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EC MICROBIOLOGY
Principle Conceptual Paper

Environmental Monitoring of Air in Clean Room and Controlled Environments

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Definitions

A clean room is an enclosed space in which airborne particulates, contaminants, and pollutants are kept within strict limits. In pharma, biotechnology and medicine, clean rooms are used when it is necessary to ensure an environment free of bacteria, viruses, or other pathogens.

FS 209E Clause 3.5 Define cleanroom as 'A room in which the concentration of airborne particles is controlled and which contains one or more clean zones'.

ISO 14644-1 Clause 2.1.1 expanded the definition of a cleanroom to 'A room in which the concentration of airborne particles is controlled, and which is constructed and used in a manner to minimize the introduction, generation, and retention of particles inside the room and in which other relevant parameters, eg temperature, humidity and pressure are controlled as necessary'.

Introduction

The purpose of the microbiological environmental air monitoring is to keep in control the critical processes within the pharmaceutical and biotechnology industries. In practice is to highlight conditions contributing to excessive microbial and particulate levels due to ineffective cleaning or staff/equipment trending issue.

Furthermore it should alert to conditions exceeding classification and to be a pro-active tool for quality assurance.
Viable airborne particulates and viable surface bound particulates on cleanroom surfaces and staff are involved.
The contamination sources in Cleanroom are people (70 - 80%), ventilation (10 - 20%), room structure (5%), instrumentation (5%).

Micro-organisms are usually found in the air of occupied rooms rafted onto skin cells. Very few present on their own.

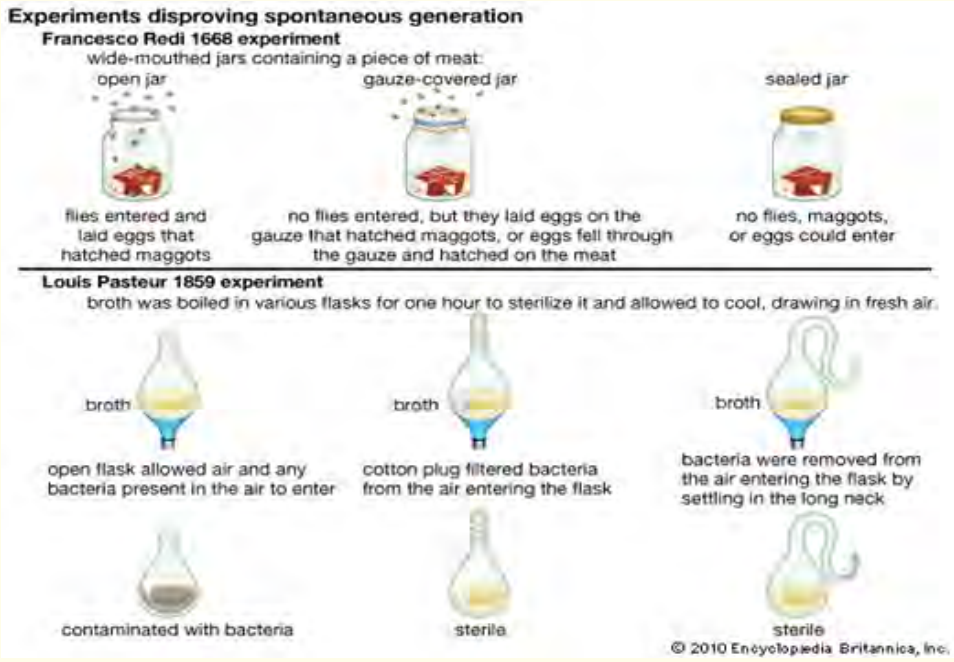
The brief History of microbial environmental test

The "spontaneous generation" theory from medieval times ended when the Italian Francesco Redi, Lazzaro Spallanzani and the French Louis Pasteur demonstrated that bacteria are present in air.

The today environmental air microbiology "follows" the same principle of Pasteur.

Until 1960 the microbiologist captured the micro-organims from the air leaving an open Petri dish with nutrient medium; the bacteria fallen down for gravity on agar. The colonies counted after incubation. The method was simple but not exact. My idea, with the co-opera-

tion of my brother Sandro, was to use the culture plate for surface and hands monitoring (called contact plate and RODAC) also for air. So, the active microbial air sampler was born!



