

# PEARLS OF LABORATORY MEDICINE

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**TITLE: Hyaline and Mucorales molds**

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**Slide 1:**

Hello, my name is Dr. Dennise Otero Espinal. I am the Assistant Director of Clinical Pathology at Lenox Hill Hospital. Welcome to this Pearl of Laboratory Medicine on “**Hyaline and Mucorales molds.**”

**Slide 2:**

Today’s objectives are: to name methods for mold identification in the laboratory, and to describe the characteristics of the most common hyaline and Mucorales molds isolated in the clinical laboratory.

**Slide 3:**

Molds can be identified in the clinical laboratory using different approaches such as direct visualization of the organisms in slide preparations. In the microbiology laboratory this is done taking a small drop or piece of specimen and staining it before setting the fungal cultures. The specimen is then stained with calcofluor white fluorescent stain as you can see in the image 1. This method is fast to perform, mold structures are easy to visualized, but is not very specific.

Direct visualization by Histology examination can be very useful for detection of mold in tissue, especially if no sample was sent to the microbiology laboratory. Hematoxylin & eosin (H&E) is the primary stain used (image 2), but Periodic acid–Schiff (PAS) and Gomori Methenamine–Silver stain (GMS), are commonly used to aid visualizing fungal structures better.

Histology slides are relatively slower to prepare than other methods, lacks specificity, and caution has to be taken since septations or lack of thereof are not always clear. Cultures or molecular identification from the paraffin block are still needed for the final identification of the organism.

### Slide 4:

Culture-based identification it is still commonly performed. The molds are identified using colony morphology (color, shape, texture) and microscopic morphology evaluation with stains like lactophenol cotton blue, as shown in the picture.

Selection of the media for culture is important: non-selective to recover all clinically significant molds, with antibiotic for samples that are non-sterile, and with cycloheximide to inhibit saprophytic molds. Care must be taken using media with cycloheximide since the growth of medically important molds can be inhibited, and media without it should be set too.

MALDI-TOF MS technology can be used for final identification of the commonly isolated molds, but it is not yet widely used. DNA sequencing is the most accurate identification technique when using isolated fungi from culture. Pan-sequencing can also be performed on patients' samples. Multiple targets are used, the most common is the Internal Transcribed Spacer (ITS) region in the ribosome. Sequencing is highly accurate, but requires a specialized laboratory and it is not widely available in clinical settings.

### Slide 5:

Hyaline molds are distinguished by their colony morphology and rapid growth rate. Microscopically they have thin, colorless, regularly septate hyphae with acute angle branching as seen in this image (blue arrows). Some of the species in this category include *Aspergillus* spp., *Fusarium* spp., and *Scedosporium* spp., which are indistinguishable by histology examination. In the next slides I will describe clinically relevant hyaline molds commonly seen in the microbiology laboratory in more detail.

### Slide 6:

Several species form part of the *Aspergillus fumigatus* complex, with *Aspergillus fumigatus* as a major human pathogen within the complex. *A. fumigatus* cause of invasive aspergillosis, allergic aspergillosis, and fungal sinusitis.

It grows fast, forming blue-green to grey colonies that have white borders, as seen on image 1, with white to tan on reverse side. Microscopically, the conidiophore ends with a dome or flask-shaped vesicle with uniseriated phialides covering 2/3 of the vesicle, as pointed by the arrows in image 2.

### Slide 7:

*Aspergillus flavus* is part of a complex and is the second most common cause of invasive aspergillosis. *Aspergillus flavus* is a major producer of aflatoxin.

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## Hyaline and Mucorales molds

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*A. flavus* grows fast, forming bright yellow-green colonies (image 1) and yellow on reverse. Microscopically, it has a rough or spiny conidiophore that it is hard to see sometimes (red arrow, image 2). The vesicle is globose and completely covered by uniseriated or biseriaded phialides.

### Slide 8:

*Aspergillus niger* is part of a complex. It can cause aspergilloma (fungus ball) and otitis externa. It forms white, fast growing colonies that darken as they age (image 1).

The colony reverse is white, which helps differentiating *A. niger* from dematiaceous molds. Microscopically has a smooth conidiophore with a globose vesicle with biseriaded phialides and brown conidia (image 2).

### Slide 9:

*Aspergillus terreus* is part of a complex. It can cause disseminated disease in the immunocompromised and is intrinsically resistant to amphotericin B. The colonies are fast growing, cinnamon-brown, and yellow on reverse. Microscopically, the upper half of the dome-shaped vesicle has biseriaded phialides, as pointed by the arrow.

