to another.

Most of the active samplers collect air in the immediate vicinity where it is expelled and consequently, part of the same air is collected again and again, producing a modification of the actual microbial density.

Moreover, the short sampling time generally used increases the unreliability of the measurement.

Evidence that what needs to be measured is really measured

The performance of an active sampler is often associated with its sampling capacity. The Andersen six-stage sampler justifies its high cost because it collects more than other devices. However, this advantage reveals one of the major weaknesses of active sampling, i.e., as far as we know, it has not been proved that an active sampler collects all the cfu/m^3, even though the results refer to this measuring unit; and as far as we know, it has not been proved that an active sampler collects a constant cfu/m^3 fraction.

Therefore it is not correct to refer to cfu/m^3 measurement or cfu/m^3 fractions.

This criticism does not concern the precision of the measurements but the fact that these measurements always yield an unknown part of the quantity supposed to be measured.

Suitability of measuring units

The cfu/m^3 measuring unit is not suitable because: the contamination is a non homogeneous process; the nearer the source, the higher the risk of contamination; and contamination is a dynamic process; the longer the surface is exposed, the higher the risk of contamination.

Most standards recommend cfu/m^3 for contamination processes. However, cfu/m^3 is a measuring unit suitable for the description of homogenous and static processes quite the opposite of biological contamination processes.

Passive sampling

Conversely, measurements performed by passive samplers meet the requirements for a reliable description of the parameter under study.

Perturbance of the parameter under investigation. There is no perturbation of the parameter under study. The only moment when it is possible to have perturbation is the moment when the operator opens and closes the plate. However, if the operator

is careful or if the sampling is performed using automatic equipment, the problem is avoided.

Reproducibility. The plates can be easily standardized.

Evidence that what needs to be measured is really measured. The plates are the mirror of what happens on the critical surface (wound, medicament, food).

Appropriateness of measuring units. The unit of colony forming unit per unit of surface and per unit of time is appropriate to describe the fallout.

Problems in measuring microbial contamination of surfaces

To illustrate the difference between passive and active measurements, we have defined some parameters referring to the microbial contamination of a surface:

Contamination: number of cfu on a determined surface;
Contamination velocity: settling velocity of cfu;
Contamination density: number of cfu present in the unit of volume of air;
Contamination flow: number of cfu which cross the unit of surface in the unit of time if the surface is imaginary, or number of cfu which are deposited on the unit of surface in the unit of time, for real surfaces.

In Table IX the symbols and the most common units of measurement are shown.

Let us now consider the measuring of microbial contamination of a laminar airflow, such as the airflow of an operating theatre, and let us suppose a homogenous diffusion of microbial contamination in the airflow. Hygienists will not attach importance to the number of cfu in the laminar airflow, but rather to the number of cfu which are deposited on the wounds in the time span of its exposure to contamination.

Table IX *Quantity and units related to microbial contamination*

Quantity	Symbols	Commonly used dimension unit
Unit of length	m	m
Unit of time	t	h
Unit of surfaces	s	dm^2
Colony forming units	cfu	cfu
Contamination	C	cfu
Contamination velocity	v_c	m/h
Contamination density	ρ_c	cfu/m^3
Contamination flow	ϕ_c	$cfu/dm^2/h$

To answer this question, users of active samplers would also need an anemometer. They would calibrate both instruments and make sure that the measurements of contamination density and air flow velocity are effected in the same place. The flow velocity at the outlet of the air conditioning unit differs from the flow velocity at the wound and so both measurements must be performed on the wound.

Since only an unknown part of contamination density can be measured with active samplers, the measurement will not assess the microbial contamination C caused by an air flow with velocity v, in time t, to a wound with surface area s. They will only indicate lower limit:

$$(\rho_c)_{measured} \cdot s \cdot v \cdot t < (\rho_c)_{actual} \cdot s \cdot v \cdot t = C \qquad (5)$$

Therefore such measurements have many disadvantages. They require many instruments for measuring and calibration; the measurements must be performed with great care; the contamination of the surfaces is underestimated.

Using settle plates and a chronometer which is easy to control for precision), microbial contamination flow can be determined and a reliable assessment of microbial contamination obtained.

$$C = \phi_c \cdot s \cdot t \qquad (6)$$

Whyte found a correspondence between the number of colonies deposited on a wound and the number of colonies deposited on a settle plate placed in the vicinity, providing the experimental evidence of Kundsin's remark, that a wound is the equivalent of a settle plate.[58]

We believe the microbial monitoring sector should include the use of settle plates to assess the contamination risk on surfaces.

