

THE SAMPLING AND ANALYSIS OF AIRBORNE FUNGAL SPORES USING THE BI-AIR FILTER CASSETTE

USE OF THE BI-AIR CASSETTE

The Bi-Air filter cassette may be used for the collection of both fungal spores and culturable fungi, in both the air and in wall cavities.

The use of the Bi-Air should be considered when a more professional level of sampling is required by your project. The benefits include:

- Long-term airborne samples (30 minutes to 7 hours) provide a more reliable estimate of spore concentrations;

Most spore samplers will only collect samples for periods of 10 minutes or less. However, longer sample times, generally referred to as time-weighted average (TWA) samples, provide a more reliable estimate of the average spore concentrations compared to short-term “grab” samples. Not every project has these requirements, but those that do will benefit from use of the Bi-Air cassette. However, the Bi-air should be considered when collecting data for legal purposes; or when sampling in sensitive environments such as hospitals.

- High airborne spore concentrations may be encountered;

The collection efficiency for a filter cassette is independent of the spore concentration, so they can be used in almost any environment. In addition, culturable concentrations of 100,000 cfu/m³ can be detected.

- Only those samples containing significant spore concentrations need to be cultured;

The Bi-Air collects two duplicate samples on one filter. This allows one sample trace to be analyzed for total spores. Only those samples with significant spore concentrations need to be cultured. This provides identification to species, and at the same time saves your client money by only culturing those samples with high spore counts.

- When information on both fungal spores and culturable fungi are desired;

Wall cavity samples can often benefit by reporting the concentrations of both total spores and culturable fungi. This information sometimes provides a partial estimate of the age of the incident that caused the mold growth.

ANALYSIS OF THE BI-AIR CASSETTE

Analysis requires the following equipment:

- Acetone vaporizer;
- Reagent grade acetone;
- Fungal stain or other wetting solution;
- Syringe, tweezers;
- Scalpel or sharp knife (to cut shrink band on cassette);
- ½-inch X-ACTO knife or equivalent (to cut the filter in half);
- Glass slides and cover slips
- Minimum 600X magnification.

Step 01. Acetone Vaporizer

- Turn on the Acetone Vaporizer;
- Fill the syringe with acetone and insert it into the Vaporizer

Step 02. Opening the cassette:

- Orient the cassette with the broad inlet cap down;
- Cut the shrink band with the scalpel;
- Use the thin, flat handle of the tweezers to pry open the cassette;
- Remove the back portion of the cassette, leaving the filter pad lying in the front portion;
- Invert the front portion of the cassette onto a clean surface, tapping it gently against the surface to dislodge the pad and filter (sample side up, with filter resting on the pad);
- Using the ½-inch knife blade, cut the filter in half between the two sample traces.

The second sample trace may be discarded, archived, or submitted for analysis. To preserve the sample, follow these steps:

- Place the filter-half sample-side down on a glass slide;
- Cover it with the filter pad, marking the back of the pad with sample information;
- Secure the pad to the glass slide with clear tape, contacting only the pad.

The sample area on the filter is recessed, so placing the filter sample-side down does not bring the sample into contact with the glass slide. Two to three samples can be archived on one side of a glass slide using this method.

Step 03. Clearing and collapsing the filter:

- Transfer one sample trace to a glass slide (trimming the excess filter surrounding the sample trace, if desired);
- Insert the sample filter (on the glass slide) into the Acetone Vaporizer, with the back of the sample under the dispensing tube in the Vaporizer;
- Dispense about 0.2 ml of acetone, gently drawing the sample towards you so the acetone wets and clears the entire area of filter;
- Add a drop of stain or wetting agent, and cover with a glass cover slip
- Analyze the sample at a minimum magnification of 600X.

The spores are not easily dislodged from the filter, so any air bubbles can be removed by inclining the slide (rest one end on a pen) and tapping on the cover slip. This can be repeated until all the bubbles are gone. However, with practice, it's possible to avoid the creation of bubbles when adding the stain.

A minimum magnification of 600X is recommended because it is difficult to distinguish between *Aspergillus/Penicillium* type spores and *Cladosporium sphearospermum* type spores at lower magnifications.

It is assumed that the diameter of a 600X objective is about 0.28 mm. The size of the sample trace is very precisely controlled to 1.7 mm x 5.5 mm, with very sharp boundaries. The size of each sample trace is therefore about 6 x 20 fields of view (FOV) at 600X magnification.

Step 04. Microscopic analysis

- Start at one corner, with the edge of the FOV just inside the edge of the sample trace;
- Move across the short dimension (from top to bottom, for example);
- Move the objective to the next adjacent traverse, not skipping any of the sample trace;
- Continue the analysis until a minimum of 10 traverses have been analyzed, or until counting rules have been satisfied.

