UNIVERSITY OF LEEDS

HumanIC

Microbiology sampling in research and Healthcare:

microbiological techniques for surface and air sampling

HumanIC CBT1

27th Feb 2025

Dr Waseem Hiwar

HumanIC project has received funding from the European Union's Horizon Europe research and innovation program under the Marie Sklodowska-Curie (HORIZON-MSCA-2022-DN-01, project no 101119726

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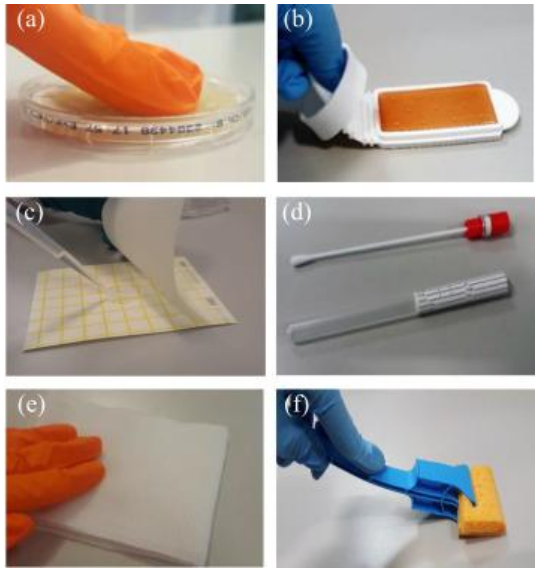
When carrying out an environmental sampling exercise you need to decide what aspects of the environment you are interested in:

Air – may be looking for a total aerobic colony count or for a specific pathogen

Hiwar et al. (2022), *Indoor Air, 32(11),* e13161

Surfaces – a whole range of surfaces can be sampled from Walls to floors and curtains and bedding.



Rawlinson et al. (2019), Journal of Hospital Infection, 103(4), 363-374

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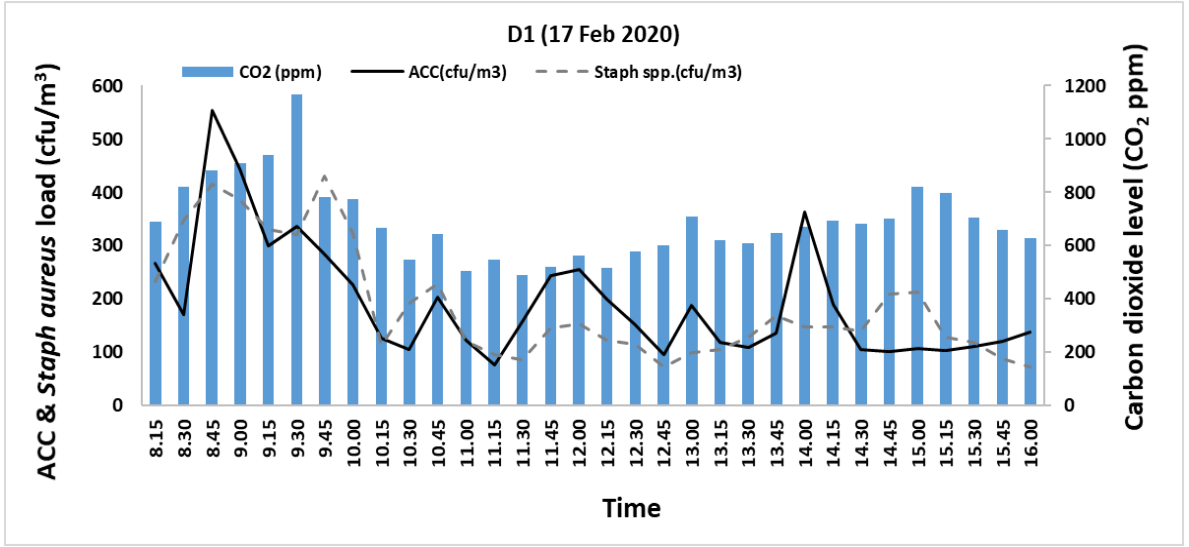
The main types of samples

1- RANDOM SAMPLING

- Provide a snapshot view of the quality of the sampled environment at a **single point** in time and at a specific location
- The results cannot be used to extrapolate to other times or locations
- Usually use for pilot study

2- SYSTEMATIC SAMPLING (Semi- continuous or continuous)

- Wide range of equipment available
- Can be programmed to take samples at fixed or variable time intervals or in response to external trigger
- Can take discrete samples at each time interval or a composite over an extended time period
- Can also be real time depending upon the parameter being measured and can be linked to a warning system



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Sampling strategy

Monitoring is of little use without a **clear understanding** of the reasons for the monitoring and the **clear objectives** that it will satisfy.

Poor sampling procedures can yield information that of little use and more importantly can contribute to the uncertainty in the results

There are a number of things that need to be taken into account before any sampling is undertaken:

- What is the **objective** of the sampling
- **How much data** and what **type of data** is required
- Is there a **budget**
- **How many samples** and what **type of samples** are needed
- What **identification and analysis techniques** are to be used (Ex. a systematic review for strong evidence)

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6th International Conference on Building Energy and Environment
Eindhoven University of Technology, The Netherlands
6 - 10 July 2025

Contribution of airborne microorganisms to surface contamination in hospital environments

W. Hiwar[1], M-F King[1], I. Clifton[2]. L. A. Fletcher[1] & C.J. Noakes[1]

Case study:

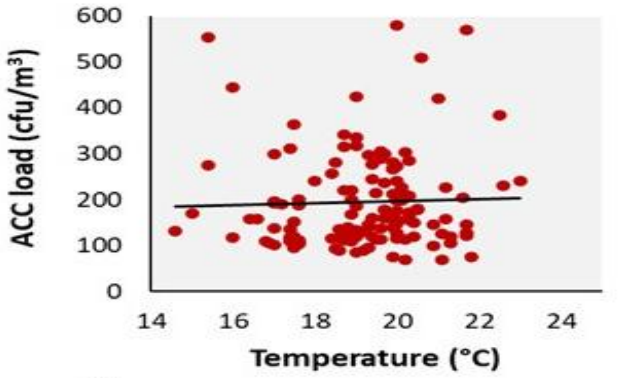
- What is the **objective of the sampling**?
  Find relationship between airborne ACC load and IAQ parameters.

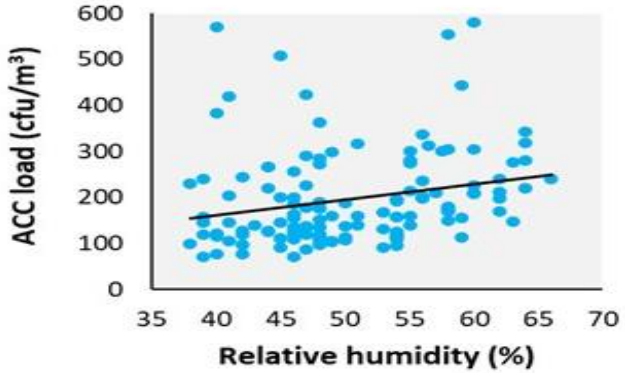
- What type of **data** and How much data is required?
  Quantitative data, Intensive sampling for 8 hours for 4 days

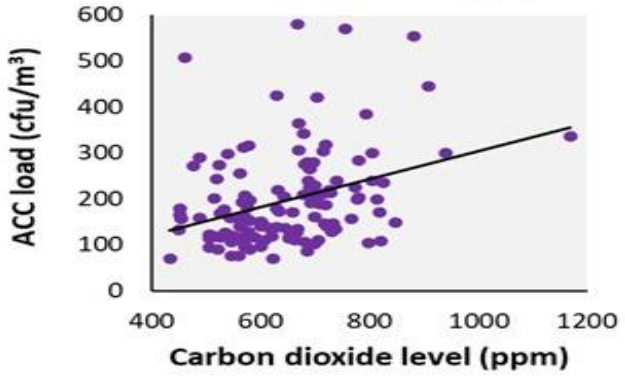
- Is there **a budget**?
  Yes, to buy materials and travel to hospitals, etc.

- What **type of samples** and How many samples are needed?
  Systematic Sampling (1/15 min)

- What **identification and analysis techniques** are to be used?
  ACC colony count Spearman and Pearson correlation test using R or SPSS. (rho=0.34 [95%CI=0.17-0.49, n=128), *P*<0.001)







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Air Sampling

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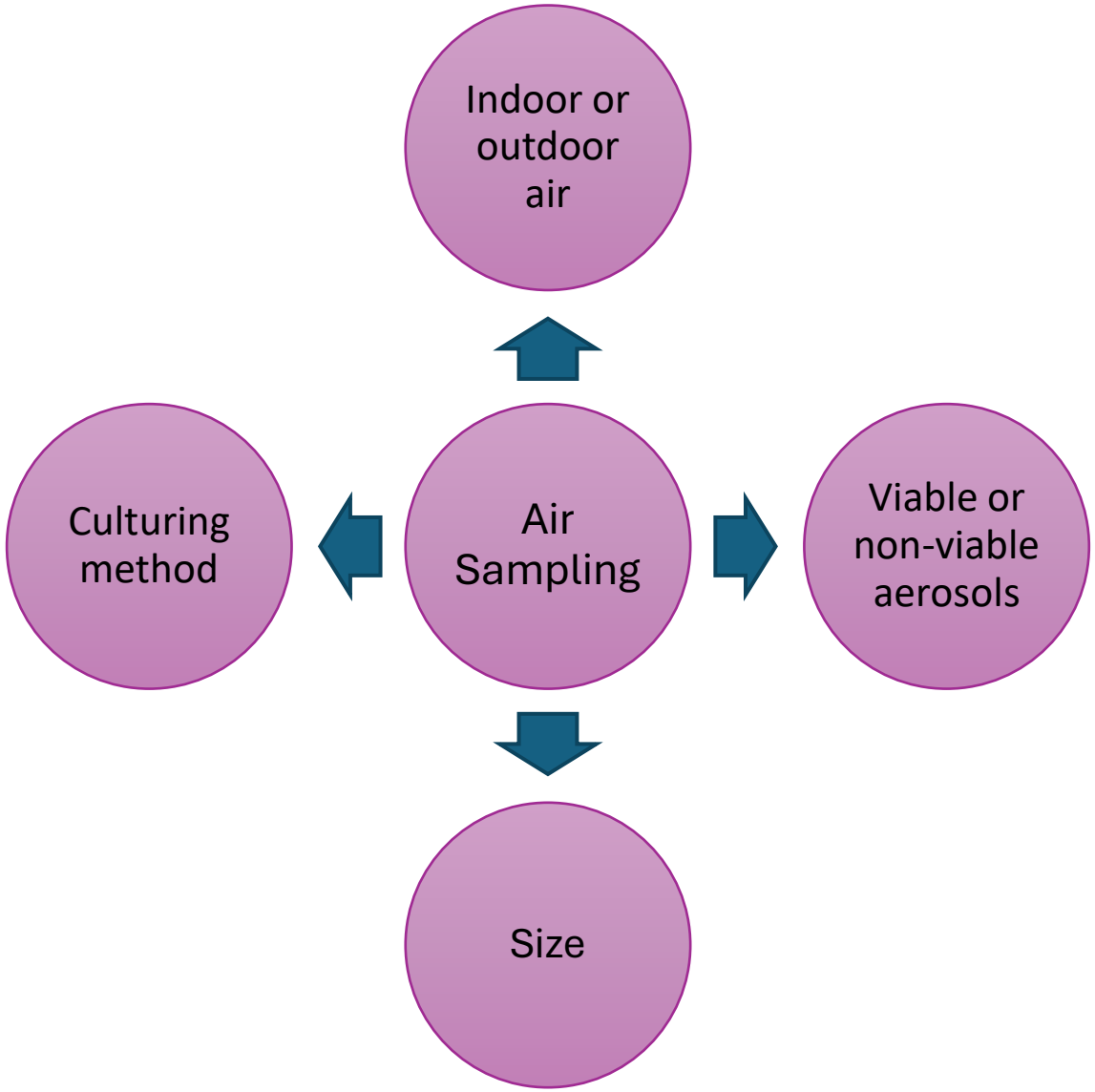

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What do you want to sample?

