PathCon® Laboratories

TECHNICAL BULLETIN 2.5

A Suggested Air Sampling Strategy for Microorganisms In Office Settings

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This Technical Bulletin 2.5 is intended to be a guide for investigating building-related complaints that might be caused by viable airborne organisms or their effluents. There are no governmental or industrial guidelines as to acceptable levels of microorganisms in indoor air.

PathCon has developed this Bulletin to describe our specific methods for collecting air samples for fungi and bacteria. These air sampling protocols were designed to yield microbiological data in order to assist the on-site investigator in making valid interpretations about the amplification of bacteria and fungi associated with indoor environmental sources. This Technical Bulletin includes two protocols: the seven-plate protocol and the four-plate protocol.

The samples should be collected by a trained environmental health professional with experience in sampling for microorganisms; therefore, this manual is not a complete sampling guide. PathCon Laboratories does not assume responsibility for selection of the appropriate sampling protocol or interpretation of the findings. These decisions must be made by the on-site investigator.

SEVEN-PLATE PROTOCOL SAMPLE COLLECTION

This protocol utilizes seven plates of microbiological media. The sampling is done with an impaction-type sampler. The samplers most often used by PathCon clients are the Andersen N6 sampler and the SAS (Surface Air System) sampler. They should be calibrated before and after each job. For the interpretation of microbiological findings, air samples must be taken at an outdoor site (preferably at the outdoor air intake of the building) and at appropriate indoor sites. The indoor sites should include those areas associated with occupant complaints and, if possible, at least one area not associated with occupant complaints. It is strongly recommended that samples at each air site be taken at multiple times during the day (i.e., morning and afternoon or at intervals at least 2 hours apart). The set of seven plates must be exposed at each sampling site as follows:

- **Two plates of Rose Bengal Agar (RBA).** The RBA plates are for isolating fungi and are designed to suppress bacterial growth. An alternative medium is Malt Extract Agar (MEA). The plates are incubated in the laboratory at room temperature (RT, 23±3°C).

- **Two plates of R2A agar with cycloheximide (R2Ac).** The R2Ac is a low nutrient medium for detection of environmental-type bacteria. The plates contain cycloheximide to inhibit fungal growth which, if present, may suppress bacterial growth. Plates are incubated in the laboratory at RT.

- **Two plates of Heart Infusion Blood Agar (BA).** The BA plates contain 5% sheep blood to enhance growth of human commensal bacteria. An alternative medium is Tryptic Soy Agar (TSA). Plates are incubated in the laboratory at 35 C.

- **One plate of Tryptic Soy Agar (TSA).** The TSA plate is a high nutrient medium incubated at 56 C. At this incubation temperature, all bacteria except thermophiles are inhibited.
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The plate numbers, types of media, sampling time (length of time plates are exposed in the sampler), laboratory incubation temperatures, and microbial groups to be charaterized on the plates are described in Table 1. The sampling times are suggested for the Andersen sampler with an air flow of 28.3 liters (1 cubic foot) per minute. Sampling times for samplers with other air flow volumes must be adjusted accordingly.

Instructions for sample collection and forms for sample identification will be provided by the laboratory. For the quantification of bacteria and fungi in air, it is required that the sampler be calibrated to provide an accurate measurement of flow rate. The paired plates 1 and 4, plates 2 and 5, and plates 3 and 6 are each evaluated as sets to determine the number of organisms per cubic meter of air and for selecting predominant microbial taxa for identification.

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Table 1: ***Seven-plate Protocol -*** *Suggested sampling times for Andersen Sampler (28.3 liters per minute)*

Plate	Medium	Sampling Time	Incubation	Microbial Group Enhanced
1	RBA	60 sec	RT	Fungi
2	R2Ac	60 sec	RT	Environmental bacteria
3	BA	60 sec	35°C	Human commensal bacteria
4	RBA	120 sec	RT	Fungi
5	R2Ac	120 sec	RT	Environmental bacteria
6	BA	120 sec	35°C	Human commensal bacteria
7	TSA	180 sec	56°C	Thermophilic bacteria

NOTE: ***Sampling times for samplers with other air flow rates must be adjusted accordingly.***

Four-Plate Protocol Sample Collection

The four-plate protocol is designed for those investigators who want to analyze for fungi and environmental bacteria only. It utilizes two types of microbiological media. The two media are Rose Bengal Agar (or Malt Extract Agar) for the fungal analysis and R2Ac Agar for the environmental bacterial analysis. The total sample exposure time is 3 minutes for each type of media divided among the plates as shown in Table 2.