The index of microbial air contamination (IMA)

Exploiting the advantages of settle plates for the measurement of microbial air contamination, we have used them since 1978 to monitor hospital environments at high or very high infection risk.

Since the beginning we were faced with the need to standardize the method and to interpret the data collected by settle plates by the definition of classes and maximum acceptable levels of contamination in places at different bio-risk.

Following the studies of Fisher, the IMA was devised in 1978[85] with the aim of unifying and

standardizing the technique of air sampling by settle plates. The 1/1/1 scheme was adopted. The IMA classes and the maximum acceptable IMA levels for each environment at risk were empirically defined by performing a large number of tests in different environments.[9,83,85]

Materials and method

The materials needed for measuring the IMA are very simple and economical. Petri dishes 9 cm in diameter must be used. For monitoring purposes, it suffices to use a nutrient medium for total microbial count, such as plate count agar (PCA) medium.

The method for measuring the IMA is also simple. A standard Petri dish 9 cm in diameter containing PCA is left open to air according to the 1/1/1 scheme, for 1 h, 1 m from the floor, at least 1 m away from walls or any relevant physical obstacle. After 48 h incubation at 36 ± 1°C the cfu are counted. The number of cfu is the IMA.

Subsequently, an automatic passive air sampler was devised.[86] The Sed-Unit device, developed at the EMPA (Eidgenössische Materialsprüfungs und Forschungsanstalt) in St. Gallen, Switzerland, allows the correct positioning of the Petri dish and makes the measurement of the IMA easier and more accurate (Figure 2). The device is held by a telescoping stand; a moving arm automatically opens and closes the Petri dish. It can be programmed to leave the Petri dish open up to 24 h, with a delay of the starting point from 2 min to 24 h. Once programmed, the Sed-Unit works in the absence of the operator: possible changes in the cfu counts by microbial shedding from his/her body are thus avoided. The IMA can be measured in empty rooms or during inconvenient times (e.g., at night).

The IMA has been measured in different environments: in hospitals, private homes, university institutes, industrial plants, in the open air, in hospitals, in museums, libraries and aboard the MIR space station.[87–97]

The amount of data collected allowed the definition of classes of IMA and the maximum acceptable IMA levels in environments at different bio-risk levels. Over time some adjustments have been made. Studies have been carried out on the correlation between counts by active air samplers (cfu/m^3) and counts on settle plates (IMA), and the evaluation of the reliability of settle plates.[21,59,82,83]

Figure 2 Sed-Unit.

IMA classes and maximum acceptable levels of IMA

IMA classes and maximum acceptable levels of IMA have been defined empirically. This has been possible thanks to the large amount of data collected in many different types of closed environments and in the open air, over a number of years.

The measurement of the IMA is meaningful in places where there is an infection or contamination risk. Therefore the lower levels of contamination have been taken into account. The maximum IMA level included in the classification is 76. Higher values, well over 1000, can be found in dirty areas or places which are not controlled. However, if there is any risk, such counts must be lowered.

Five classes of IMA have been devised: 0–5 very good; 6–25 good; 26–50 fair; 51–75 poor; >76 very poor. IMA classes have been also normalized to cfu/dm^2 (Table X).[9]

Each class represents a different increasing level of contamination. In practice, this choice proved useful for the aim to which it was intended.

Maximum acceptable values of IMA have been established, related to different infection or contamination risks. These are 5, 25 and 50, in places at very high, high and medium risk, respectively (Table XI).[9]

The operators have simple but clear guidelines for defining the acceptable IMA level in the places under their responsibility and for controlling the preventive means applied. It is up to whoever is in

charge to state the level of the infection risk and to adopt the corresponding maximum acceptable IMA level.

Table XII shows the comparison among the classes of contamination taken by FS 209E,[34] NASA,[35] EU GMP,[75] IMA[9] and ISO.[76]

Table X *IMA classes and their application*

IMA value	cfu/dm^2/h	Performance	In places at risk
0–5	0–9	Very good	Very high
6–25	10–39	Good	High
26–50	40–84	Fair	Medium
51–75	85–124	Poor	–
⩾76	⩾125	Very poor	–

Table XI *Maximum acceptable levels of index of microbial air contamination (IMA) in environments at risk*

Environment at risk	Maximum acceptable level of IMA
Very high*	5
High†	25
Medium‡	50
Low§	75

*Ultra clean rooms: reverse isolation; operating room for joint replacement; some procedures of the electronics and pharmaceutical industries;
† Clean room: conventional operating theatres, continuous care units, dialysis unit;
‡ Day hospital, hospital wards, food industries, kitchens;
§ Facilities.