- **Indoor or outdoor air** – is important to consider practicalities of **setting up** and **operating a sampler e.g. power supply** – may be necessary to have battery power.

- **Viable or non-viable aerosols** – if you are interested in the viability of a bioaerosols which will often be related to infectivity you need to use a sampling method which collects a live sample for culturing.

- **If collecting a live sample** the choice of sampler may be determined by the subsequent culturing method.

- **Do you want to get information on bioaerosol size**?

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Bioaerosol Sampling:

- Can be difficult to collect a '**representative**' sample
- Very **labour intensive**
- Difficult to find **specific microorganisms** as the concentration may be very low
- Bioaerosol concentration may **fluctuate** widely
- Easy to **miss various microorganisms** if you are not looking for them
- Bioaerosols can be extremely **susceptible** and frequently die during the sampling process.
- Although **particle size** is important to determine it is very difficult to establish

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Bioaerosol Samplers

There are many samplers available for the collection of bioaerosols and they are categorised according to their collection method.

Five main types of sampler commonly in use:

1. Gravitation / Settle Plates
2. Impactors / Andersen six-stage impactor
3. Filters / Gelatine filters for virus collection
4. Impingers / AGI-30 impinger
5. Centrifugal / Biocapture 650

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- Each device has its own **advantages/disadvantages**
- Same collection **principle as for non biological particles**
- More concern on ensuring **survival** and maintaining biological activity during and after collection
- Different **handling, storage and analysis** methods
- **Purpose** usually verify and quantify presence of bioaerosols in air
- **No single methods** is appropriate for all bioaerosol types
- **Choice of sampler** will depend upon location and information required

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Bioaerosol sampler efficiency

- Ability to extract particles from ambient environment without bias regarding **particle size**, **shape** or **aerodynamic behaviour**

- Ability to remove the particles from the air stream and **deposit** them into on onto the collection medium

- Ability to remove particles without altering their viability or **biological activity**

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1- Gravitation / Settle Plates

- The simplest form of collection of airborne particles
- Can be a coated microscope slide or agar plate
- Surface is exposed face upwards to the atmosphere to collect particles settling by gravity
- Does not require specialist equipment
- Passive non-volumetric and therefore does not give information on the volume of air sampled
- Over **represents larger particles** due to their faster sedimentation rate
- Collection in turbulent air is seriously affected by shadowing or turbulent deposition
- Not recommended for hospital sampling unless there is a mathematical model to calculate the relationship between air and surfaces?

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2- Impaction Methods

6-stage Andersen sampler

Single stage SAS sampler

Single stage MicroBio MB2

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2- Impaction Methods

- Impaction is used to separate a particle from a gas stream based on the **inertia** of the particle.

- Particles **larger** than a particular aerodynamic size will be impacted onto a collection surface due to inertia while **smaller** particles proceed through the sampler

- Samplers may be **single stage** (Microbio MB2, SAS) or **multiple stages** (Andersen sampler) which allow data to be collected on particle size

Advantages:

- Portable and easy to use – especially true of the single stage hand held devices
- Multiple stage impactors allow particle size distributions to be determined

Disadvantages:

- Impactors capture only a small fraction of the microorganisms present in the sampled air, as the impaction process can cause significant damage, reducing the number of viable microorganisms collected.
- Can only determine one species with one sample

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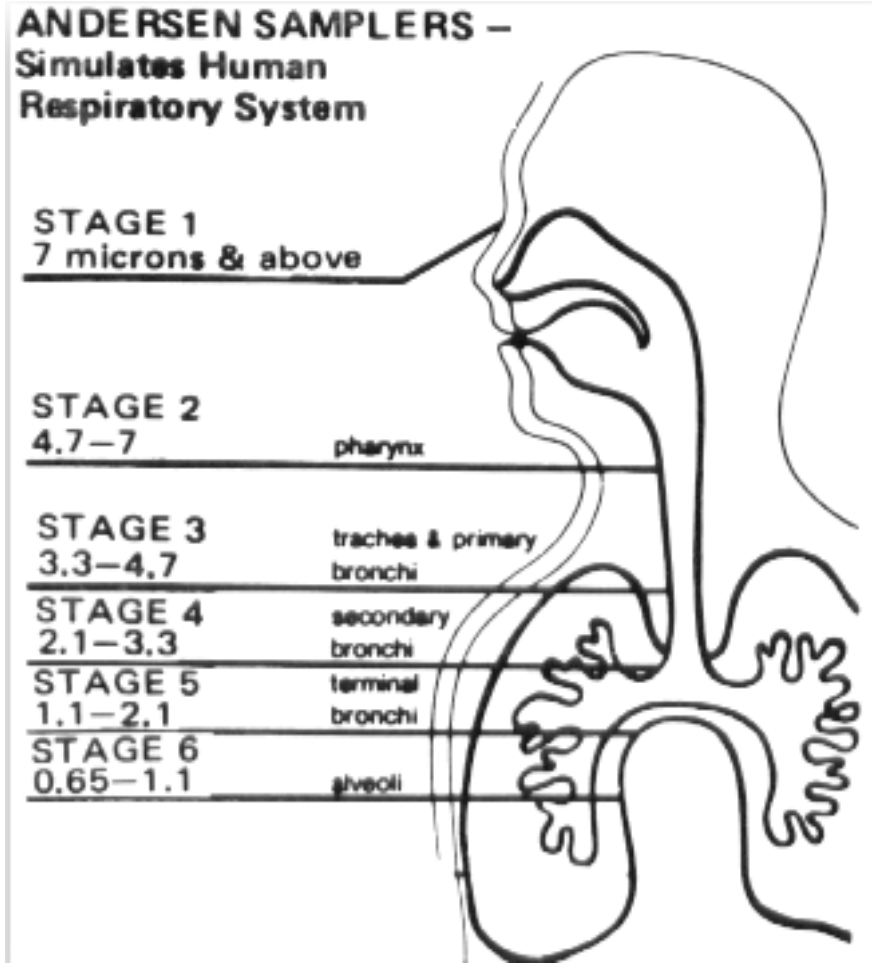

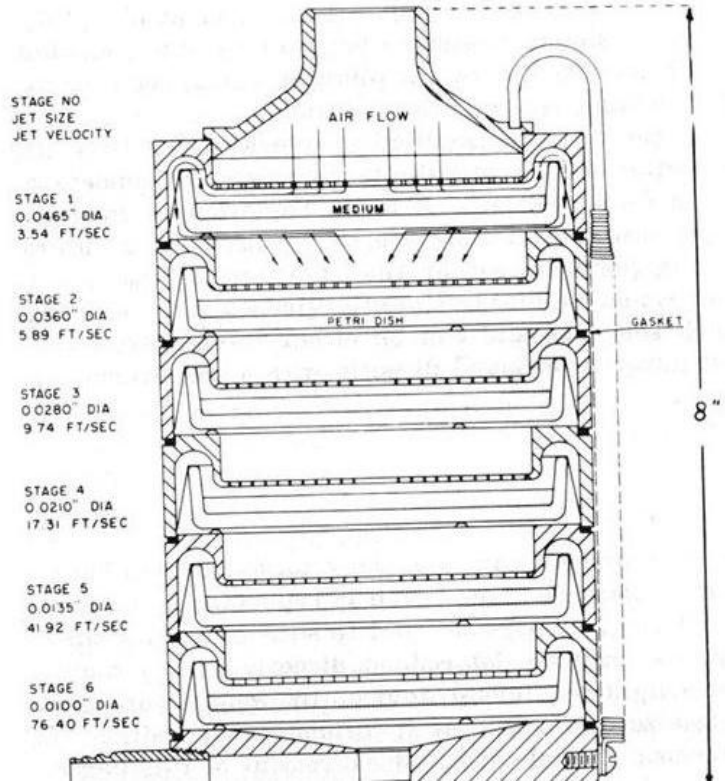

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3- Filtration Methods

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3- Filtration Methods

- Deposition occurs when particles **impact and are intercepted** by the fibres or surface of filter
- Particles smaller than the pore size may be **efficiently collected**
- The efficiency of **removing particles** from the air depends on the face velocity
- The sampled organisms are washed from the surface of smooth-surface polycarbonate filters, serially diluted if necessary and **cultured**

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Advantages:

- Easy to use
- After washing the filter microorganisms caught on the filter are then in solution. This can be plated out onto a variety of media to enumerate different species from one sample

Disadvantages:

- The air flow rate through the filter will reduce as it get dirtier and therefore the actual volume of air sampled may be difficult to determine
- The size distribution of the sampled particles cannot be determined.
- Error incurred at sampling and recovery stage

