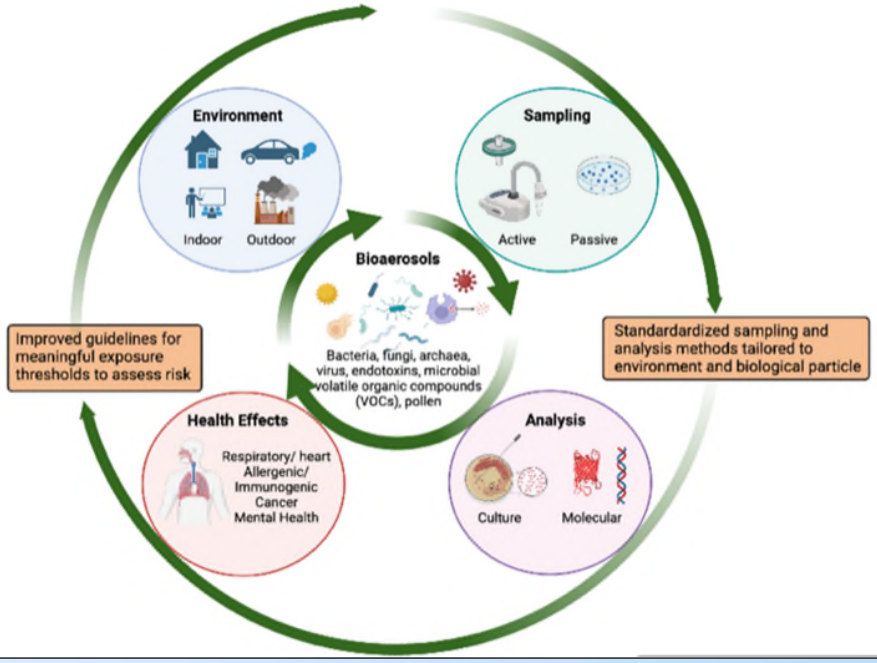
Summary: Compendium of analytical methods for sampling, characterisation and quantification of bioaerosols. Whitby C, Ferguson RMW, Colbeck I, Dumbrell AJ, Nasir ZA, Marczylo E, Kinnersley R, Douglas P, Drew G, Bhui K, Lemon M, Jackson S, Tyrrel S, Coulon F (2022). In D. A. Bohan, & A. Dumbrell (Eds.), *Functional Microbiomes* (pp. 101-229). Adv in Ecol Res; Vol. 67. 10.1016/bs.aecr.2022.09.004

What are bioaerosols and why are they important? Bioaerosols are suspensions of airborne particulate matter of biological origin (BioPM), which includes microorganisms (bacteria, fungi/mould, viruses) and their products (e.g. endotoxins, cell fragments, and microbial volatile organic compounds (MVOCs)). They are very diverse, found in both indoor and outdoor air, and are an important transmission route for infectious and allergenic agents[1]. Following inhalation, viruses, bacteria, and fungi can colonise the respiratory tract or other parts of the body, inducing infections and/or allergic disease[2,3]. However, exposure to a wide range of microbes, particularly during early life, also promotes normal development of the immune system. This complexity means we do not fully understand the negative and positive impact of bioaerosols on human health.

What are the challenges and needs? Existing standards and guidelines rely on short 'snap-shot' sampling, often using culture and microscopy for fungal and bacterial bioaerosols as they are simple and relatively low-cost. But there are several inherent limitations with culture-based methods, such as limited data, an underestimation or misrepresentation of the communities present making it hard to determine the health effects[4]. Molecular tools, (e.g. High Throughput Sequencing), have significantly advanced bioaerosol research, enabling more robust associations between bioaerosols and health to be made. Yet, these bioaerosol sampling and analysis methods are being used with no clear guidelines and recommendations.

> There is an urgent need for a suite of universally accepted protocols that are reliable, tailored to each environment and biological target to enable cross-comparisons to be made globally.

What is the solution? A set of guidelines applicable for different environments has been described[5], but more research is needed to properly assess exposure.