### Slide 10:

*Penicillium* spp. are considered to be contaminants. *Talaromyces marneffe* formerly *Penicillium marneffe* was considered the only pathogen in this group. It is a thermally dimorphic fungi that cause invasive mycosis in immunocompromised in endemic areas

*Penicillium* spp. forms fast growing, powdery blue-green colonies. Microscopically it has branched and unbranched conidiophores with clusters of phialides topped with round conidia in chains.

### Slide 11:

*Paecilomyces* spp. is considered a contaminant, but also can cause keratitis. *Paecilomyces* spp. forms fast growing, flat, yellow-brown, colonies. Microscopically shows branched conidiophores with clusters of phialides with delicate tapering ends and oval conidia in chains.

### Slide 12:

*Penicillium* and *Paecilomyces* are very similar microscopically, but *Paecilomyces*, which is a pathogen, has long tapering phialides and oval conidia in comparison with the blunt phialides and round conidia from *Penicillium*. The colony color is also a key distinguish feature, since *Paecilomyces* do not form blue-green colonies.

### Slide 13:

*Fusarium* spp. can cause a wide array of infection, from nail infection to disseminated disease, mostly in immunocompromised patients. It forms fast growing wooly colonies that can range from white or cream, to pink or purple with light or deeply colored reverse. Micro and macro conidia can be seen. Phialides produce 1-2 celled microconidia and the macroconidia are curved (banana or sickled shaped), septate, usually in clusters, as seen in the image.

### Slide 14:

*Acremonium* spp. can cause white-grain mycetoma, keratitis, and nail infection. Macroscopically, it can be confused with *Fusarium* due to the pink to white colonies, but it grows at a slower rate. Microscopically, it has narrow hyphae and the conidia form clusters at the end of narrow, delicate phialides, as pointed by the red arrow.

### Slide 15:

Mucormycosis is caused by fungi from the Mucorales order such as *Rhizopus* spp., *Mucor* spp., *Lichtheimia* (previously *Absidia*) spp., *Rhizomucor*, and *Apophysomyces* spp and others rarely. Zygomycetes is an obsolete term and shouldn't be used anymore. These fungi can cause rhinocerebral, pulmonary, cutaneous, and systemic invasive disease, especially in patients with diabetes, iron overload, immune suppressed as in solid organ or stem cell transplant, and hematological malignancies.

### Slide 16:

Mucorales fungi are characterized by a rapidly growing mycelium and pauciseptated, ribbon-like or broad based hyphae. Wide-angle branching is usually seen in histology slides.

### Slide 17:

*Rhizopus* spp. is the most common cause mucormycosis.

It forms white, cottony, fast growing colonies that darken with age and the reverse is white to pale grey or brown. Microscopically is characterized by having rhizoids, root like structures, pointed by red arrow. The sporangiophores are mostly brown, not branched and directly connect to rhizoids. The sporangia are multispored, spherical, and the conidia can be brown.

### Slide 18:

*Mucor* spp. is a less common cause of mucormycosis.

It forms white-yellow to grey, cottony, fast growing colonies that darken with age, as seen in image 1. The reverse is white. Microscopically the sporangiophores are mostly hyaline,

multispored, spherical sporangia can be seen (red arrow image 2), but it lacks rhizoids as we can see in the red arrow. Other Mucorales need to be excluded to reach this diagnosis.

### Slide 19:

Here are some of the differential characteristics between *Lichtheimia*, *Rhizomucor*, and *Apophysomyces*.

*Apophysomyces* have unbranched grey-brown sporangiophores with a bell-shaped apophysis. The columella is domed-shaped and the sporangia pyriform. *Lichtheimia* have similar columella and sporangia but the sporangiophores are branched and hyaline terminating in a conical apophysis. *Lichtheimia* have primitive or indistinct rhizoids. *Rhizomucor* has branched brown sporangiophores with globose sporangia.

*Rhizomucor* rhizoids can be at the base of the sporangiophores or in between. *Lichtheimia* and *Apophysomyces* sporangiophores arise from the stolon, but *Apophysomyces* have septation between the sporangiophore and the rhizoids.

### Slide 20: References

### Slide 21: Disclosures

### Slide 22: Thank You from [www.TraineeCouncil.org](http://www.TraineeCouncil.org)

Thank you for joining me on this Pearl of Laboratory Medicine on “Hyaline and Mucorales molds.”