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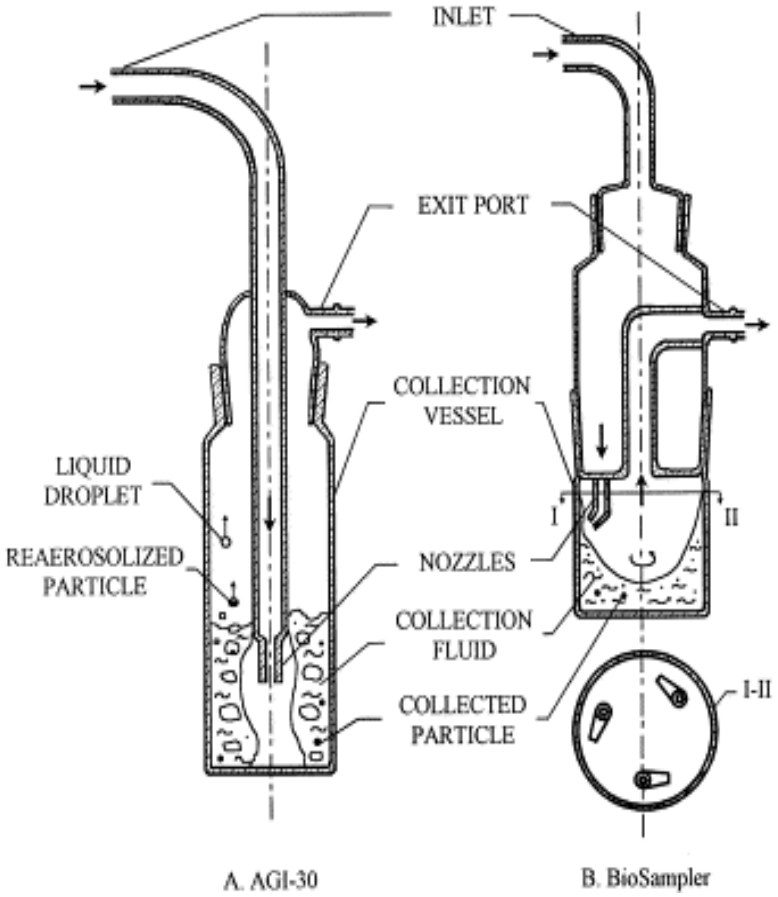
4- Impingers



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4- Impingers

- Air is drawn by vacuum through a body of liquid and particles leave the airstream and are collected by **impingement into the liquid**

- Most are made from **glass** with a single collection chamber and suction is applied via a side arm

- More modern versions have jet raised above base of vessel and also some have jet entering at an angle – led to **increased sampling efficiency**

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Advantages:

- High sampler efficiency
- Single sample can be used to detect a range of species
- No limitation on sample time other than evaporation of collection fluid

Disadvantages:

- The size distribution of the sampled particles cannot be determined
- Requires additional pump and power supply
- Post sampling culturing is required

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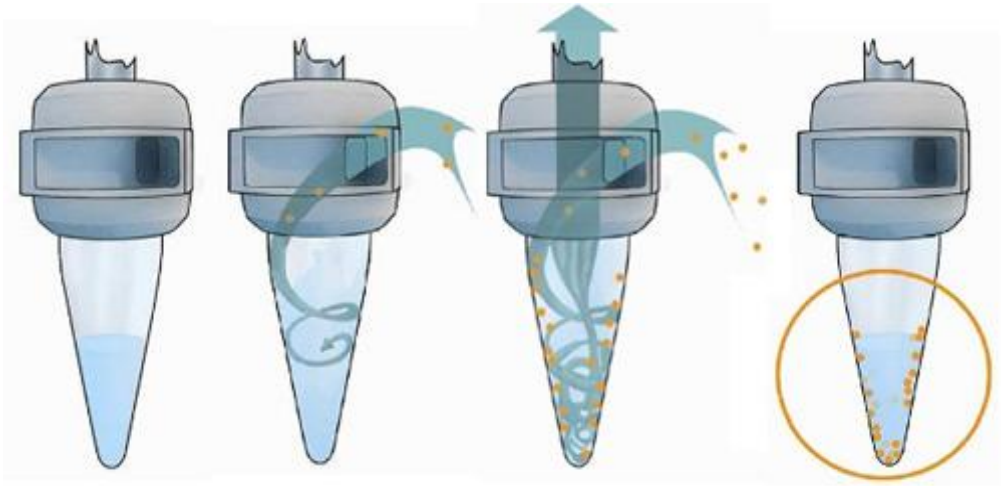
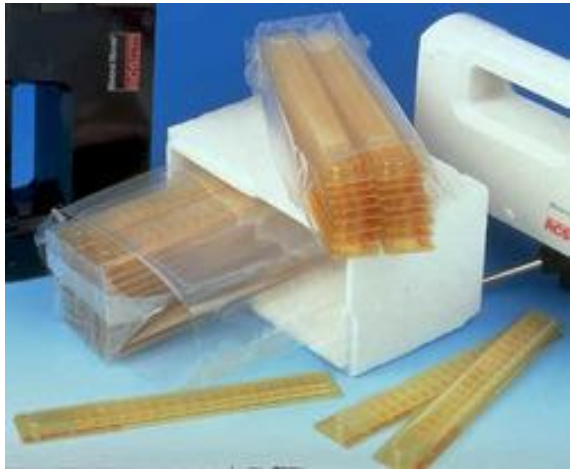
5-Centrifugal samplers



https://uk.vwr.com/store/product/17030105/air-sampler-coriolis



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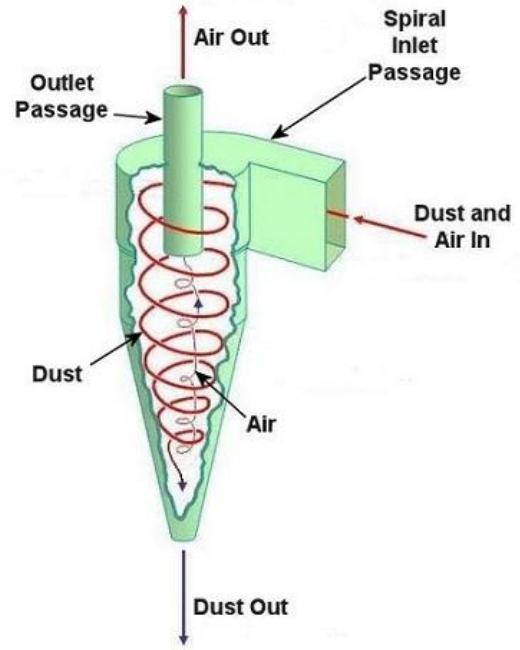
5-Centrifugal samplers

- **Creation of a vortex** in which particles with **sufficient inertia** leave the airstream to be impacted upon a collection surface
- Can collect **dry** samples or into a **liquid** (cyclone) or onto **semi-solid** media (centrifugal air sampler)
- Cyclone – air is drawn in tangentially near the top of a cylindrical or inverted cone shaped chamber and air flow spirals down chamber following the inner wall. Particles are deposited on the walls of the chamber
- Centrifuges – air is drawn in using an impeller in a shallow drum and air is forced towards the inner wall of the drum which is often coated in a layer of agar onto which particles are impacted



http://www.engineeringexpert.net/Engineering-Expert-Witness-Blog/

GAP EnviroMicrobial Services Ltd.

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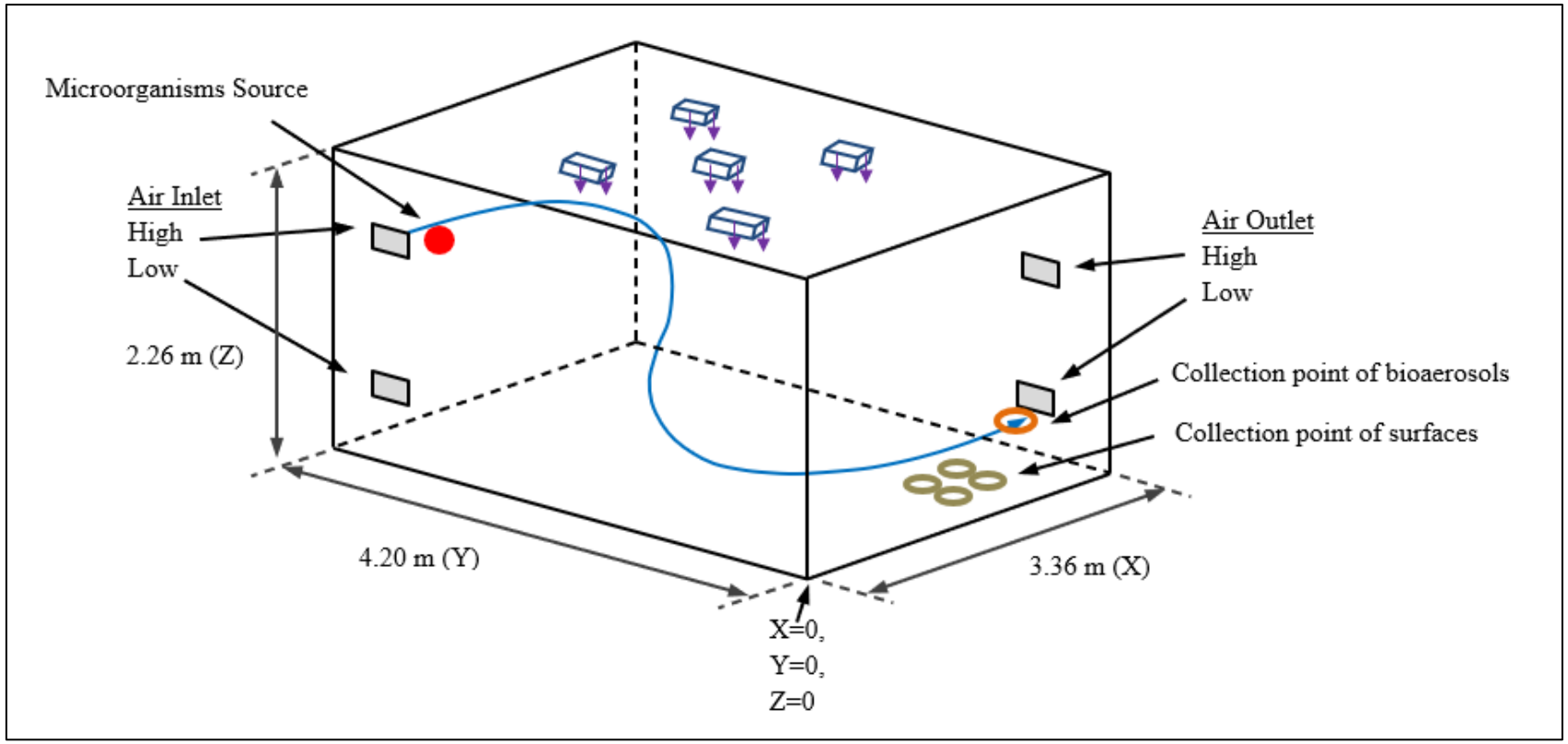
In research, we often need to improvise tools in order to adapt to the research needs.
For example, to determine the deposition loss rate of airborne microorganisms in the **steady state condition** in a controlled environment.

Which method do we need to use for air and surface sampling?