BioAirNet developed a compendium of current techniques, workflows, and technologies for bioaerosol sampling, characterisation, and monitoring for different environments to support a framework for developing meaningful standards for better bioaerosol monitoring.

Top 20 Recommendations:

1. **Bespoke standardised methods are needed for each environment and biological target.** (e.g. for viruses, particle size should be included, given the link to particle size and virus viability).
2. **Clearer definitions and terminology accessible across disciplines - a common language**.
3. Exposure to microbial products (e.g. endotoxins, mycotoxins, allergens etc) is an important consideration[3].
4. Combining culture and molecular methods provides a fuller image, but their respective biases must be noted.
5. Simultaneous measuring of particulates, chemicals (e.g. MVOCs) and bioaerosols from ***both indoor and outdoor air***[2,6,7] **is important to assess exposure levels**.
6. More sampling data is needed to validate dispersion modelling, including long-term monitoring with real-time analysis methods[8,9]**.**
7. Metadata on occupancy (including a diversity of individuals), activity airflow, environmental conditions and ventilation.
8. Bioaerosol data repository is needed.
9. Development and use of appropriate model systems is needed to better assess environmental and health risks.

For Sampling:

10. Multiple factors should be considered (e.g. sampler efficiency, sampling time (dependent on location and flow rates (impingement, or faster filters are recommended), sensitivity, recovery, biological target, environment (indoor or outdoor), sampling duration, sample preservation and storage, cost).
11. Sampling method should consider bioaerosol analysis method (e.g. for combined molecular and cultivation, samplers into liquid collection is recommended so samples can be split).
12. Air sampler should ideally be portable, sample large volumes, fractionate by particle size, efficient and allow for greater recovery of nucleic acids (if molecular methods are being used).
13. Where peak exposures are a concern, multiple samples should be collected.
14. Particle losses should be kept to a minimum and accounted for during data analysis.
15. Sampling the inhalable fraction (and over longer time-scales) is important so a combination of personal and static sampling is recommended (e.g. polycarbonate filters with an IOM Multidust sampler head to select for health-relevant fractions).

