



0704-3 *Aspergillus niger* (invasive type)

HISTORY This sample was sent as an ear biopsy isolate.

CMPT QA: Pure growth of 4+ *Aspergillus niger*, viable for 29 days.

Reference Laboratory: Growth of 4+ *Aspergillus niger* confirmed.

All laboratories correctly reported the genus; identifications received and media noted by participants are shown in Table 1.

IDENTIFICATION Please refer to the critique for CMPT Mycology Plus 0609-3 *A. flavus* that included a review of *Aspergillus* species.

When isolated on Sabouraud dextrose agar, aspergilli tend to reproduce in the asexual form, therefore isolates are usually inoculated (at three points) on Czapek dox agar and potato dextrose agar and incubated at 25°C¹. Most species sporulate within 7 days. Both macroscopic morphology, primarily based on colony pigmentation, and microscopic morphology of the conidial head are required for identification. Microscopic mounts are best made using a cellotape flag or slide culture preparation mounted in lactophenol cotton blue. A drop of alcohol is usually needed to detach the cellotape flag from the stick, and to act as a wetting agent.

Colony morphology²⁻⁴ Colonies on potato dextrose agar at 25°C are woolly initially white, quickly becoming black with conidial production. Reverse is white to pale yellow and growth may produce radial fissures in the agar.

Microscopic morphology²⁻⁴ Hyphae are septate and hyaline. Conidial heads are radiate initially, splitting into columns at maturity. The species is biserial (vesicles produce sterile cells known as metulae that support the conidiogenous phialides). Conidiophores are long (400-3000 µm), smooth, and hyaline, becoming darker at the apex and terminating in a globose vesicle (30-75 µm in diameter). Metulae and phialides cover the entire vesicle. Conidia are brown to black, very rough, globose, and measure 4-5 µm in diameter. *Syncephalastrum racemosum*, generally considered a contaminant, may at first resemble *A. niger*, but careful examination reveals tubular sporangia and the absence of phialides².

Other Methods PCR-based identification systems could provide a powerful tool to control invasive fungal infections and to speed the application of effective treatment⁵.

CLINICAL SIGNIFICANCE^{1,6,7} *A. niger* is the most frequently encountered agent of otomycosis (otitis externa) a condition that can cause pain, temporary hearing loss and, in severe cases, damage to the ear canal and tympanic membrane. In addition, *A. niger* is the third most common species associated with invasive pulmonary aspergillosis. It is commonly associated with "fungus ball", a condition wherein fungus actively grows in the human lung, forming a ball, without invading lung tissue. It is important to note that *A. niger* may also be a common laboratory contaminant resulting in reporting false-positive cultures.

This *A. niger* was described as "invasive type". The diagnosis of invasive aspergillosis relies on the histologic demonstration of fungal invasion and the isolation of *Aspergillus* from normally sterile clinical samples. However, the culture yield of an *Aspergillus* or another septate mold from infected tissue has been shown to be generally low, ranging from 30% to 50%⁶. For example, a patient who was not immunocompromised and presented with a mycetoma in the right maxillary sinus caused by *A. niger*, the speciation of the fungus was done by examination of the morphology of the hyphae and the conidial heads, and pigmentation noted on histologic sections stained with hematoxylin and eosin as the fungal cultures were negative⁷.

Most cases of hyperoxaluria and oxalosis are an inborn metabolic defect (http://www.ohf.org/about_disease.html).

Perhaps the most well-known application of certain strains of *A. niger* is as the major source of citric acid; this organism accounts for over 99% of global citric acid production, or more than 4.5 million tonnes per annum. *A. niger* is also commonly used for the production of native and foreign enzymes, including glucose oxidase and hen egg white lysozyme.

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Table 1. 0704-3 <i>Aspergillus niger</i> reports received and media noted.		
Report received	No. of labs	Media reported
<i>Aspergillus niger</i>	7	SAB, BAP, PDA, 30C; IMA, BHIA, 28 C; IMA 30C; IMA, 25 C, 37 C; BHIA w/ antibiotics, 25 C; SABHI, Littman oxgall, Mycosel, 25 C; IMA, Mycobiotic agar, SAB, 25C; IMA, BHI w/ chloramphenicol, gentamicin, cycloheximide; BHI w/ chloramphenicol, gentamicin, 10% sheep blood, 29 C
<i>Aspergillus</i> species	2	FSA, SAB, Cornmeal Agar, PDA, 25C; SAB, BHI + SB, BHI + chlor & gent 30C
Ear specimens not normally processed, refer	1	n/a
Total	10	

TREATMENT Susceptibility testing should be performed either in-house or by forwarding the isolate to a reference laboratory¹. Susceptibility testing may be useful if the patient is failing treatment and the fungus is isolated repeatedly.

In a case of a 73-year-old diabetic man with malignant otitis externa due to *A. niger* cure was achieved with a 3-week course of intravenous amphotericin B, followed by oral itraconazole for 3 months⁸. In this same reference 13 cases of malignant otitis externa caused by *Aspergillus* sp. are reviewed.

REFERENCES

1. Verweij PE, Brandt ME. 2007. p. 1802-1838. *Aspergillus*, *Fusarium*, and other opportunistic moniliaceous fungi. In PR Murray et al. (ed.) *Manual of Clinical Microbiology*. Ch. 121. 9th ed. ASM Press. Washington, DC.
2. Larone DH. 2002. p. 175 and 266. *Medically Important Fungi*. 4th ed. ASM Press. Washington, DC.
3. [http://www.mycology.adelaide.edu.au/Fungal_Descriptions/Hyphomycetes_\(hyaline\)/Aspergillus/niger.html](http://www.mycology.adelaide.edu.au/Fungal_Descriptions/Hyphomycetes_(hyaline)/Aspergillus/niger.html)
4. http://www.doctorfungus.org/thefungi/Aspergillus_niger.htm
5. Chise Sugita C, Makimura K, Uchida K, et al. 2004. PCR identification system for the genus *Aspergillus* and three major pathogenic species: *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus niger*. *Med Mycology*. 42:5. p. 433 – 437.
6. Tarrand JJ, Lichtenfeld M, Warraich I, et al. 2003. Diagnosis of invasive septate mold infections: a correlation of microbiological culture and histologic or cytologic examination. *Am J Clin Pathol*. 119:854- 858.
7. Zaman SU, Sarma DP: 2007. Maxillary sinus mycetoma due to *Aspergillus niger*. *Internet J Otorhinolaryngology*. 6:1. <http://www.ispub.com/ostia/index.php?xmlFilePath=journals/ijorl/vol6n1/niger.xml#e2e2>
8. Bellini C, Antonini P, Ermanni S, et al. 2003. Malignant otitis externa due to *Aspergillus niger*. *Scand J Infect Dis*. 35:4. p. 284 – 288.

Suggested reading:

Mycology Plus 0609-3 Simulated bronchial aspirate: *A. flavus*; http://www.cmpt.ca/pdf_mycology/0609_3_aflav.pdf

Mycology Plus 0409-3 Simulated Blood isolate: *A. flavus*;

Mycology Plus 0405-3 Simulated Skin: *Aspergillus fumigatus*