Up to 100 cfu/m^3, corresponding to grades A, B and C of the EU GMP and to 100 and 10 000 classes of FS 209E, there is some acceptable compliance among the values suggested by different sources. At grade D (100 000 of the FS 209E) NASA assigns 88.4 cfu/m^3 and a value of 15 on settle plates, while EU GMP assigns 200 cfu/m^3 and a value of 25 on settle plates exposed to air for 1 h. In the same way, IMA at grade D assigns a value of 25.

Conclusion

Regarding bio-risk in the food processing industry, regarding the measurement of the microbial air contamination, Favero *et al.* pointed out that the first and most important decision is whether air sampling at any level is required. If it is, then quantitative and qualitative guidelines should be established which relate numbers and types of micro-organisms per volume of air to critical levels of product contamination.[98]

This statement that can be applied to every place in which an infection or microbial contamination risk exists. It underlines the need to relate, quantitatively and qualitatively, the number of cfu/m^3 of air to the number of contaminating micro-organisms, i.e., falling out, on a product or a surface at risk.

Wherever a bio-risk is present, air sampling is required.[1–8] Once this is accepted, Favero's suggestion implies a difficult problem in that cfu/m^3 have to be correlated with the cfu falling out. Volumetric samplers will measure the total number of micro-organisms in the air, but this is an indirect

Table XII *Correlation among the microbial contamination classes suggested by the US FS 209E, the NASA, the EU GMP, the IMA and the ISO, based on cfu/m^3 and settle plates*

EU GMP*	FS209E†	NASA‡		EU GMP*		IMA§	ISO¶
grades	classes	cfu/m^3	s.p. ‖	cfu/m^3	s.p.**	s.p.††	
A	100	3.5	0.6	<1	<0.25	0	5
B	100	3.5	0.6	10	1.25	5	5
C	10 000	17.6	3.0	100	12.50	–	7
D	1000 00	88.4	15.0	200	25	25	8

*European Union Good Manufacturing Practice;
† Federal Standard for air contamination by inert particles;
‡ National Aeronautics and Space Administration;
§ Index of microbial air contamination;
¶ International Organization for Standardization;
‖ Settle plates 73.5 cm^2 wide exposed to air for 1 h;
** cfu on settle plates 9 cm in diameter expected after 1 h exposure, calculated from the results obtained by settle plates exposed for 4 h;
†† cfu counts on settle plates 9 cm in diameter exposed to air for 1 h.

measurement of the likely microbial contamination of a surface at risk through fallout.[40,57,59]

Conversely, IMA measurement by settle plates, related as it is to the level of the microbial contamination of the surrounding atmosphere, immediately gives an objective and accurate representation of both conditions: the extent of air contamination and the amount of micro-organisms falling out in the area at risk. For routine monitoring purposes, the quantitative approach suffices. If needed, at the same time a qualitative study can be carried out, using settle plates with differential nutrient media or subculturing and analysing the isolates.

The general acceptance of the IMA would allow the comparison of results obtained by different persons in different places in the study of the microbial air contamination, which currently is not possible. At the same time, it could provide an easy and generally valid parameter for official guidelines, particularly in view of the low cost and the ease of the test.

The widespread use of both active and passive air sampling methods is responsible for the lack of clearness in this topic. The cfu/m^3 count is intuitive but difficult to measure. Not one of the many active samplers produced is above criticism.[5,99] As a consequence, every year new designs are marketed but always with the same basic drawbacks. On the other hand, settle plates, which offer considerable advantages, are frequently considered not reliable or useful. Our research up to now has yielded a number of data that support the usefulness of settle plates, provided they are used in a standard way to measure the IMA.

In all the different environments tested, the IMA always gave a clear answer, in accordance with the real conditions and has proved to be a valuable tool as a complement to, rather than a replacement for, the volumetric measure.

In time the measurement of a microbial population by the count of cfu will be certainly surpassed by other techniques, such as molecular biology. But there will still be a need for the correct collection of air samples. Settle plates are likely to remain in use and the IMA will be all the more meaningful, changing from counts of micro-organisms to counts of their genomes.[100,101]

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