The four-plate protocol may be used in place of the seven-plate protocol when data on human commensal and thermophilic bacteria is not required. PathCon technical staff members are available for phone consultation with clients. We offer assistance to the on-site investigator in developing an appropriate air sampling strategy suited to each specific situation. However, the final decision about sampling strategy must be made by the on-site investigator.

Table 2: ***Four-plate Protocol*** *- Suggested sampling times for Andersen Sampler (28.3 liters per minute)*

Plate	Medium	Sampling Time	Incubation	Microbial Group Enhanced
1	RBA	60 sec	RT	Fungi
2	R2Ac	60 sec	RT	Environmental bacteria
3	RBA	120 sec	RT	Fungi
4	R2Ac	120 sec	RT	Environmental bacteria

NOTE: ***Sampling times for samplers with other air flow rates must be adjusted accordingly.***

ANALYZING DATA

When the seven-plate protocol is implemented, results are obtained as described below. The four-plate protocol yields data on fungi and environmental bacteria only.

• **Fungi.** The number of fungi per cubic meter of air is determined. All types of fungi present are identified to the genus level. Fungi of the genera *Aspergillus* and *Stachybotrys* are speciated.

• **Bacteria cultured on R2Ac agar incubated at room temperature.** R2Ac agar is designed to culture environmental bacteria; however, human commensal bacteria may also grow on it. The number of colony-forming units per cubic meter of air is determined. The three most predominant genera of bacteria are identified to aid in determining the probable source of possible bacterial amplification. Some organisms may be identified to the species level.

• **Bacteria cultured on blood agar incubated at 35°C.** Blood agar is designed to culture human-associated bacteria, but environmental bacteria may also grow on it. The number of colony-forming units per cubic meter of air is determined. The three most predominant genera in their group are identified to aid in determining the probable source of possible bacterial amplification. Some organisms may be identified to the species level

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• **Thermophilic Bacteria.** The number of thermophilic bacteria (56° C incubation) and the three most predominant genera in this group are determined.

INTERPRETATION

By comparing the microbiological profiles at the sites associated with health complaints to those at outdoor air sites and those at indoor sites where no health complaints have been reported, it may be possible to determine if amplification of microorganisms is occurring at the indoor air sites in buildings.

Fungal amplification should be considered when the indoor concentration is above 200 colony-forming units per cubic meter of air and substantially exceeds that detected in the outdoor air. Selective fungal amplification may exist when specific fungal taxa that are absent or in low numbers in the outdoor air are found in substantially higher numbers in the indoor air.

Bacterial amplification should be considered only for environmental-type bacteria and not human commensal bacteria. Amplification should be considered only when counts are above 200 and substantially exceed other sites within the building and/or that found in the outdoor air. An increase in the concentration of human shed bacteria is expected over the work day. Increased numbers of human commensal bacteria at indoor sites may indicate high occupant density and/or poor ventilation. Areas with high concentrations of human commensal bacteria may suggest an environment where airborne-transmitted pathogens may be more easily spread from person-to-person.

Samples are screened for potential pathogens such as *Aspergillus fumigatus, Pseudomonas aeruginosa, Staphylococcus aureus*, etc. The analytical results from water and other bulk samples collected from building systems have much greater value if they can be related to amplification of microorganisms in air.

ANALYTICAL LIMITATIONS

There are no governmental or industrial regulations concerning permissible levels of fungi and bacteria in indoor air. The protocols described here are designed to detect abnormal microbiological amplification. The purpose is not to suggest associations with health complaints in building occupants nor to suggest any remediation procedures.

The protocols are suggested to assist the on-site investigator in developing a strategy for overall assessment. Decisions regarding the selection of protocol and overall interpretation of findings must be made by the on-site investigator.

In determining the existence and source of microbial amplification in a building, visible evidence of microbial contamination must weigh heavily on the decision. Air sampling represents a "snapshot in time". In order to make a good interpretation of microbiological air sampling data, it is strongly recommended that air sampling be done at multiple times. If the multiple samplings must be done in a single day, we suggest that separate samples be taken in the morning and afternoon at intervals at least 2 hours apart.

A negative result does not indicate that the source of the sample was free of fungi or bacteria. A negative result indicates only that if present in the sample, the number of fungi or bacteria was below the detection limits of the test. These protocols are unlikely to detect environmental mycobacteria and *Legionella* bacteria. These bacteria require special analyses.