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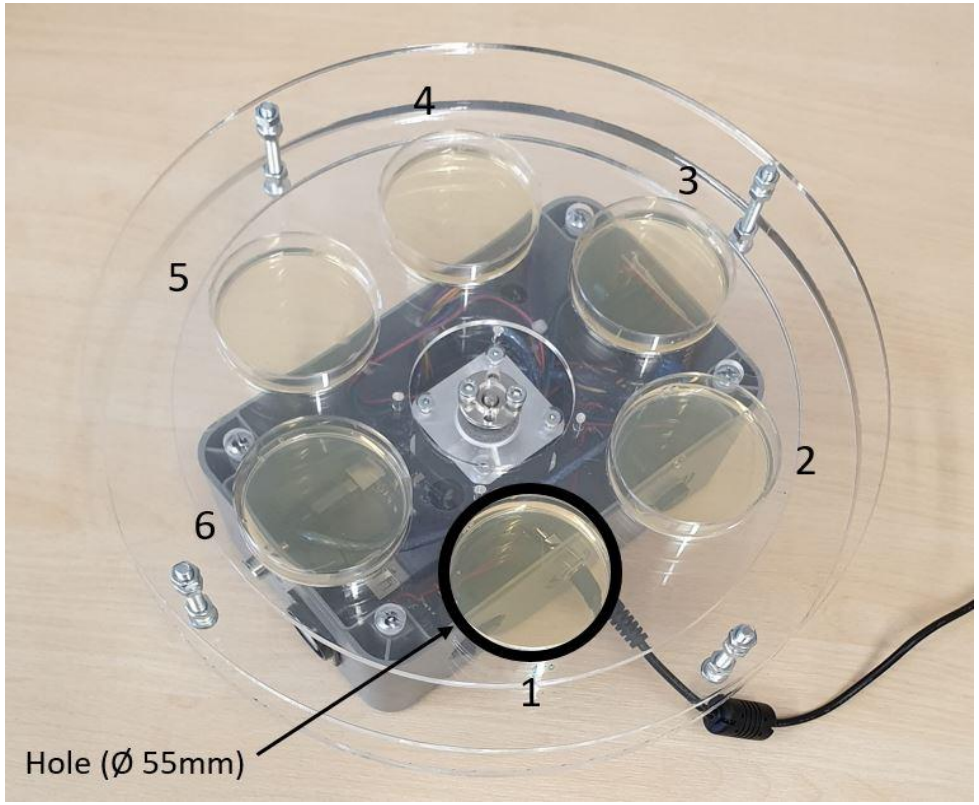
Methodology

An automated multiplate passive air sampler (AMPAS) was created to performed time series surface samples, a novel configurable device that can expose a 55 mm diameter plate to the air for a pre-determined interval, cover it, and autonomously expose a different one (Hiwar et al., 2020).



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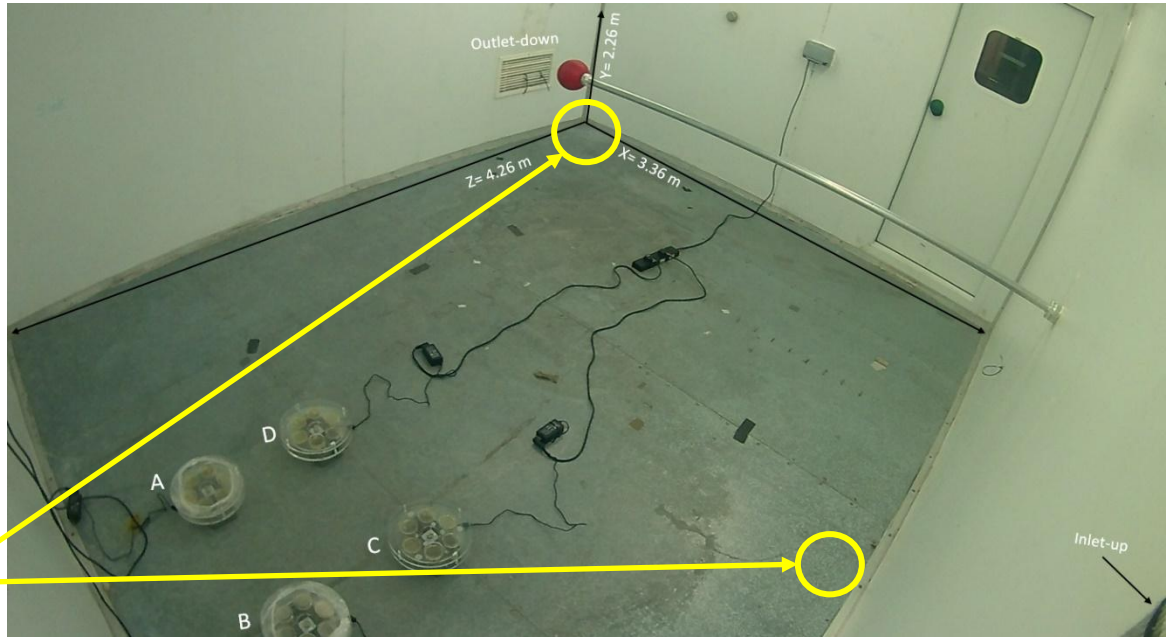
Methodology

Collection point of Air



Anderson
6 stage

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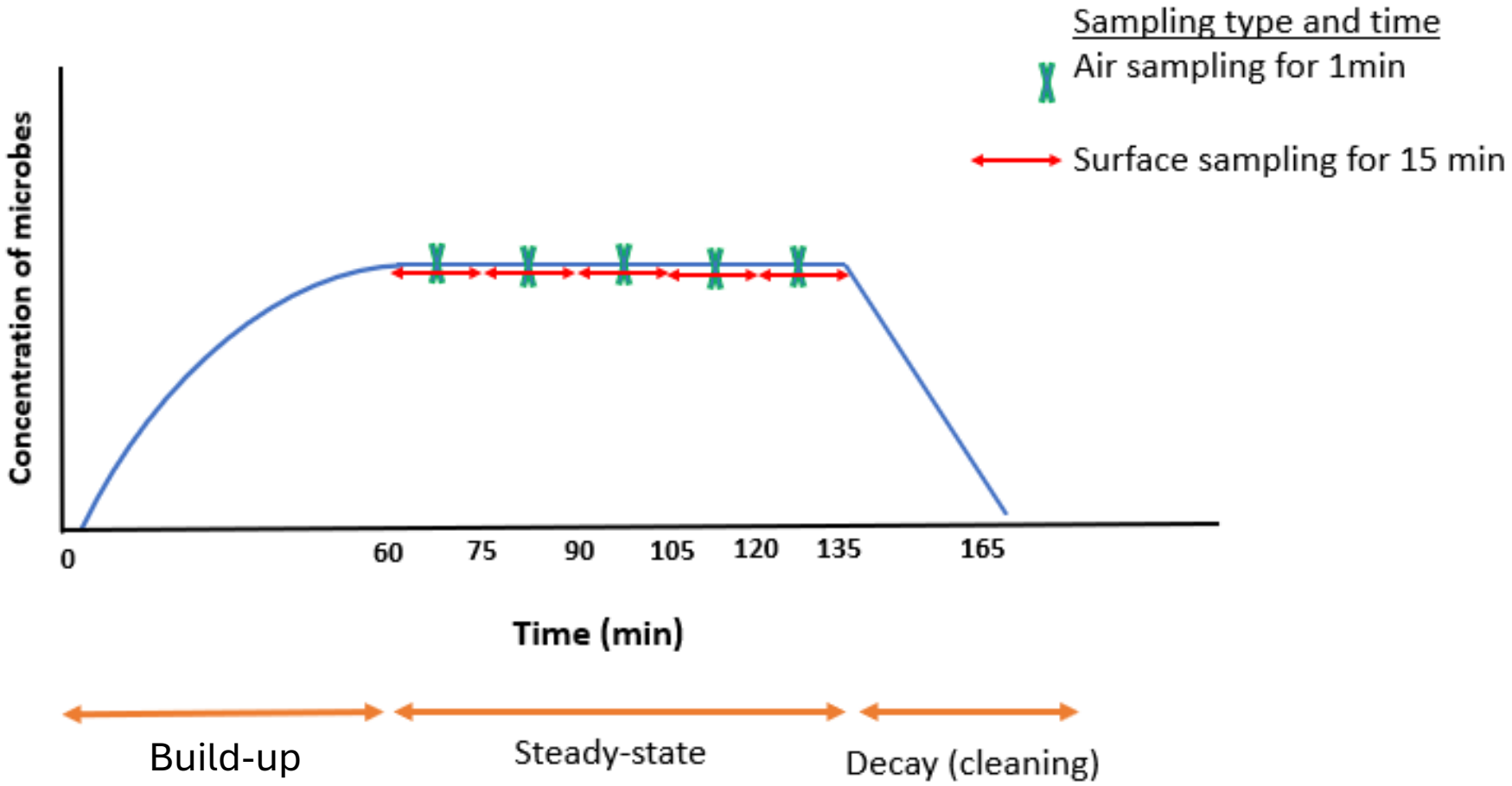
Methodology