It is recommended that 10 adjacent traverses, starting at one end of the sample, be analyzed instead of randomly picking traverses for analysis. There is sometimes an end-effect, not only with the Bi-Air cassette, but with adhesive-strip impaction samplers as well. This sometimes causes the spore concentrations near the ends of the sample trace to be higher than in the middle. The recommended method of analysis provides an improved estimate of the spore concentration in those cases.

The spore counts will be analyzed by AEMTEK using a specially prepared Excel spreadsheet.

PHOTOS SHOWING THE ANALYSIS OF THE BI-AIR CASSETTE



Figure 1. Calibration of the Bi-Air cassette.



Figure 2. The MCE filter, with dual sample traces.

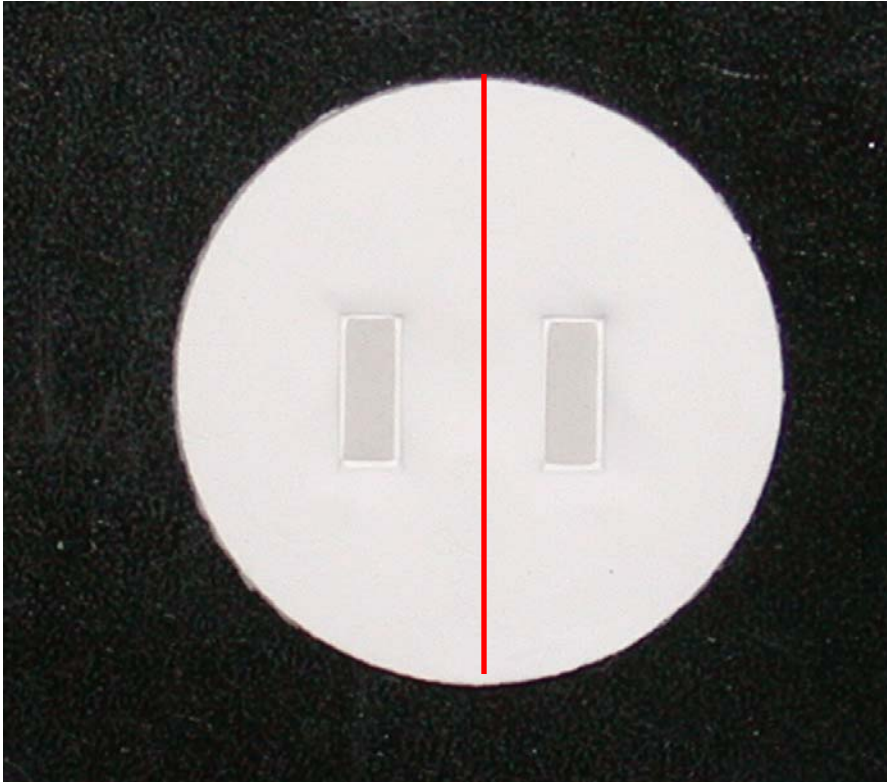


Figure 3. Filter with dual sample traces shown (actual appearance, sample traces are visible). Red line indicates where the filter is cut in half to separate the two sample traces.

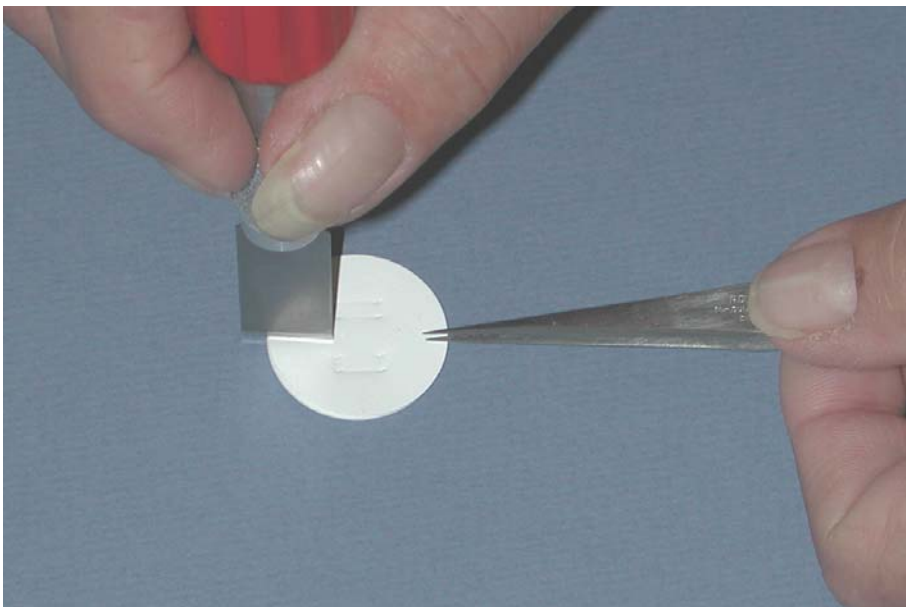


Figure 4. Cutting the filter between the two sample traces.

