



0609-3 *Aspergillus flavus*

HISTORY This sample was sent as a bronchial aspirate isolate.

CMPT QA: Pure growth of 4+ *Aspergillus flavus*, viable for 33 days.

Reference Laboratory: Growth of *Aspergillus flavus* confirmed.

All laboratories correctly reported the genus *Aspergillus*. Identifications and media reported are shown in Table 1.

Czapek Dox Agar may also be used for the routine cultivation of fungi, especially *Aspergillus*, *Penicillium*, and non-sporulating moulds².

IDENTIFICATION¹ *Aspergillus* belong to a group of fungi, which includes those conidial fungi which are not darkly pigmented, but with colonies that may be colorless (hyaline) or brightly colored also termed moniliaceous. These fungi show distinctive conidial heads with flask-shaped phialides arranged in whorls on a vesicle. **Identification of the hyphomycetes** is primarily based on microscopic morphology including; (a) conidial morphology, especially septation, shape, size, color and cell wall texture; (b) the arrangement of conidia as they are borne on the conidiogenous cells, e.g.,solitary, arthrocatenate, blastocatenate, basocatenate or gloiosporae, (c) the type conidiogenous cell, e.g.,non-specialized or hypha-like, phialide, annellide or sympodial and (d) other additional features such as the presence of sporodochia or synnemata. Culture characteristics, although less reliable may also be useful. These include surface texture, topog-

raphy and pigmentation, reverse pigmentation and growth at 37°C. For identification, potato dextrose agar and cornmeal agar are two of the most suitable media to use and exposure to daylight is recommended to maximize culture color characteristics.

Aspergillus colonies are usually fast growing, white, yellow, yellow-brown, brown to black or shades of green, and they mostly consist of a dense felt of erect conidiophores. Conidiophores terminate in a vesicle covered with either a single palisade-like layer of phialides (uniseriate) or a layer of subtending cells (metulae) which bear small whorls of phialides (the so-called biseriate structure). The vesicle, phialides, metulae (if present) and conidia form the conidial head. Conidia are one-celled, smooth- or rough-walled, hyaline or pigmented and are basocatenate, forming long dry chains which may be divergent (radiate) or aggregated in compact columns (columnar). Some species may produce Hülle cells or sclerotia.

For identification, isolates are usually inoculated at three points on Czapek dox agar and potato dextrose agar and incubated at 25°C. Most species sporulate within 7 days. Descriptions are primarily based on colony pigmentation and morphology of the conidial head. Microscopic mounts are best made using a cello tape flag or slide culture preparation mounted in lactophenol cotton blue. A drop of alcohol is usually needed to detach the cello tape flag from the stick, and to act as a wetting agent.

Unlike most fungi, *A. flavus* is favored by hot dry conditions. The optimum temperature for growth is 37°C, but the fungus readily grows between the temperatures of 25-42°C and will grow at temperatures from 12-48°C. Such a high temperature optimum contributes to its pathogenicity in humans. *Aspergillus fumigatus* is thermotolerant and grows at temperatures up to 55°C.

Colony morphology^{2,3} *A. flavus* colonies are yellowish-green, consisting of a dense felt of conidiophores or mature vesicles bearing phialides over their entire surface. Colonies on potato dextrose agar at 25°C are olive to lime green with a cream reverse. Rapid growth. Texture is woolly to cottony to somewhat granular. Sclerotia, when present, are dark brown. A clear to pale brown exudate may be present in some isolates.

Effuse, lime green colonies with rough conidiophores and smooth to very finely roughed conidia distinguish *A. flavus* from the similar *Aspergillus parasiticus* that produces very rough conidia (and also aflatoxin). *A. fumigatus* show

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Table 1. 0609-3 <i>Aspergillus flavus</i> reports received and media noted.	
Report and (No.) received	Media and Incubation Temperatures
<i>Aspergillus flavus</i> (n=5)	SAB, BAP, SAB-Dx, 30°C
	IMA, BHIA, 30°C
	IMA, 25°C, 37°C; BHIA w/ chloramphenicol, cyclohexamide, 25°C; BHI 25°C
	IMA, Mycosel, SAB, 25°C
	IMA, BHI w/ chloramphenicol, gentamicin, cycloheximide; BHI w/ chloramphenicol, gentamicin, 10% sheep blood, 29°C
<i>Aspergillus fumigatus</i> (n=1)	Sabhi, Littman, Mycosel, 25°C
<i>Aspergillus versicolor</i> (n=1)	BHI2, BHI3, IMA, 30°C
<i>Aspergillus</i> sp., 1, refer (n=4)	FSA, SAB, Cornmeal Agar, 27°C
	BAP, Mycosel, PDA, 30°C
	IMA, BHIA w/ chloramphenicol, cyclohexamide, gentamicin, 10% sheep blood, 28°C
	SAB, BHI w/ chloramphenicol, gentamicin, 30°C

typical blue-green surface pigmentation with a suede-like surface consisting of a dense felt of conidiophores. *A. versicolor* colonies on potato dextrose agar at 25°C are as the name “versicolor” suggests variously colored and may range from very pale dull green, to greenish-beige, pinkish-green, salmon green, or dark green. Reverse is reddish to uncolored. Exudate, when present, is pink to reddish-brown. The growth rate is slow to moderate.