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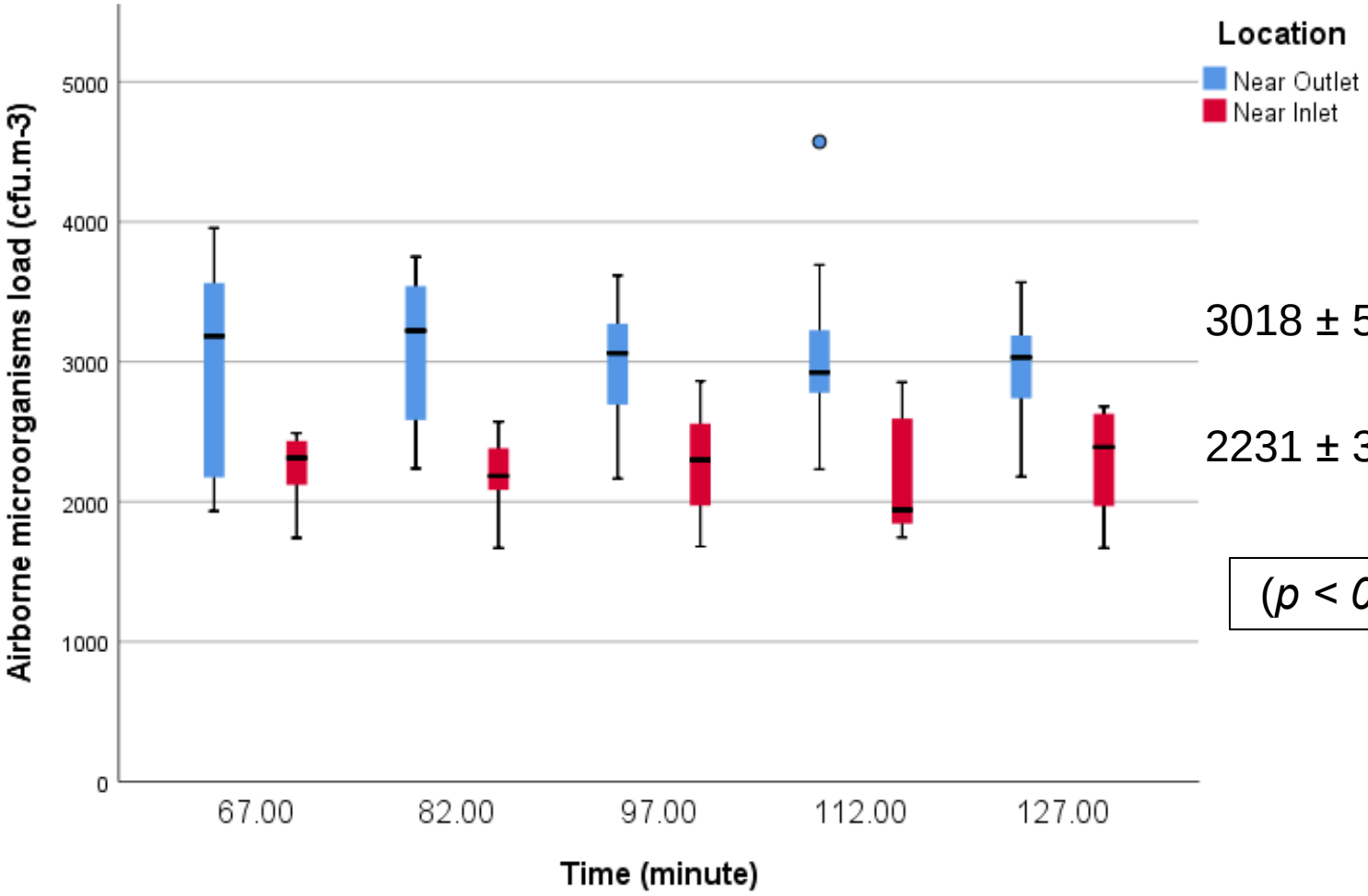
Results



3018 ± 549 (cfu.m^{-3})

2231 ± 340 (cfu.m^{-3})

($p < 0.01$), n=60

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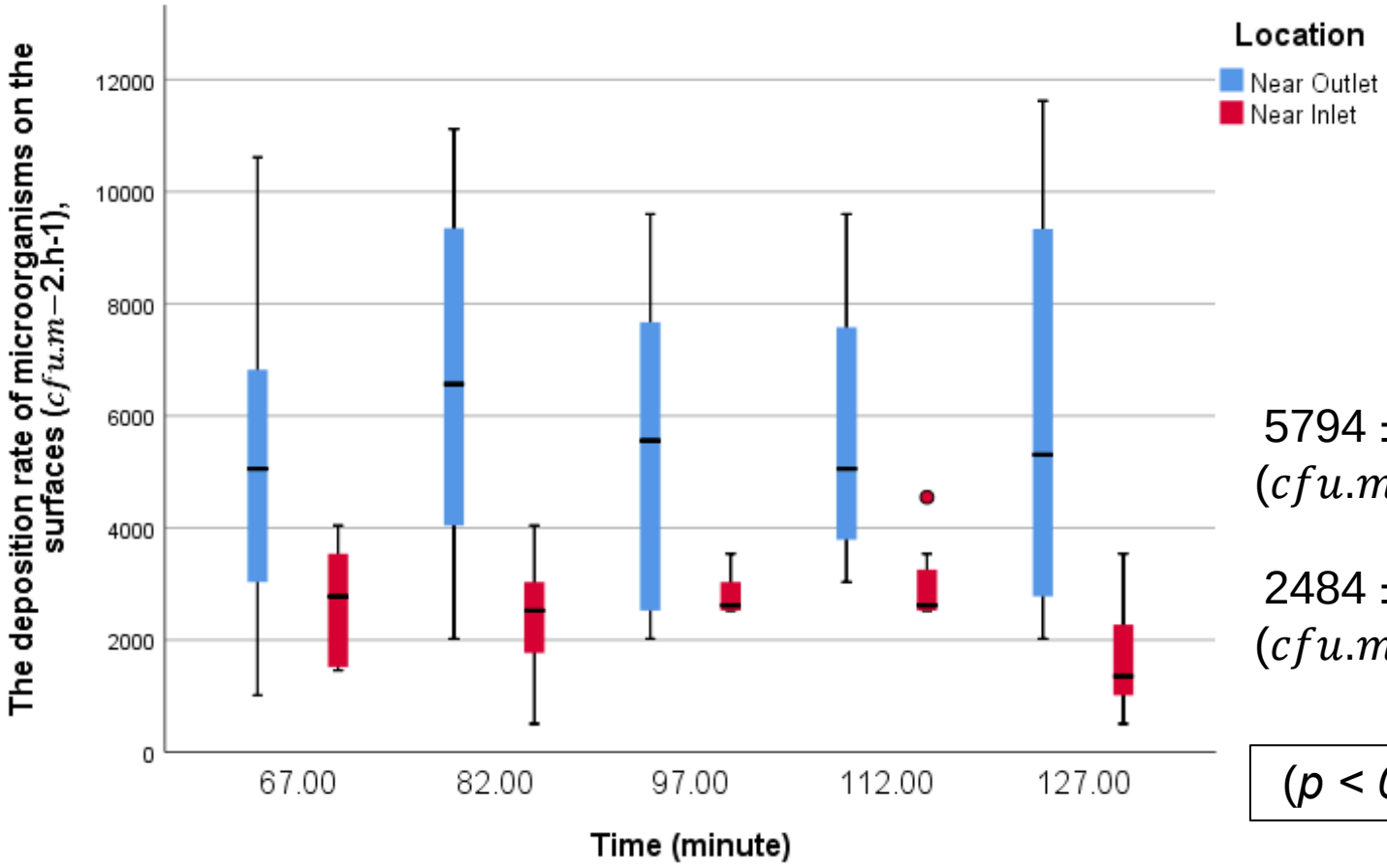
Results



5794 ± 2908
$(cfu.m^{-2}.h^{-1})$

2484 ± 936
$(cfu.m^{-2}.h^{-1})$

($p < 0.01$), n=60

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Surface Sampling

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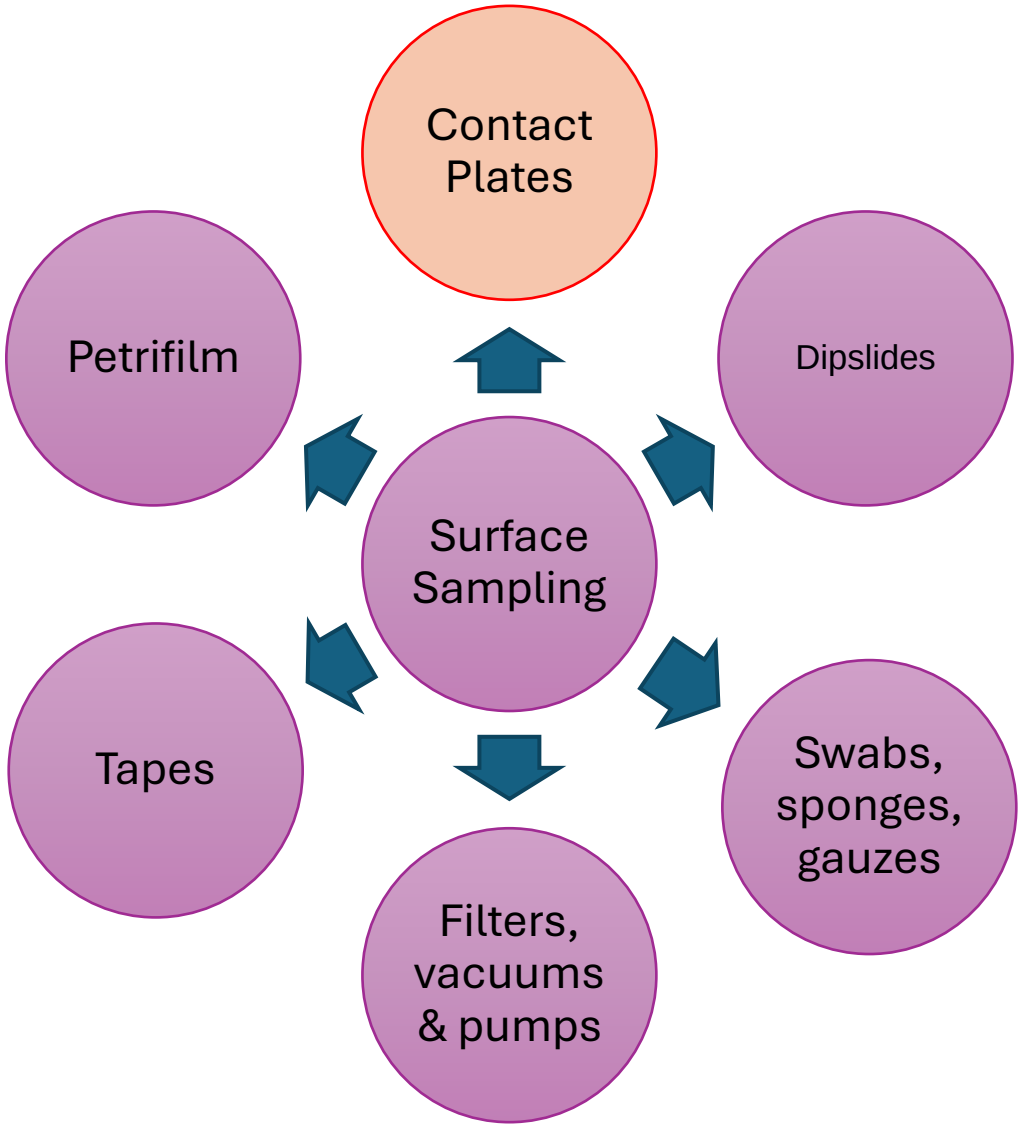

