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Selecting methods for airborne microbial detection: from isolated signals to interpretable evidence

Abstract

Airborne microorganisms occur within a complex particulate matrix and are commonly present at low and variable concentrations. Consequently, no analytical technique can independently resolve abundance, identity, viability, and biological relevance. This mini review discusses method selection as a sequence of linked decisions: defining the study question, preserving the target during sampling, matching detection to the desired evidence, and interpreting results within the limits of the analytical workflow. Culture, microscopy, nucleic-acid-based methods and biosensors provide different types of information and should therefore be considered complementary rather than interchangeable. A practical strategy is to combine a quantitative or rapid screening tool with a confirmatory method and suitable controls. This focused approach can improve comparability and reduce overinterpretation without requiring an unnecessarily complex analytical platform.

Keywords: airborne microorganisms; bioaerosols; particulate matter; method selection; viability; molecular detection.

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Introduction

The atmosphere is a dynamic microbial habitat rather than a passive transport route. Bacteria, fungi, viruses, and other biological particles are emitted from soil, vegetation, water, waste, animals, and human activities, and their abundance changes with location, season, and meteorological conditions.[1–4] These particles may influence ecosystem connectivity, atmospheric processes and exposure to biological agents.[5] However, the biological fraction of particulate matter cannot be described adequately by a single measurement because the term 'detection' may refer to several distinct outcomes: observing a particle, recovering a viable organism, detecting a molecular marker, identifying a taxon or estimating a functional risk.[6,7]

This distinction is central to study design. Results are shaped not only by the analytical method, but also by the sampler, collection substrate, air volume, storage conditions, extraction efficiency, and controls.[8] A highly sensitive assay cannot compensate for loss or damage during collection, and a broad taxonomic profile does not necessarily demonstrate viability or infectivity.[12,13] This mini review therefore presents a concise framework for selecting and combining methods for airborne microbial studies. It intentionally emphasizes decision criteria and interpretation rather than providing an exhaustive catalog of instruments, protocols, or performance values.

Methodology

A focused narrative synthesis was prepared from 23 representative studies on atmospheric microbial ecology, bioaerosol sampling, culture-based analysis, molecular detection, and biosensors. Targeted searches combined terms related to the matrix ("bioaerosol," "airborne microorganism," "airborne bacteria," "airborne fungi," "airborne pathogen" and "biological particulate matter") with terms representing the principal decision domains: sampling and recovery ("air sampling," "impaction," "filtration," "impingement," "passive sampling" and "collection efficiency"); analytical endpoints ("culture," "microscopy," "PCR," "qPCR," "sequencing," "metagenomics," "transcriptomics" and "biosensor"); and interpretation ("quantification," "identification," "viability," "infectivity," "quality control," "exposure" and "risk"). Detailed comparisons of devices, operating conditions, assay protocols and emerging platforms were outside the scope of this brief review.

Begin with the evidence required

Method selection should begin with the claim that the study is intended to support. Culture-based approaches are useful when recovery of viable and cultivable organisms is required, but they represent only the fraction able to grow under the chosen conditions. Microscopy provides information on morphology and particle association, although taxonomic resolution is often limited. PCR and quantitative PCR offer sensitive, targeted detection, while sequencing expands taxonomic coverage but detects genetic material regardless of whether the source organism remains viable.[10,14–16] Rapid optical, electrochemical, or bioluminescent systems can support screening and near-real-time monitoring, although specificity and matrix effects require careful validation (Table 1).[17–19]

Table 1 Evidence provided by major analytical approaches

Approach	Most defensible evidence	Main interpretive limit
Culture	Recovery of cultivable, viable microorganisms	Excludes non-cultivable or stressed cells
Microscopy	Particle morphology, counts and associations	Limited taxonomic specificity
Targeted PCR/qPCR	Presence or abundance of selected genetic markers	DNA/RNA signal is not equivalent to viability
Sequencing	Community composition and relative diversity	Sensitive to extraction, contamination, and database bias
Biosensors/ rapid assays	Fast response to a defined biological signal	Performance may change in complex air-sample matrices

Sampling and analysis form a single workflow

Sampling cannot be treated as a neutral step. Passive sedimentation favors particles with sufficient settling velocity and is useful for simple surveillance, whereas active impaction, filtration and liquid

collection provide greater control over sampled air volume and particle capture.[8,9,20] The selected mechanism can alter cell integrity, culturability, nucleic-acid recovery, and size representation. Filters may suit particulate matter studies and molecular analysis, while impaction onto culture media facilitates direct recovery of cultivable microorganisms. Liquid collectors can simplify downstream extraction but may introduce dilution. The same environment may therefore yield different microbial profiles when sampled using different principles.[11]

A coherent workflow links the sampler to the downstream endpoint. If viability is central, collection stress, transport time and culture conditions must be controlled. If sequencing is planned, field blanks, extraction blanks and low-biomass contamination controls become indispensable. When particle-size distribution is relevant, the sampler must preserve aerodynamic information and the analysis should report the size fraction explicitly.[10,21] In every case, metadata on air volume, duration, meteorology, site characteristics and sample handling are part of the result rather than optional background information.