Microscopic morphology ^{2,3} *Aspergillus* hyphae are septate and show dichotomous, 45° angle branching. Larger hyphae may resemble hyphae of the Zygomycetes. Conidial heads are radiate to loosely columnar with age. Conidiophores are coarsely roughened, uncolored, up to 800 µm long x 15–20 µm wide, vesicles spherical (20–45 µm), metulae (8–10 x 5–7 µm) covering nearly the entire vesicle in biseriate species. Conidial heads radiate, uni- and biseriate; however, some isolates may remain uniseriate, producing only phialides (8-12 x 3–4 µm) covering the vesicle. Conidia are pale green and conspicuously echinulate, smooth to very finely roughened, (sub)spherical, 3.5 - 6 µm in diameter. *A. versicolor* conidiophores measure 120-700 µm in length, are hyaline to pale brown, smooth-walled and brittle (can see where many have "snapped off") ⁴. Vesicles are small (approximately 9-16 µm in diameter) and variably shaped, with metulae and phialides covering most of the vesicle. Reduced conidial structures resembling those seen in *Penicillium* species are common.

CLINICAL SIGNIFICANCE ¹⁻⁶ *Aspergillus* is a group of molds which is found world-wide, especially in the autumn (harvest time) and winter in the Northern hemisphere. The most commonly isolated species is *Aspergillus fumigatus*, followed by *Aspergillus flavus* and *Aspergillus niger*. Other species less commonly isolated as opportunistic pathogens include *Aspergillus clavatus*, *Aspergillus glaucus* group, *Aspergillus nidulans*, *Aspergillus oryzae*, *Aspergillus terreus*, *Aspergillus ustus*, and *Aspergillus versicolor*.

A. flavus is predominately a saprophyte and grows on dead plant and animal tissue in the soil. For this reason it is very important in nutrient recycling. However, *A. flavus* can also be pathogenic on several plant and animal species, including humans and domestic animals. *A. flavus* is particularly found in the Americas. It can infect seeds of corn, peanuts, cotton,

and nut trees. Growth of the fungus on a food source often leads to contamination with aflatoxin, a toxic and carcinogenic compound that can cause harmful effects, including liver cancer, when ingested by humans or animals. Four different aflatoxins, B1, B2, G1 and G2, have been identified with B1 being the most toxic, carcinogenic, hepatotoxic and potentially mutagenic, while also being the most prevalent.

Patients infected with *A. flavus* are often immunocompromised or neutropenic. The fungus also causes allergic diseases in asthmatics and patients suffering from cystic fibrosis. It is one of the main agents of human allergic bronchial aspergillosis (hypersensitivity pneumonitis) and paranasal sinusitis. It also occurs in external ears and may be involved in otitis; systemic infections have been reported in leukemic patients.

TREATMENT Susceptibility testing should be performed either in-house or by forwarding the isolate to a reference laboratory^{1,2}. Susceptibility testing may be useful if the patient is failing treatment and the fungus is isolated repeatedly either from deep pulmonary samples or from blood. See the Table shown below adapted from reference 2.

REFERENCES

1. Sigler L, Verweij PE. 2003. p. 1726-1760. *Aspergillus, Fusarium*, and other opportunistic moniliaceous fungi. In PR Murray et al. (ed.) *Manual of Clinical Microbiology*. Ch. 116. 8th ed. ASM Press. Washington, DC. 2003.
2. [http://www.mycology.adelaide.edu.au/Fungal_Descriptions/Hyphomycetes_\(hyaline\)/index.html](http://www.mycology.adelaide.edu.au/Fungal_Descriptions/Hyphomycetes_(hyaline)/index.html) Includes a Glossary of Mycological Terms (examples shown below.)
3. [http://www.mycology.adelaide.edu.au/Fungal_Descriptions/Hyphomycetes_\(hyaline\)/Aspergillus/flavus.html](http://www.mycology.adelaide.edu.au/Fungal_Descriptions/Hyphomycetes_(hyaline)/Aspergillus/flavus.html)
4. http://www.doctorfungus.org/thefungi/Aspergillus_versicolor.htm
5. Larone DH. 2002. p.36-37. *Medically Important Fungi*. 4th ed. ASM Press. Washington, DC.
6. http://www.doctorfungus.org/thefungi/Aspergillus_flavus.htm

Glossary of Mycological Terms (from reference 2)

Echinulate: Covered with delicate spines.

Sclerotium (sclerotia): A mass of thick-walled cells formed by the vegetative hyphae that function as an organ of perennation (survival).

Organism (From ref 2)	AmB	Flu	Vori	Itra	Keto	Terb	5FC	Caspo
<i>Aspergillus fumigatus</i>	S	R	S	SDD	R	S	R	S
	1.0 µg/ml	>100 ug/ml	0.5 ug/ml	0.5 ug/ml	8.0 ug/ml	0.5 ug/ml	>64 ug/ml	
<i>Aspergillus flavus</i>	S-R	R	S	SDD	R	S	R	S
	1-2 ug/ml	>100 ug/ml	0.5-1 ug/ml	0.5-1 ug/ml	8.0 ug/ml	0.5 ug/ml	> 64 ug/ml	

Key: AmB, amphotericin B; Flu, fluconazole; Vori, Voriconazole; Itra, itraconazole; Keto, Ketoconazole; Terb, terbinafine; 5FC, 5-fluorocytosine; Caspo, Caspofungin.