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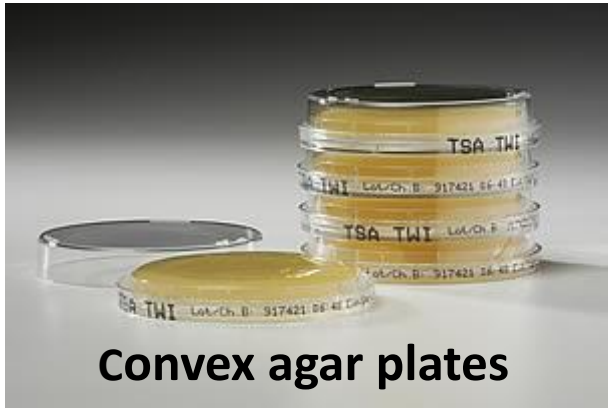
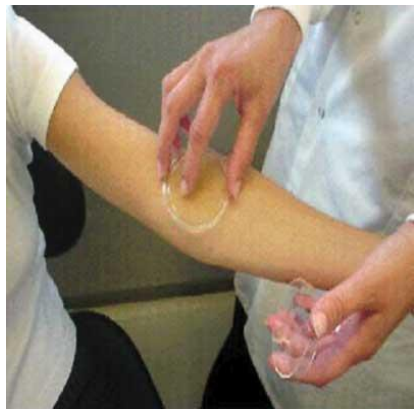
Contact Plates



Convex agar plates



They are easy to use

Quantitative – No need for sample template

No additional transfer stage just sample and incubate

Can only get information on one species with one plate

More expensive than swabs

Surface has to be relatively flat

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		Day 1 (17 Feb 2020)	Day2 (21 Feb 2020)	Day3 (3 Mar 2020)	Day4 (10 Mar 2020)	Average
		cfu/55mm plate (Mean ± SD [Min-Max], Sample size)				
Surface bioburden in four-bed ward at 8am (cfu/plate)	ACC	37.35±26.72 (4-75), n=20	32.40±24.07 (1-86), n=20	67.80±50.27 (6-160), n=15	42.75±40.86 (2-139), n=20	42.53±37.49 (1-160), n=75
	Staphylococcus spp.	25.40±16.02 (3-65), n=20	24.50±17.23 (2-65), n=20	30.47±33.37 (2-129), n=15	18.47±20.99 (1-69), n=20	24.74±21.99 (1-129), n=75
Surface bioburden in four-bed ward at 4pm (cfu/plate)	ACC	35.35±24.65 (6-62), n=20	19.80±21.58 (1-75), n=20	37.55±28.31 (6-106), n=20	23.47±22.37 (6-78), n=15	29.41±23.68 (1-106), n=75
	Staphylococcus spp.	23.60±13.39 (1-53), n=20	14.75±10.67 (1-43), n=20	20.95±21.03 (1-70), n=20	13.80±11.97 (1-41), n=15	18.49±15.28 (1-70), n=75

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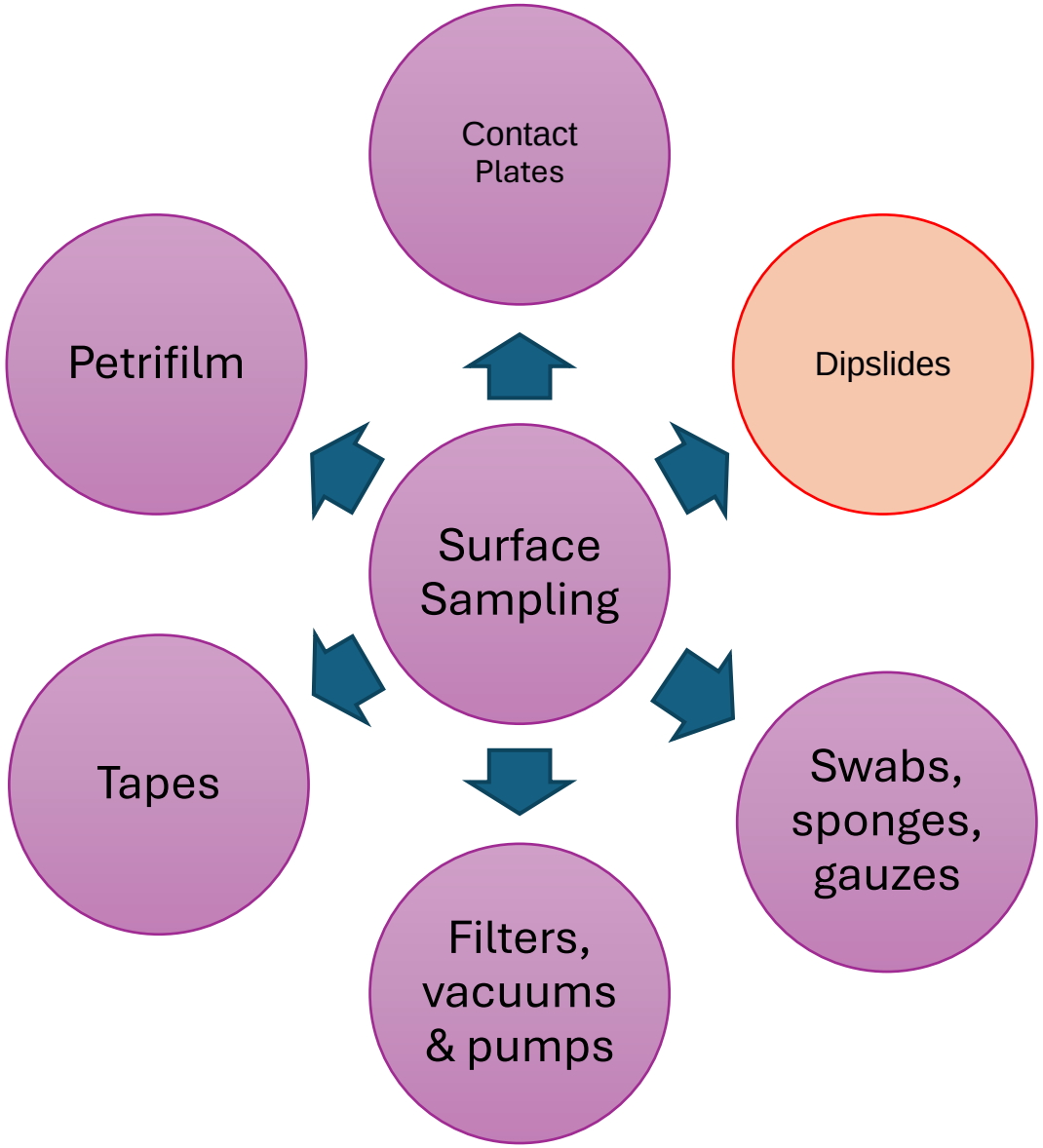

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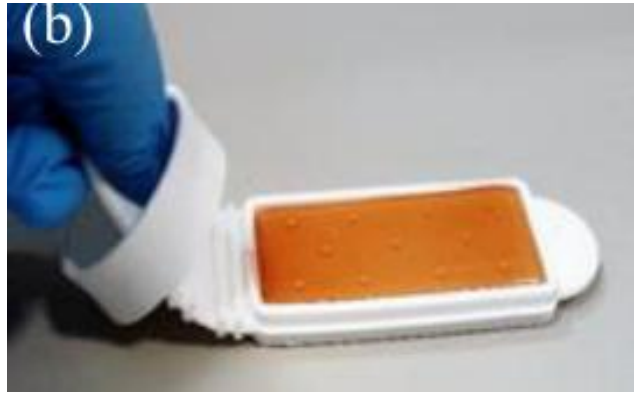
Dipslides



(Rawlinson et al., 2019)

They are easy to use

No additional transfer stage just sample and incubate

Can only get information on tow species with one dipslides

Surface has to be relatively flat but could manage with uneven surfaces

Semi quantitative – No need for sample template

More expensive than swabs

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Is there an association between airborne and surface microbes in the critical care environment?

J. Smith [a], C.E. Adams [b], M.F. King [c], C.J. Noakes [c], C. Robertson [d,e,f], S.J. Dancer [a,g,*]

Table I
Microbial soil categories for five hand-touch sites on intensive care unit

Site ($N = 100$)	No growth	Scanty growth (<2.5 cfu/cm^2)	Light growth ($\geq 2.5-12$ cfu/cm^2)	Moderate growth ($>12-40$ cfu/cm^2)	Heavy growth (>40 cfu/cm^2)	No. of hygiene fails (>2.5 cfu/cm^2)
Infusion pump	16	47 MSSA	22	13 MSSA	2	37/100 (37%)
Cardiac monitor	45	28	16 MSSA	9	2	27/100 (27%)
Right bedrail	6	38	17	27	12 MSSA	56/100 (56%)
Over-bed table	13	35	33 MSSA	16 MSSA	3	52/100 (52%)
Left bedrail	6	31	26	25 MSSA $\times$ 2	12 MSSA and MRSA	63/100 (63%)

MSSA, meticillin-susceptible *Staphylococcus aureus*, and MRSA, meticillin-resistant *S. aureus* isolated on ten occasions only.
Hygiene standard for surfaces: <2.5 cfu/cm^2 [7].
Average surface fail: 47% (range: 27–63%).

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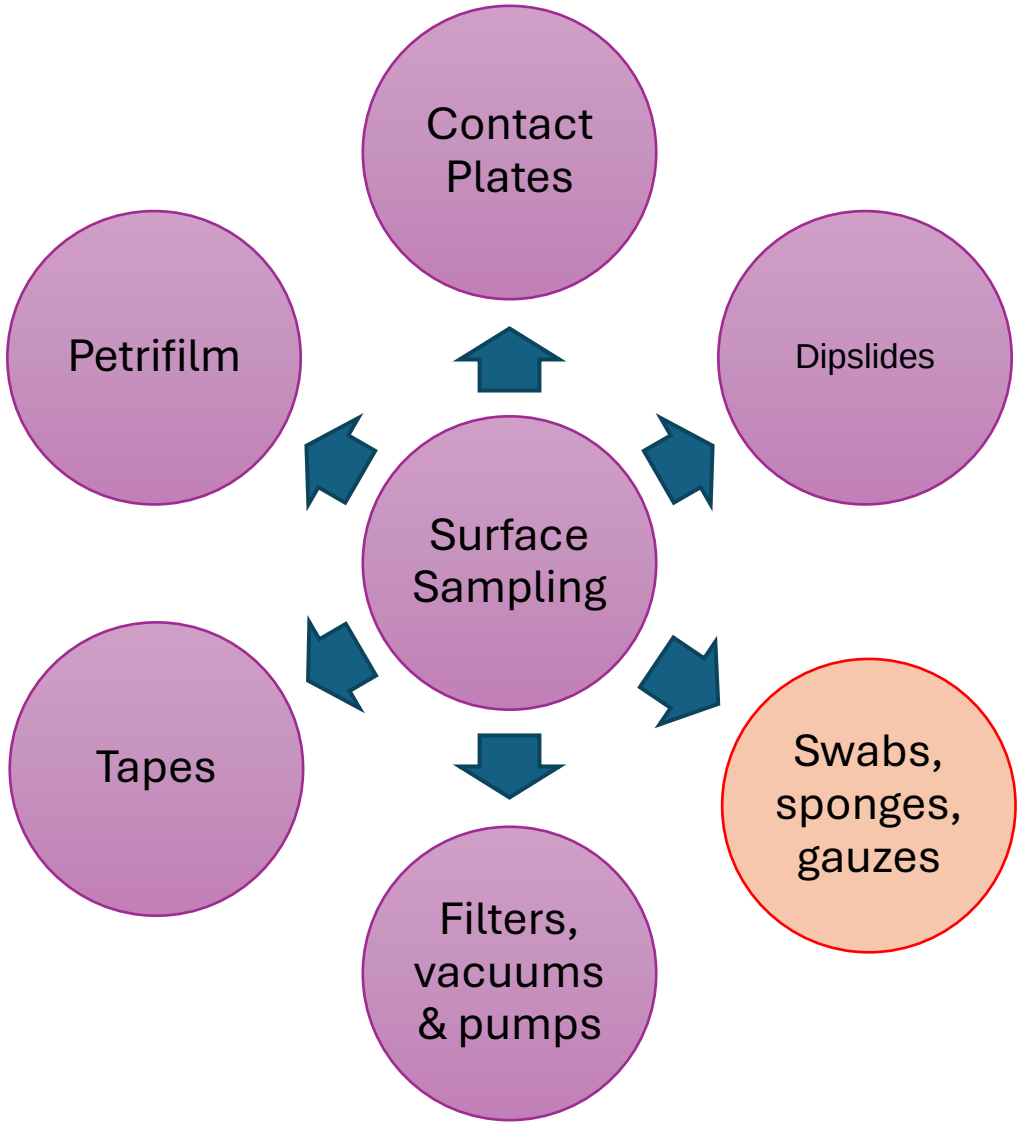