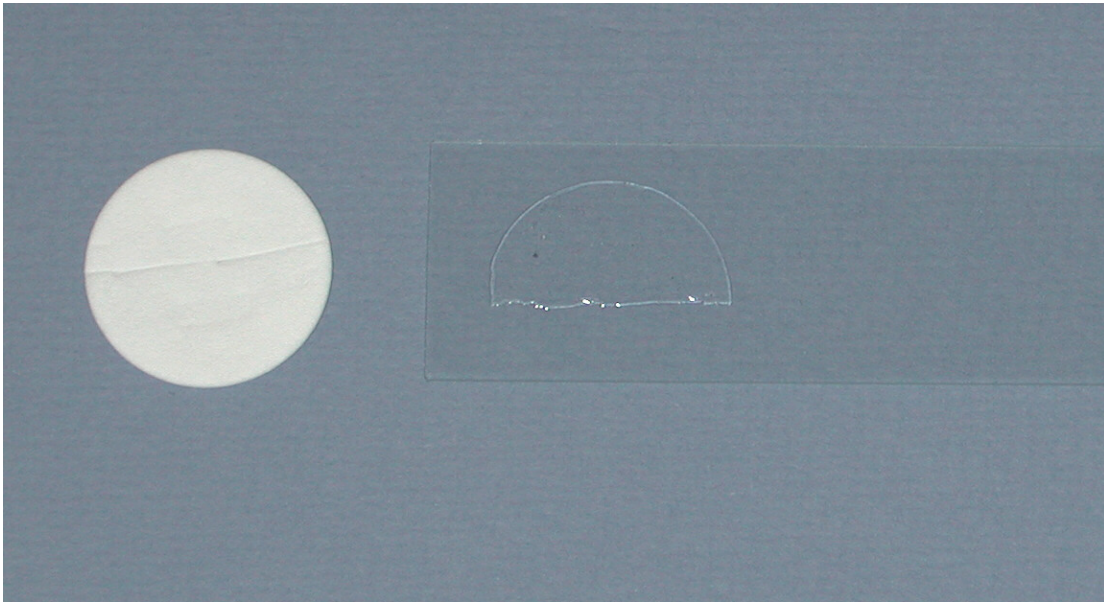


Figure 5. Sample trace on the glass slide collapsed and cleared with Acetone Vaporizer. Second trace available for archiving or further analysis.

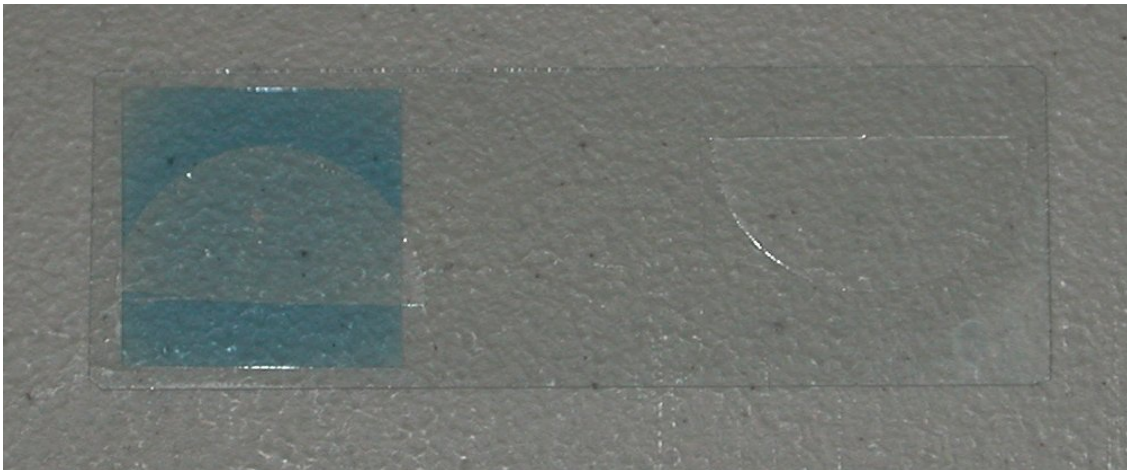


Figure 6. Sample trace stained and ready for microscopic analysis.