The principle of "active" air sampler

The air containing viable particles are aspirated through very thin holes and directed on the culture medium. The growth medium has low selectivity to be capable of supporting a broad spectrum of micro-organisms including bacteria, fungi, yeast and molds. TSA (Tryptone Soya Agar) with incubation at 30 - 35°C for 48 - 72 hours supports general microbial colonies; SDA (Sabouraud Dextrose Agar) with incubation at 20 - 25°C for 5 days supports yeast and fungal colonies.

If necessary to detect or search for a particular type of micro-organism a selective culture medium should be used. The micro-organims are counted and the results are reported as the number of CFU (Colony Forming Unit) per 1000 litres of air (=1 cubic metre).

Typically, to apply the Good Laboratory practice, two positive controls and two negative control are used:
B. subtilis and *C. albicans* for positive. Unoped plate for negative.

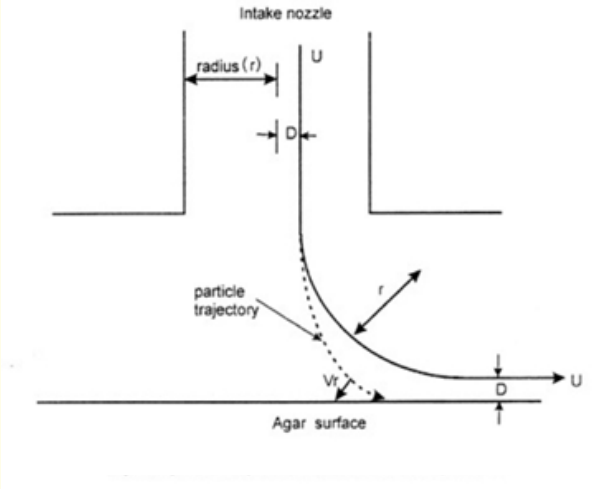


Figure 2: *Principle of active air samplers and a petri dish.*

Time of testing

Air sampling in the static conditions should be performed at an agreed frequency to monitor baseline contamination levels.

Air Sampling in the operational conditions (staff present and normal operations being carried out) should be performed at each shift.

Type of Aseptic Operation

Grade	ISO Definition	Classification	CFU/M^3 of Air	Type 0f Operation
A	5	100	< 1	Aseptic preparation and filling
B	6	1.000	10	Room Conditions for activity requiring Grade A
C	7	10.000	100	Preparation of solutions to be filtered
D	8	100.000	200	Handling of components after washing

Sampling location

The selection of sampling location depends on the room classification, design, layout of the manufacturing process.
Each process should be evaluated in order to identify the actual and potential sources of contamination.

Out of specifications

- Alert Level – Is a quality level that, when exceeded, signals a possible deviation from normal operating conditions and may not require action, but may need to be monitored more closely.
- Action Level – is a quality level that, when exceeded, signals an apparent deviation from normal operating conditions and requires immediate action.

The following actions are suggested when levels are breached:

- Identify: possible cause; contaminating micro-organisms
- Investigate: whether isolated sample or whole area involved; level of training, health, technique, wash up of operators; cleaning protocols; changing protocol; integrity of HEPA filter; previous test results for trends.

Action Level for Microbiological Air Contamination	
Classification of the Environment	CFU/1 cubic metre
ISO Class 5	> 1
ISO Class 7	> 10
ISO Class 8 or over	> 100

The Standards

The standards for viable airborne particulates are:
ISO 14644-1:2015 Clean rooms and associated controlled environments -
Fed Std-209E-
USP <1116> Microbial control and monitoring of aseptic processing environments
(Figure 3)

SOP and Quality Control

A specific SOP should be available for a correct microbial air sampling execution.

It Is here reported a simple scheme:

- SOP
- Number and identification
- Responsibility
- Title
- Purpose
- Glossary
- Standards
- Safety
- Protocol
- Non Conformity
- Corrective Actions

Conclusions

A complete and exaustive air monitoring plan is a must. It should be prepared with great care so that it can be defended during the inspection of regulatory authorities and compliance audit.

What is reported in this paper refers mainly to the pharma and biotechnology industries, but should be integral part also for hospital and medical centre for the patient health.

Museum of Microbial Air Samplers

The air samplers were part of my life and I decided to organize a museum in Villa Cella Milan, where are collected all the air samplers from different producers (PBI Surface Air System, Merk, Biotest, Millipore, Sartorius, IUL, Microflow, USDA, Oxoid, Air Ideal, MAS, Andersen, Casella, G1000 Bracco, Western Sennon, Microbio Parrett, Bellco, TRIO.BAS).

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