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Swabs, sponges & gauzes

- They are quick and easy to use
- Quantitative- if through the use of a sterile sample template
- Non destructive
- Relatively cheap
- Swabs can be used to sample a whole range of surfaces including non-flat surface

Sample needs further transfer and culture stage to determine level of contamination

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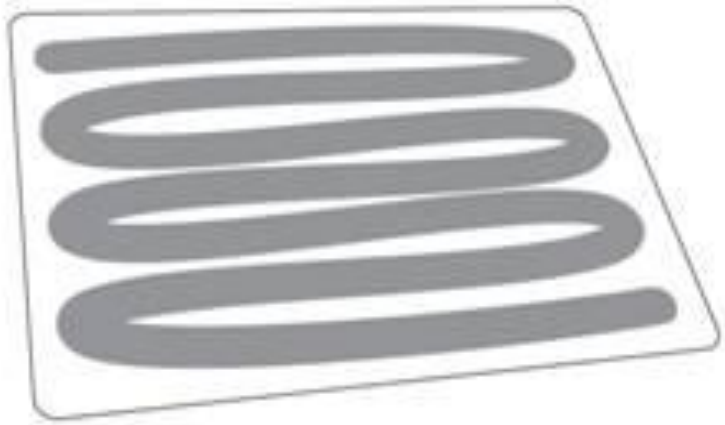
Use an overlapping ‘S’ pattern to cover the entire surface with horizontal strokes.



Rotate the swab and swab the same area again using vertical ‘S’-strokes

Rotate the swab once more and swab the same area using diagonal ‘S’-strokes

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Review

How to carry out microbiological sampling of healthcare environment surfaces? A review of current evidence

S. Rawlinson [a], L. Ciric [a], E. Cloutman-Green [a, b]

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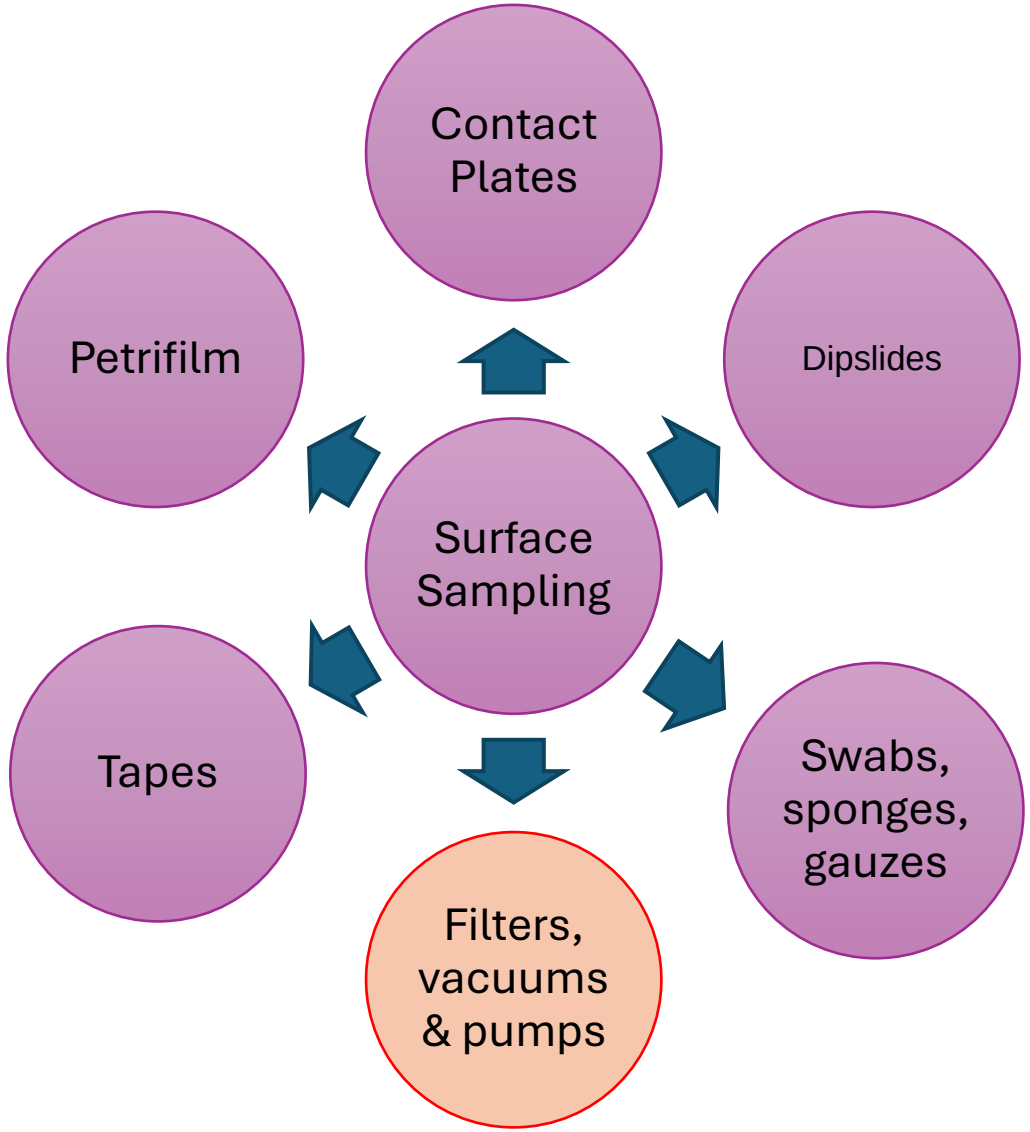

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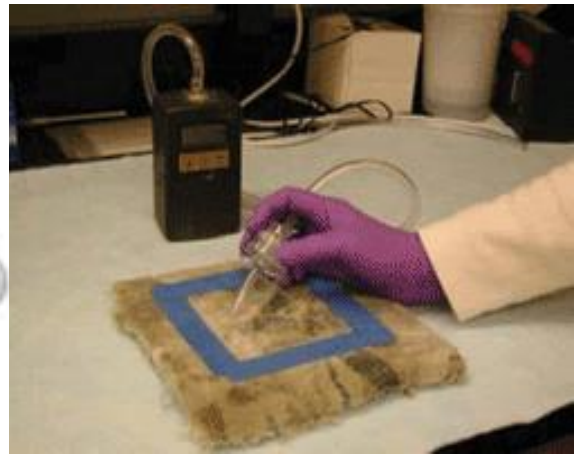
Filters, vacuums &Pumps



Creek et al. (2006), Journal of Environmental Monitoring, 8(6), 612-618.

Quick and relatively easy to use

Quantitative

Can be used on range of surface types including soft furnishings

More expensive than other options

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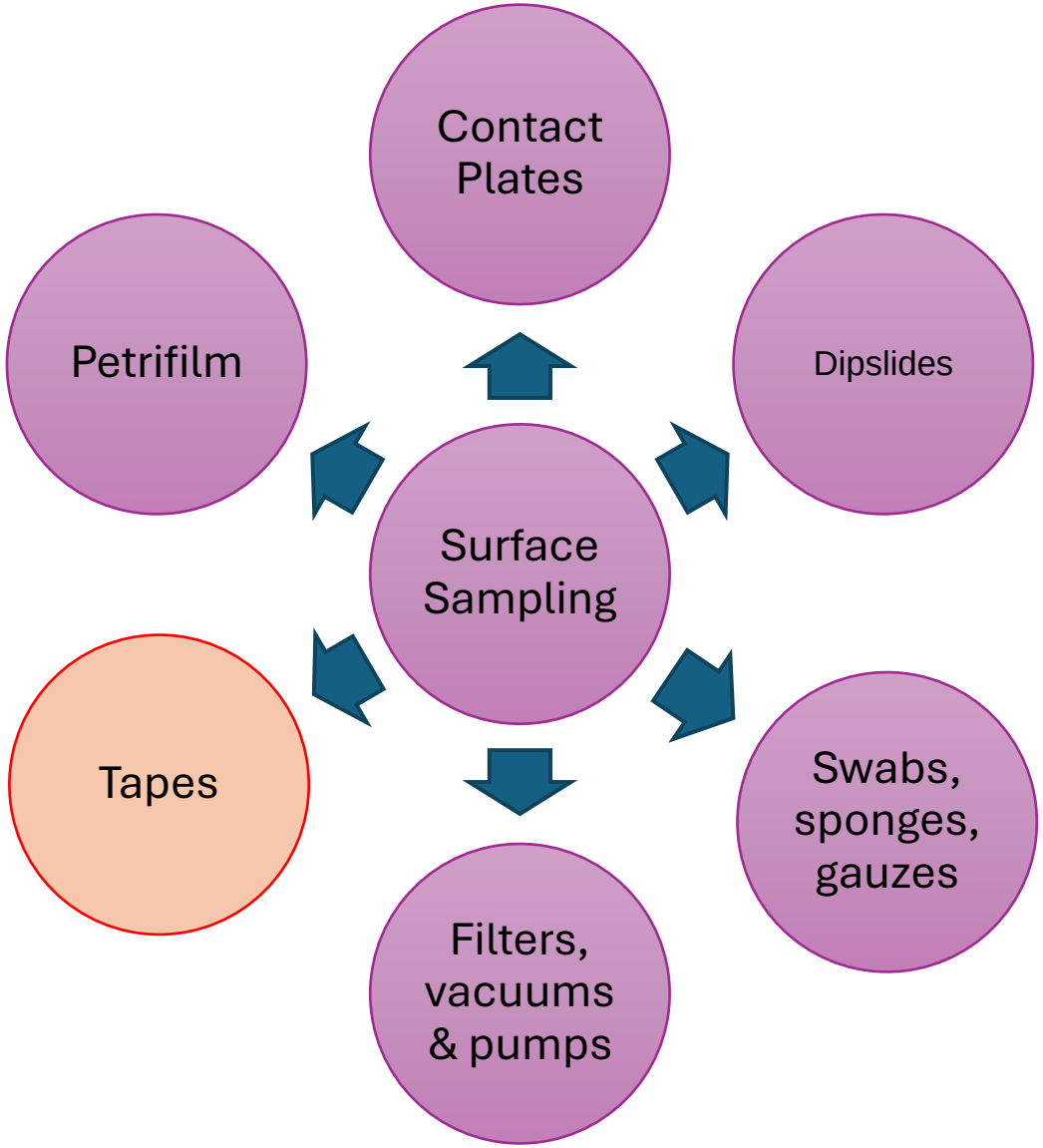