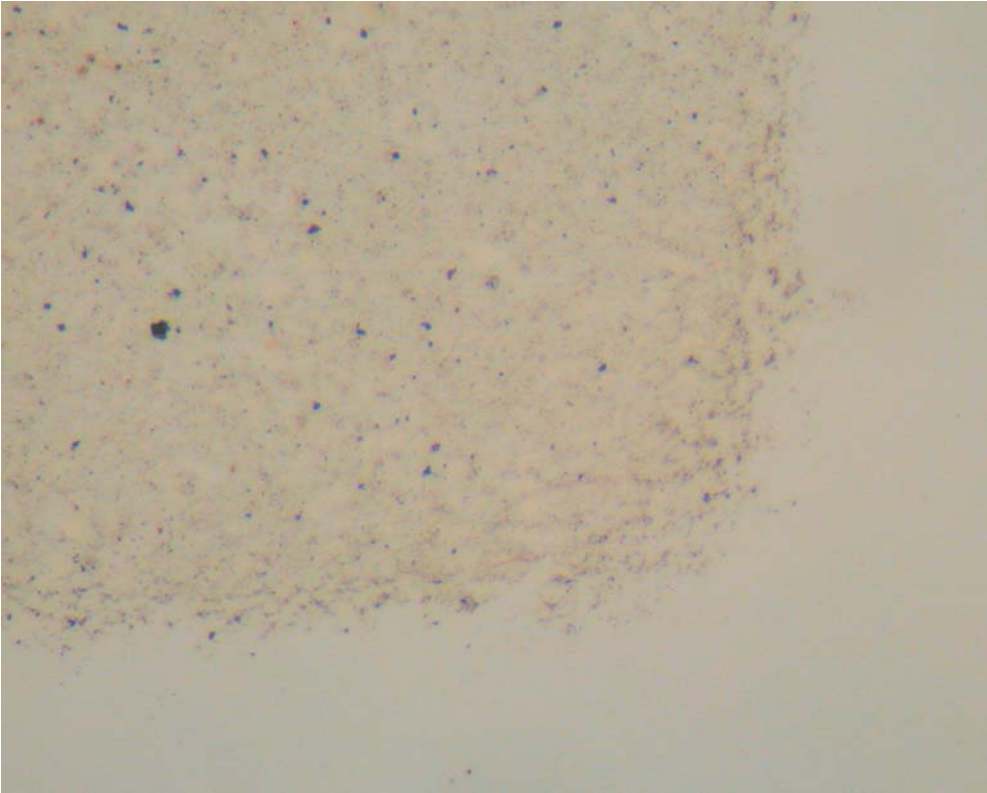


Figure 7. Corner of a Bi-Air sample trace, showing the well defined boundary of the sample. The defined area of the sample makes analysis much easier and more reliable.

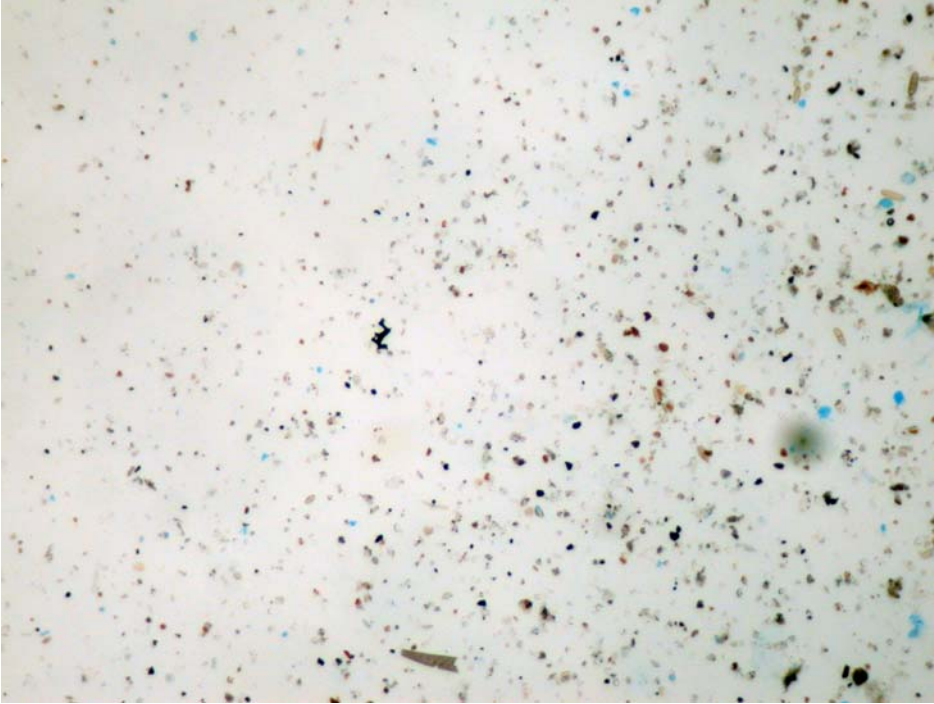


Figure 8. Corner of an Air-O-Cell sample trace. The boundary of the sample trace is not well defined, and finding all the spores is sometimes difficult for the analyst.