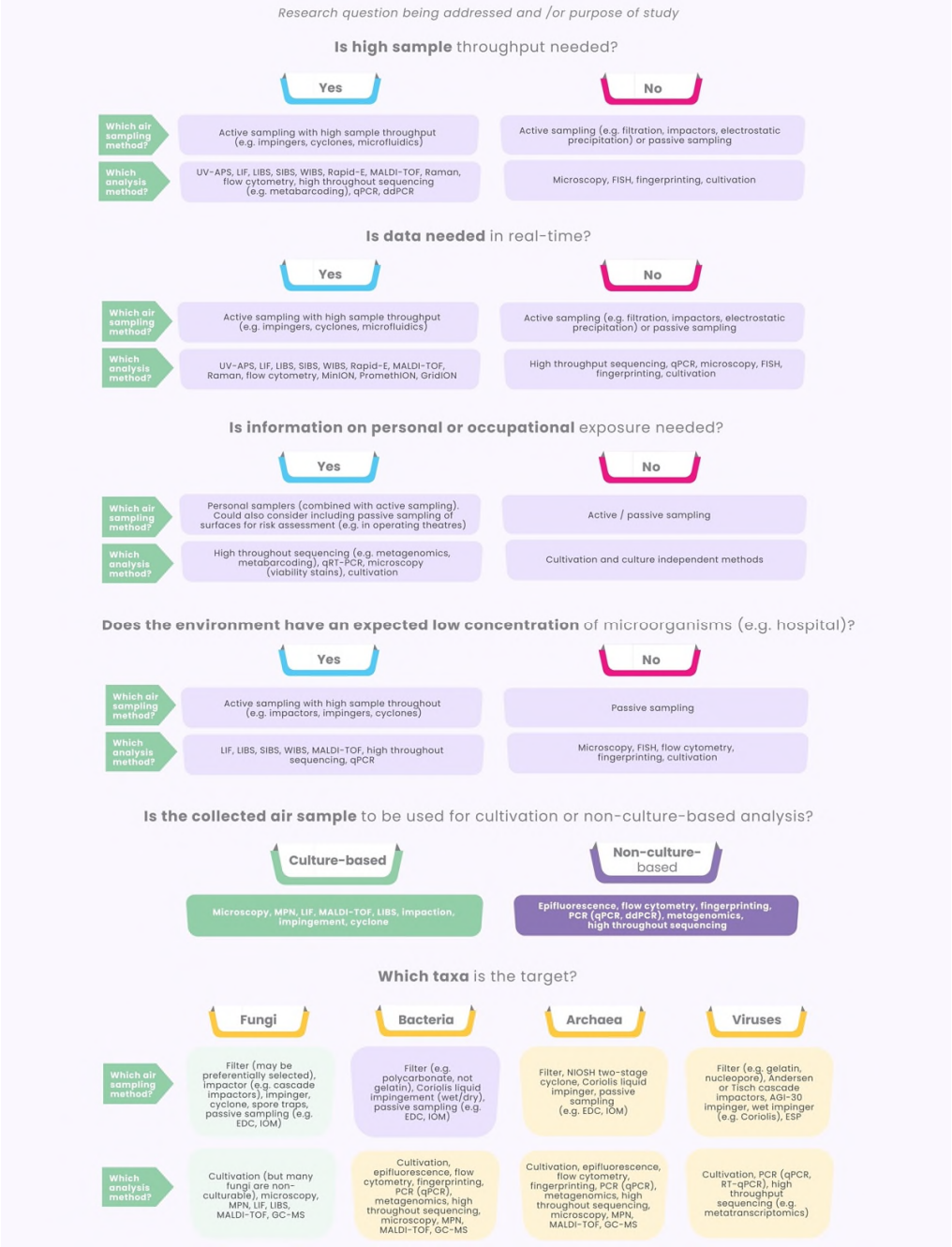
For culture-based analyses:

16. Agar volume should be standardized and reported.
17. Avoid gelatine filters for examining exposure in high humidity environments.

For molecular analyses:

18. Air filtration using polycarbonate filters is recommended, as it gives the highest DNA recovery, should be used for viruses or where metagenome analysis e.g. antimicrobial resistance (AMR) is required. However, impingement is better for shorter sampling periods using centrifugation to concentrate cells from the liquid matrix[5].
19. Current sequence databases including reference databases need improvement (especially with regard to viruses) plus best practice for sequence data analysis and storage.
20. Combining metagenome or metabarcoding (amplicon sequencing) and metatranscriptomics will provide information of function in relation to taxonomy. High-throughput sequencing is recommended for epidemiological studies to identify long-term associations between the microbiome, specific environment, biomarkers, and the health effects.

Decision Framework:



References 1. Douglas P et al (2018). *Int J Hygiene and Environ Health* 221(2):134–173. **2.** Ferguson RMW et al (2021). *Environ Int* 147:106327. **3**. Goode EJ, Marczylo E. (2023) Clin Transl Allergy, 13(6):e12252. **4.** Colbeck I, Whitby C (2019). Issues in Environmental Science and Technology No. 48 Indoor Air Pollution, Edi R.M. Harrison and R.E. Hester. Published by the Royal Society of Chemistry. **5.** Ferguson RMW et al (2019). *Mol Ecol Res* 19:672-690. **6.** Grydaki N et al (2021). *Environ Int*, 146:106186. **7.** Pankhurst LJ et al (2012). *FEMS Microbiol Ecol*, 79(1), 229–239. **8.** Nasir ZA et al (2018). *Atmosphere* 9(10):379. **9.** Nasir ZA et al (2019) *Sci Total Environ* 648:25–32. **Acknowledgements:** BioAirNet (NE/V002171/1), RAMBIE (NE/M010813/1) and BioSkyNet (NE/V008293/1).