

## Air Sampling in Wards and Hospitals: What we Know and What we do not

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### Abstract

Bacterial sampling of air for microbes is very important in order to prevent airborne infections. Techniques for this air sampling can be divided into active and passive ones. With time new methods and techniques have been devised. Studying these methods and their relevance is very important and new methods are expected to appear in near future.

**Keywords:** Air sampling; Sampler; Aerosol.

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### Introduction

Microbes are ubiquitous in air, soil and water, especially air via which it can produce many airborne infections; it can come from both endogenous and exogenous sources. Hence microbial sampling of air and containment or purification of the air is important.<sup>1</sup> Post-operative infections comprise about one-third of all the hospital-acquired infections, and a large number of them arise from the contaminated air in the operation theatres.<sup>2</sup>

#### *Need of air sampling*

In any closed workplace airborne microbes are important source of infections and hence the bioload of microbes in ambient air needs to be monitored.<sup>1</sup>

#### *Air sampling:*

Methods of air sampling for pathogenic microorganisms can be active and passive.<sup>1</sup> In

passive method different basal and enriched culture media plates are used as "settle plates". Results can be expressed as CFU/plate/time or in CFU/m<sup>2</sup>/hour.<sup>3</sup>

In active sampling, by suction a fixed volume of air is drawn in, and colonies are expressed as CFU per cubic metre of air.<sup>1</sup>

Passive method depends on many factors like gravitational field, electrical gradient, thermal gradient and others, which governs the way bioaerosols would settle down; some of them are taken advantage of in passive sampling.<sup>3</sup>

**Settle plate method:** Here, petri plates containing culture media are placed in the "1/1/1 scheme", i.e. for 1hour, 1metre above the floor, and at least 1metre away from walls or any obstacle.<sup>4</sup> In this method, agar coated slides can also be used in place of plates.<sup>3</sup> Typically media like Tryptone soya agar can be used for settle plate method, also called sedimentation method, and duration of keeping the plates open for aerosols to settle down is 4 hours.<sup>5</sup>

#### *Advantage of the settle plate method:*

According to Charnley, this method reproduces the actual process by which infectious aerosols deposit on biological and non-living surfaces by gravity, and hence are better than active sampling which estimates all suspended bioaerosols.<sup>6</sup>

- (a) Media used: Blood agar, MacConkey agar or Tryptone soya agar are the common media used.

- (b) Newer media used: Bhattacharyya et al have used successfully milk air for air sampling by settle plate method, and this medium also helped in identification of pathogens by supporting good growth and Staphylococcal pigment production.<sup>7</sup>

*Disadvantage and limitations of settle plate method:*

The main disadvantage of passive sampling, according to Humphreys, is that this method is not quantitative and selects out only large airborne particles.<sup>8</sup> Hence it is not suitable for operation theatres, but is to some extent useful in these places because it can measure the airborne bacteria nearest to operation site, as reported by Friberg et al.<sup>9</sup>

*Active sampling:*

Active sampling is better in the sense that it is quantitative, but the results are variable with individual equipments.<sup>10</sup> Literature mentions that the Anderson's sampler is better than Anderson's impactor and other equipments.<sup>10</sup>

*Slit sampling:*

Centrifugal air sampler: This is a very useful technique where media is in strips at edges of sampler and by centrifugal force particle are impinged on the media. Reuters centrifugal sampler is a very useful equipment where colonies are expressed as CFU per strip per unit time.<sup>11</sup>

Disadvantages of active air sampling: These methods are expensive and noisy, and are also heavy and difficult to sterilize.<sup>12</sup>

Other methods: A 6-stage Anderson's impactor containing media in petri plates has been devised to accurately assess the load of bacteria and fungi in ambient air.<sup>10</sup> However it is not so popular now.

Newer methods: Flow cytometry and Fluorescent in situ Hybridisation have been shown to be useful but lack sensitivity and specificity.<sup>11</sup> Also, microbial metabolites like ATP and DNA can also be studied, but are not very specific.<sup>12</sup>

Electrostatic precipitation: Electrostatic precipitation using ionisers have been studied since many years for air sampling.<sup>13</sup> However, powerful electric field may damage bacteria, humidity may alter the results, and recovery rates for *Pseudomonas fluorescens* and *M.bovis* BCG are low.<sup>13</sup>

Thermal precipitation: In this technique which is also old, air is forced on a heated surface and then onto membrane filter or petri plate with media on

to the cooler surface.<sup>14</sup> There it precipitates and colonies are observed the next day.

Impingement in liquids: This is also an old method and has also been tried later, and can be devised as air inlet and outlet with a liquid culture medium with sieve in between. However this method is also not suitable for *P. fluorescens*.<sup>15</sup>

Air sampling for fungi: Using different methods of active sampling, Sabouraud's dextrose agar has been used for assessing fungal load in air. DNA isolation and sequencing has also been done from the colonies for accurate identification.<sup>16</sup> *Aspergillus* spp. are generally found in air inside and outside wards.<sup>16</sup> Passive sampling methods like settle plate method are not recommended for fungal air sampling because fungal spores can remain suspended in air and not settle down indefinitely.<sup>17</sup>

Air sampling for viruses: Newer methods like electrostatic precipitation (ESP)-based bioaerosol sampler, coupled with downstream quantitative polymerase chain reaction (qPCR) analysis, have been found to be very effective in finding Influenza virus in ambient air of hospitals and other places.<sup>18</sup>

Sampling of air for *M. tuberculosis*: In a study by Mastorides et al, Micropore membrane air sampling, assisted by suction pump, along with PCR analysis has been found to be very effective in detecting *M. tuberculosis* in air especially near the infected patients' beds.<sup>19</sup>

*One health approach:*

One health envisages safe human health, animal health and environmental health, and clean and safe air is an essential component of one health.<sup>20</sup>

Future directions: New methods and techniques are awaited that might simplify the air sampling for microorganisms.

Summary and Discussion: Hence the basic methods for studying microbial load in ambient air are:

- a. impingement in liquids,
- b. impaction on solid surfaces,
- c. sedimentation,
- d. filtration,
- e. centrifugation,
- f. electrostatic precipitation, and
- g. thermal precipitation.

