

FUNGI CULTURE

Culture differs from a microscopic exam in that spores are placed on agar (petri plate) and allowed to incubate for growth. The microscopic exam can determine the genus level of most fungi found in the indoor air. It can not determine if the spores are viable (alive) or non viable (not able to grow).

Culture can determine both the genus and species of a fungi. The only fungi that will be reported are the viable ones.

Many indoor investigations require a combination of microscopic exam and culture testing.

Anderson and SAS air sampling

Air sampling involves using an agar plate for the impaction of the fungi on a surface and a device such as an Anderson or SAS.

Air is collected based on total volume, and impacted on an agar plate. The plate is delivered to the laboratory and after a week of incubation, the fungi on the plate are identified and quantified.

Common plate types for fungi recovery are Malt Extract Agar 2% (general recovery agar) and Dichloran Glycerin 18 (DG18) for the recovery of *Aspergillus* and *Penicillium* species. Potato Dextrose Agar (PDA) can be substituted for MEA 2%.

Culture air sampling is a good companion to the spore trap. Spore traps can not differentiate between most of the spores that are classified as *Aspergillus* / *Penicillium*. Culture will identify the genus (ex. *Aspergillus*), as well as the species. An example is *Aspergillus fumigatus*, which is the most common cause of Aspergillosis. As many of the *Aspergillus* and *Penicillium* species produce mycotoxins, it is a good practice to use some form of culture testing in an investigation.

Anderson

- The Anderson N6 air sampler involves using an agar plate that has 400 impaction sites.
- A pump that is capable of drawing 28.3 liters of air per minute is used.
- sample duration is generally 2 – 5 minutes.



Anderson Air Sampler

Procedure

- Put on a pair of sterile examination gloves.
- Using an alcohol wipe, clean all of the inside surfaces of the Anderson and allow to air dry.
- Mark the bottom of the agar plate with your sample ID.
- Remove the top cover of the agar plate and place it inside of a clean sandwich bag.
- Place the Anderson near the breathing space to be sampled.
- Place the agar plate inside of the Anderson and clamp it closed.
- Make sure it has an even seal all the way around.
- Attach the air hose to the Anderson, coming from the pump. Make sure the pump rotometer is adjusted to approximately 28.3 lpm.

- Turn on pump for 2 minutes.
- Take plate out and check for a “wagon wheel” shape of impaction on the agar. Cover the plate with the top that was originally put into the sandwich bag.
- Wrap plate with parafilm and place inside the sandwich bag.
- Record volume and length of time of sampling on the COC.
- Deliver the plates to the laboratory same day or next day. If shipping in warm weather, ship with ice pack.



This is what the agar plate will probably look like one week after delivery to the laboratory.

SAS Surface Air Sampler

Various SAS types of samplers are available that use 55 mm or 90mm diameter plates containing 220 impaction sites. A pump that is capable of drawing 90-180 liters of air per minute is used for a sample duration of 1- 2 minutes.



SAS Sampler

Procedure

- Unscrew and remove head.
- Remove top of agar plate and insert onto pump, agar side up. Replace head.
- Set appropriate volume and start pump.
- After time has expired, remove head.
- Place cover on agar plate, and wrap with parafilm.
- Wash head, inside and out with alcohol wipe between samples.
- Deliver the plates to the laboratory same day or next day. If shipping in warm weather, ship with ice pack.

Fungi Culture

Bulk, Dust, Swab, Rodac Contact Plates

Samples delivered to the laboratory will be plated on appropriate media to provide growth for the common fungi found in the indoor environment. At the least, the media will include MEA 2%, DG18 and Cellulose. Results will include counts and identifications to the genus level and, if requested, the species level.

The major advantage of culture identification is the ability to separate out the species of *Aspergillus* and *Penicillium*. These are the most common allergenic and/or toxigenic fungi found in the indoor environment. Between the 2 genera there are approximately 400 species.

Bulk samples can be any material, although the most common submitted are the paper part of gypsum board, wet carpet, wet insulation, ceiling tile and wood products. The laboratory needs only a small piece of material to work with. It can be a few ounces or grams, or if using area, one to two inches, centimeters square.

Dust samples are particularly useful when coming from carpet, as the carpet generally provides a history of the environment, while an air sample only provides the most current conditions.

Swabs can be used in almost any situation to collect what appears to be fungal growth from wall board, carpets, HVAC coils, supply and return vents, surfaces, liquids. The area swabbed should, whenever possible be measured in square inches, centimeters, feet or meters and recorded on the COC.

Rodac contact plates are raised agar surfaces that can be pressed gently on flat surfaces that contain fungal growth. The colonies are enumerated and identified to the genus level.



Rodac Contact Plates