

## Identification of *Aspergillus fumigatus*

### I. Principle and Clinical Significance

*Aspergillus fumigatus* is the most pathogenic of the *Aspergillus* species, commonly causing pulmonary aspergillosis. It is a thermotolerant organism, growing well at 45-50°C or higher. Many *Aspergillus* species are opportunistic invaders, infecting various sites in immunocompromised individuals; however, these species are not thermotolerant. This principle allows the distinction of *Aspergillus fumigatus* from other *Aspergillus* species that are widespread in the environment and are common culture contaminants.

### II. Specimen

- A. A culture of a rapidly growing colony with a surface that is at first white and then any shade of yellow, green, brown or black, depending on the species.
- B. Characteristics
  1. Texture: velvety or cottony
  2. Reverse is white, gold-toned, or brown.
  3. Hyphae are septate; the conidiophore is enlarged at the tip, forming a swollen vesicle.
  4. Vesicles are partially or completely covered with flask-shaped phialides.
  5. Phialides produce chains of roundish conidia.

### III. Reagents and Equipment

- A. Lactophenol cotton blue, store at room temperature until the stated expiration date.
  1. Supplied by manufacturer; use as is
  2. Avoid skin contact. Rinse thoroughly with water if contact occurs.
  3. Protect from light
  4. Label all boxes of LPCB with a Chemical Hazard sticker: 3 for Health, 2 for flammability, and 0 for reactivity.
- B. 6 Potato Flake Agar plates
- C. Incubator at 47° to 50°C
- D. Clean glass slides and 22 X 22 mm cover slips
- F. Marking pen
- G. Sterile wooden sticks

### IV. Quality Control

- A. Quality control is performed each day of patient testing.
- B. Use *Aspergillus fumigatus* and *Aspergillus niger* as positive and negative controls, respectively.
  1. Remove the organisms from the -70°C freezer or use the stock culture.
  2. Label 2 PF plates for each control organism.
  3. Inoculate the plates with the control organisms. Two plates are inoculated with *Aspergillus fumigatus*, and two plates are inoculated with *Aspergillus niger*.
  4. Incubate one plate of each organism at 47-50°C. Incubate the other set of plates at room temperature.

5. *A. fumigatus* should grow at room temperature and at 47-50°C. *A. niger* should grow only at room temperature.
- C. Record the control results in LIS.
- D. Do not report the patient results if the quality control organisms do not yield the expected results. Notify the supervisor, and repeat the procedure using new controls from the -70°C freezer.

## V. Procedure

- A. Perform a lactophenol cotton blue mount on any suspected *Aspergillus* colonies (refer to "**Lactophenol Cotton Blue Preparations**" procedure). Record the microscopic morphology in the workup. Include the color of the surface and reverse of the colony as well as the microscopic characteristics. Rounds may be consulted to determine if temperature studies are necessary.
- B. Label 2 PF plates for each patient isolate. Include the accession number and date.
- D. Label 2 PF plates for each control.
- E. Inoculate the culture plates with the appropriate patient isolate or control using a sterile wooden stick.
- F. Place 1 set of plates (patient, *Aspergillus fumigatus* QC and *Aspergillus niger* QC) into the 47-50°C incubator.
- G. Incubate the second set of plates at room temperature on the Mycology counter.
- H. Incubate all of the plates for 4 days, and examine daily (M, W, and F).
  1. Compare the growth on the room temperature plate with the growth on the corresponding plate incubated at 47-50°C.
  2. Record the results of patient and controls.

## VI. Interpretation

- A. *Aspergillus fumigatus* will grow at room temperature and at 47°C or higher.
- B. *Aspergillus niger* will grow only at room temperature.
- C. *Aspergillus* sp. not *fumigatus* will grow only at room temperature.

## VII. Result Reporting

- A. If the isolate exhibits typical colonial and microscopic morphology and grows at room temperature **and** 47-50°C, report: "*Aspergillus fumigatus*".
- B. If the isolate exhibits typical colonial and microscopic morphology and grows only at room temperature, report "*Aspergillus* sp. not *fumigatus*".

## VIII. References

- A. Haley, LD and Carey, CS. 1978. Laboratory Methods in Medical Mycology. 4th ed. HEW publication no. (CDC) 78-8361, p 153-154.
- B. Larone, DH. 1995. Medically Important Fungi: A guide to identification, 3rd edition American Society for Microbiology, Washington DC, pp 190-192.
- C. Koneman, EW, Robert, GD and Wright, SF. 1978. Practical Laboratory Mycology, 2nd ed., Williams and Wilkins, p 59-61.

**IX. Document Control**

Medical Director Approval: Reviewed by Dr. Schappert 3/10/2010.

Microbiology Director Approval: Dr. Ann Robinson 05/19/2000

Microbiology Supervisor Reviews: Jerry Claridge 06/2001, 04/2002, 03/2003, 04/2004, 07/2005, 06/2006, 06/2007, 05/2008, 07/2009, 03/31/2011, 04/01/12, 03/2013, Jason Ammons 07/2015

Revisions & Updates: updated procedure for incubator rather than water bath.