Complementary methods reduce overinterpretation

The strongest designs do not necessarily use the largest number of techniques. They use two or more methods that answer different parts of the same question. A culture result can be paired with a targeted molecular assay to distinguish recoverable organisms from a broader genetic signal. Microscopy can verify the presence and physical context of biological particles, while sequencing can describe community structure. A rapid sensor can identify temporal changes, which can then be confirmed using a more specific laboratory method (Table 2).[17–19]

Table 2 Examples of focused method combinations

Study objective	Primary method	Complementary confirmation
Routine surveillance	Rapid optical or biochemical screening	Targeted qPCR or culture of selected organisms
Viable microbial burden	Culture-based enumeration	Molecular identification of representative isolates
Community characterization	Amplicon sequencing or metagenomics	Microscopy and targeted quantification
Size-resolved biological PM	Size-selective active sampling	Molecular assay plus particle or ATP measurement

Interpretation should remain proportional to the evidence. Detection of a pathogenic taxon or marker does not by itself establish infectivity, exposure dose, or health risk. Relative sequence abundance is not an absolute concentration, and colony counts cannot be extrapolated to the full airborne community. Clear wording that distinguishes detection, viability, culturability and risk improves scientific accuracy and makes results easier to compare across studies.

Quality controls and minimum reporting

Air samples frequently contain low microbial biomass, making them particularly vulnerable to background contamination and stochastic variation. Select field blanks, transport blanks, extraction blanks, positive controls, and replicate samples according to the workflow. Recovery tests or internal standards can help identify losses and inhibition. For molecular studies, reporting the extraction procedure, target region, primer or probe information, sequencing and bioinformatic criteria is essential. For culture-based work, the medium, incubation conditions, and calculation of air concentrations should be specified.[8,10,16]

At minimum, reports should identify the sampling principle and model, flow rate, sampled air volume, duration, collection substrate, particle-size range, storage conditions, time to analysis, analytical target, units and controls. These elements are more useful for reproducibility than broad statements that a ‘standard method’ was used. Harmonized reporting is also necessary before data from different locations or seasons can be integrated meaningfully.[2,3,22]

Future direction: fit-for-purpose monitoring

Technological development is moving toward portable, automated, and real-time detection. These advances may improve temporal resolution and support early warning, but deployment should be guided by the intended decision rather than novelty alone.[17–19] A practical monitoring system may use a robust screening method continuously and reserve high-resolution molecular analysis for selected events or samples. This tiered design can balance cost, speed and interpretability.

Future progress will also depend on reference materials, interlaboratory comparisons, standardized metadata and validation in real atmospheric matrices. Particular attention is needed for viability assessment, low-biomass contamination and the relationship between measured signals and actual exposure.[4,10,23] These priorities can be addressed without assuming that one platform will replace all others.

Conclusion

Airborne microbial detection is most reliable when the research question, sampling strategy, analytical method, quality control, and interpretation are designed as parts of a connected workflow. Each decision influences the type and quality of the evidence obtained. The sampling method determines which particles and microorganisms are collected and how well their integrity is preserved, while the analytical approach defines whether the result supports detection, quantification, identification, viability, or another specific endpoint.

No single method can simultaneously establish abundance, identity, viability, infectivity, and health risk. Culture-based methods provide evidence of cultivability under defined conditions, whereas molecular methods can detect and identify microbial markers with greater sensitivity but do not necessarily demonstrate that the detected microorganisms remain viable or infectious. Similarly, rapid assays and biosensors may improve temporal resolution and support routine screening, but their results require validation and, when necessary, confirmation using more specific analytical methods.

Therefore, complementary methods should be selected according to the study objective and the level of evidence required. Their interpretation must consider sampling efficiency, recovery losses, analytical sensitivity, contamination, inhibition, and the controls applied throughout the workflow. Detection of a microorganism, genetic marker, or biological signal should not be interpreted automatically as evidence of viability, infectivity, exposure dose, or health risk. The framework presented in this mini review provides a concise guide for aligning methodological decisions with defensible conclusions. It does not replace standardized protocols or method-specific validation, but it may improve reporting, facilitate comparison among studies, and reduce conclusions that extend beyond the evidence generated.

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Conflicts of interest

The authors declare no conflict of interest in writing the manuscript.

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