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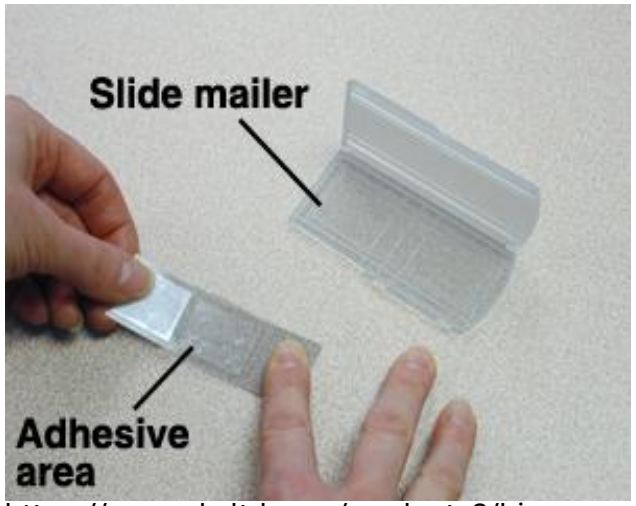
Tapes



https://www.skcltd.com/products2/bioaerosol-sampling/stick-to-it-lift-tape.html

- Easy to use
- Non destructive
- Little or no sample loss
- Quantitative
- Can be used to sample irregular surfaces
- Ensures consistent sample area for better data interpretation

- More expensive than swabs
- The type of analysis is limited to direct observation as it is extremely difficult to extract the samples for further analysis using culturing techniques

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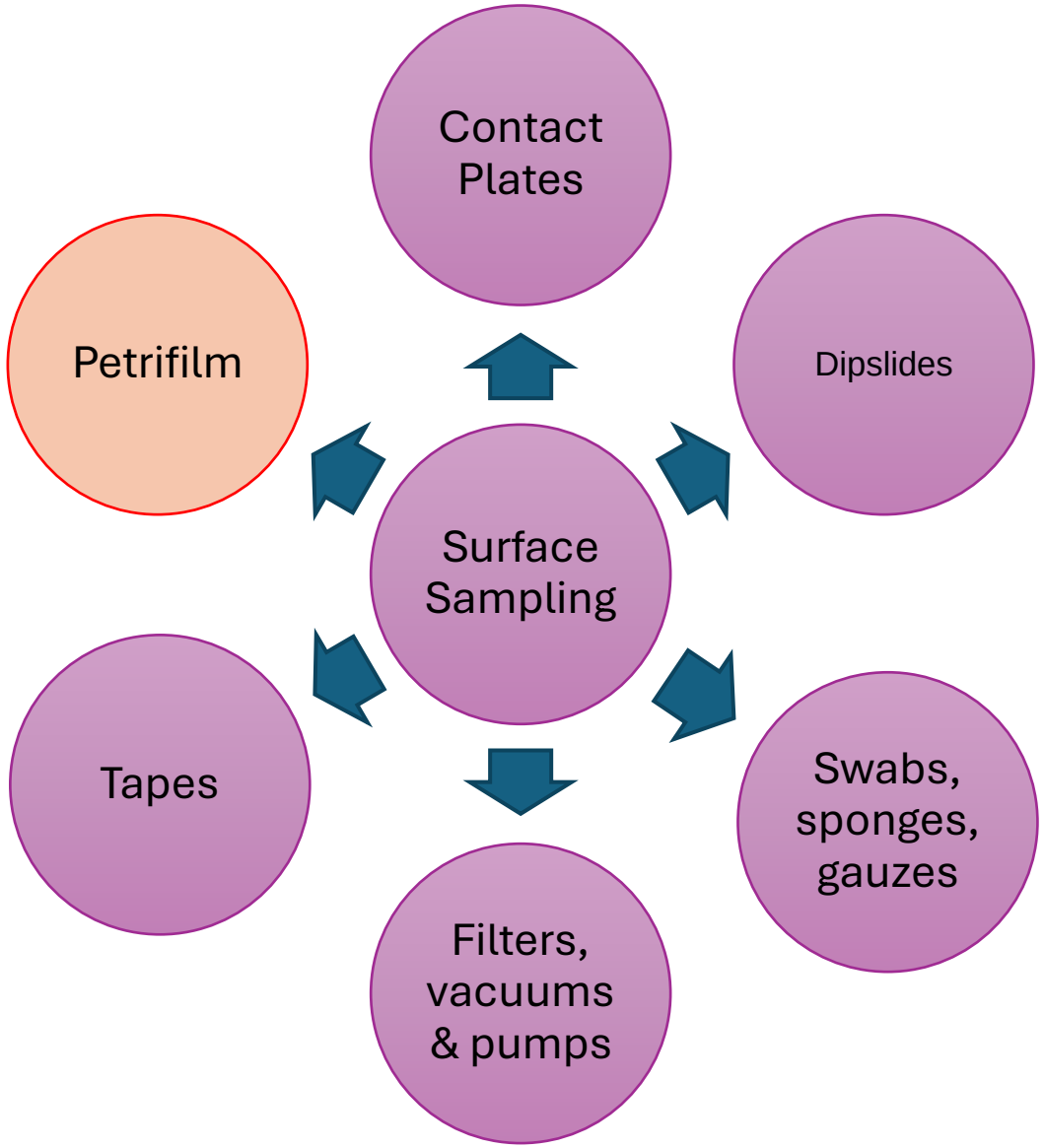

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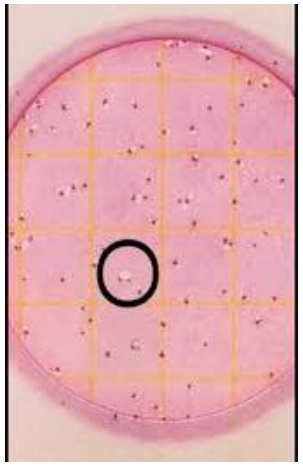
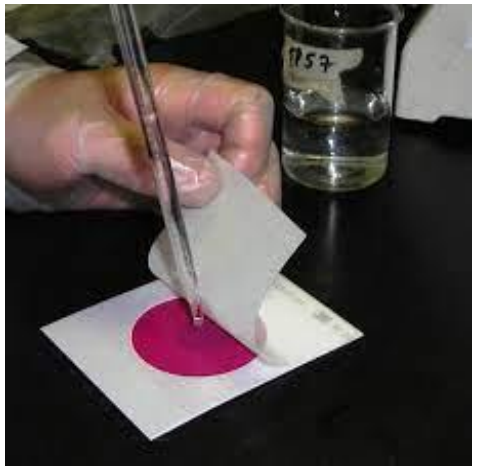
Petrifilm





https://en.wikipedia.org/wiki/Petrifilm

https://uk.vwr.com/store/product/33799761/rapid-yeast-and-mold-count-plates-petrifilmtm

They are easy to use

Quantitative – No need for sample template

No additional transfer stage just sample and incubate

Petrifilm can be used to sample a whole range of surfaces including non-flat surface

Can only get information on one species with one plate

More expensive than swabs

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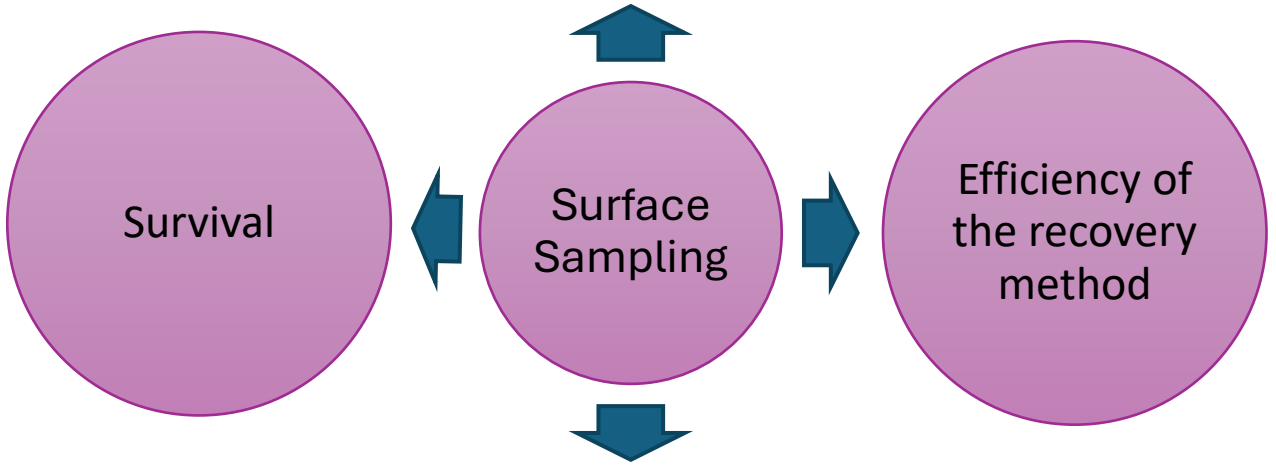
Ensuring survival of the microorganisms between sampling and recovery

Sampler removal efficiency

The ability of the sample method to absorb or capture the surface contaminants when moved across the surface



if you need to be specific about the concentration of microorganisms per unit area then it is necessary to use sterile sampling templates.

Qualitative vs Quantitative

It is unlikely that 100% of the microorganisms contained on the sampler will be recovered and this will be method specific

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Q&A

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