

Challenge MY1709-3

September 2017

Cornea: *Aspergillus flavus*

HISTORY

This challenge was sent as a simulated cornea sample. Laboratories were expected to isolate and identify *Aspergillus flavus*.

CMPT QC/QA/Statistics

All Mycology samples are produced at CMPT according to CMPT internal protocols.

The samples are assessed for homogeneity and stability using in-house quality control methods and random selection of samples before and during production, and post sample delivery. The number of random samples selected is 15% of the total production batch.

The sample was verified by a reference laboratory. *Aspergillus flavus* was isolated as a pure culture after 48 hours incubation at 30C on Phytone medium.

The challenge sample lot was confirmed to be homogeneous and stable for at least 35 days.

All challenge components have in-house assigned values based on the most clinically appropriate result; the most clinically appropriate result is determined by expert committee evaluation. No further statistical analysis is performed on the results.

SURVEY RESULTS

7/9 participants reported the presence of *Aspergillus flavus*. Two laboratories reported *Aspergillus* species. All participants were given an acceptable grade. (Table 1).

Table 1. Identification Results

Reported	Labs	Grade
<i>Aspergillus flavus</i>	7	Acceptable
<i>Aspergillus</i> species , refer	1	Acceptable**
<i>Aspergillus</i> species	1	Acceptable**
Total	9	

IDENTIFICATION

Corneal scrapings obtained from the eye are the preferred sample to diagnose microbial keratitis. These samples are collected by ophthalmologists and they should be inoculated on several media such as blood agar, Sabouraud agar and potato dextrose agar so that an early diagnosis can be made. ^{1, 2}

Aspergillus hyphae in clinical specimens can be readily detected by direct microscopic examination. .

Aspergillus species grow well on a variety of conventional agar media and most species form a mature colony within 3 days.

Some species are sensitive to cycloheximide thus Mycosel agar should not be used. Most pathogenic aspergilli reach optimal growth at a temperature of 30 to 37°C, but sporulation may be more abundant at 30°C.

Aspergillus flavus

Colony

A.flavus produces rapidly growing, velvety to cottony, yellowish-green to olive, colonies (Figure 1). Reverse is yellowish to tan.

Grading

Reporting *Aspergillus flavus* was graded acceptable.

Reporting *Aspergillus* species was graded 'acceptable**'.

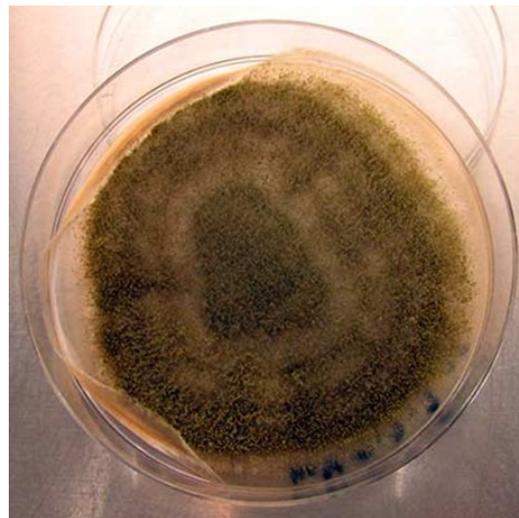


Figure 1. *Aspergillus flavus*. Colony on Sabouraud's agar.

A. flavus has septate hyphae. The conidiophores are hyaline, thick-walled, and appear fine to coarsely roughened and spiny. The conidial heads are both radiate and loosely columnar.

The vesicles can be elliptical, subglobose, or globose, with a diameter of 10 to 65 µm; both uniseriate and biseriata phialides cover either the entire or three fourths of the vesicle surface (Figure 2).³⁻⁵



Figure 2. *Aspergillus flavus*. Lacto-phenol cotton-blue stain under magnification of 400X.

Because pathogenic *Aspergillus* species are common contaminants, it is necessary to be cautious when examining cultures. A more reliable diagnosis can be made when cultures correlate with positive results on direct examination of the clinical sample or when the same organism is isolated from multiple clinical samples.⁴

CLINICAL RELEVANCE

Aspergillus flavus is the second most common species (after *A. fumigatus*) isolated from invasive aspergillosis.

The intact ocular surface is an effective barrier against most microorganisms but once breached, the host defenses are less than sufficient to prevent infection which can lead to eventual loss of vision⁶

Mycotic keratitis is a mycosis of the cornea and can be caused by a wide variety of fungi. They may account for more than 50% of all culture-positive microbial keratitis especially in tropical and sub-tropical countries.⁶ Keratitis due to filamentous fungi (*Fusarium*, *Aspergillus*, phaeohyphomycetes and *Scedosporium apiospermum*) most commonly occurs after trauma although previous use of corticosteroids and contact lens wear are gaining importance as risk factors. Mycotic keratitis can also be

caused by yeasts; in this case, there is usually some systemic or local (ocular) defect.¹

As a minimum, topical antifungal therapy is started for all cases on the finding of a positive corneal scraping. Systemic and intraocular therapy are also required.⁷

REFERENCES

1. Thomas PA, Kaliamurthy J. Mycotic keratitis: epidemiology, diagnosis and management. *Clin Microbiol Infect.* 2013;19:210-220.
2. Baron EJ, Miller JM, Weinstein MP, et al. A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: 2013 Recommendations by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM). *Clinical Infectious Diseases.* 2013.
3. Ellis D, Davis S, Alexious H, Handke R, Bartley R. *Descriptions of Medical Fungi.* 2nd ed. Adelaide, Australia: Ellis, D; Davis, S; Alexious, H; Handke, R; Bartley, R.; 2007.
4. Kwon-Chung K.J., Bennet J.E. Aspergillosis. In: *Medical Mycology.* Malvern, Pennsylvania: Lea & Febiger; 1992:201.
5. Larone Davise H. *Medically Important Fungi. A Guide to Identification 5th Ed.* 4th ed. Washington DC: ASM Press; 2011.
6. Lakhundi S, Siddiqui R, Khan NA. Pathogenesis of microbial keratitis. *Microbial Pathogenesis.* 2017;104:97-109.
7. Rautaraya B, Sharma S, Kar S, Das S, Sahu SK. 2011. Diagnosis and treatment outcome of mycotic keratitis at a tertiary eye care center in eastern India. *BMC Ophthalmol.* 2011 Dec 22;11:39.

Mycology Grading scheme

Acceptable: Report is technically correct and clinically appropriate. *Slide or Culture or Susceptibility is correctly identified.*

Acceptable:** Report contains deficiencies, but within the limits of acceptable. *Culture may be identified correctly only to the class, or genus level. Nomenclature errors would not likely lead to errors in clinical judgement or decision making.*

Unacceptable: Report contains errors that could result in incorrect interpretation and actions. *Significant error.*

Ungraded: Report cannot be fairly assessed.