PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

Department of Microbiology
Caffeic Acid Disk Procedure
For *Cryptococcus neoformans* Differentiation

I. Purpose and Test Principle

Caffeic Acid disks are a rapid test to detect the ability of an organism to produce the enzyme phenyloxidase, which is useful for the identification of *Cryptococcus neoformans*. The disks are impregnated with caffeic acid and iron citrate. When the disks are inoculated with a test isolate of *C. neoformans*, phenyloxidase produced by the organism reacts with caffeic acid in the presence of iron. This reaction results in the production of a brown pigment called melanin. *C. neoformans* is the only species known to produce phenyloxidase.

II. Specimen Information

Test isolates should be 48-72 hours old and growing on a non-dextrose containing medium, such as Corn Meal with Tween 80 Agar.

III. Reagents & Equipment

- Hardy Disk™ Caffeic Acid Disks
- On receipt, store at 2 - 8°C away from direct light. Product should not be used if there are any signs of deterioration, discoloration, or if the expiration date has passed. Do not use if disks have any brown, gray, or black discoloration. Protect product from excessive heat and moisture.
- Wooden applicators
- Sterile water
- Glass slides
- Filter paper
- Sterile petri plate
- Aerobic incubator set at 35 ± 2°C

IV. Procedure

Prior to use, allow the disks to equilibrate to room temperature.

A. Inoculation

Place a disk on a slide with one drop of sterile water and place the slide into a petri dish containing a moistened piece of filter paper to prevent the disk from drying out during incubation. Alternatively, the disk can be placed on the surface of a non-dextrose containing medium. Inoculate the disk with five to six yeast colonies from a 48-72 h old culture to yield a visible paste on the disk surface.

B. Incubation

Replace the plate lid and incubate the disks aerobically at 35°C in the dark. Observe for the development of dark brown pigmentation at 30 min intervals for up to 4 h.

V. Interpretation and Reporting

A. Positive Test

Development of dark brown to brown-gray color on the disk surface within 4 h is a positive result indicative of phenyloxidase activity found in *C. neoformans*.

B. Negative Test
Yeast other than *C. neoformans* will not typically produce any pigmentation. Very light non-specific pigmentation may be produced by *Cryptococcus albidus* and *Cryptococcus laurentii*. The intensity of this non-specific reaction will remain the same even after 24 h of incubation.

VI. Quality Control

Each new lot or shipment of disks should be examined for product deterioration and tested with the following control strains.

Control strain	Expected Results
Cryptococcus neoformans ATCC 14116	Dark brown to brown-gray color
Cryptococcus albidus ATCC 10666	No color change or very light tan; nonspecific reaction.
Candida albicans ATCC 90028	No color change.

VII. Limitations

A. It is recommended that physiologic tests be performed on colonies from pure culture for complete identification.
B. The addition of more than one drop of sterile water to the disk will delay the results or may produce false-negative results.
C. Rare strains of *C. neoformans* may not produce a positive reaction. Negative results on isolates suspected of being *Cryptococcus* spp. should be confirmed by testing the isolate on Bird Seed Agar.
D. As noted above, other species of *Cryptococcus* may produce light tan, non-specific pigmentation.
E. Dextrose inhibits the activity of phenyloxidase. Testing isolates growing on media containing dextrose will lead to false-negative results.

VIII. Verification of Test Method

The Hardy Disk™ Caffeic Acid Disks were evaluated by using clinical isolates, ATCC strains and isolates from past CAP surveys. A total of thirteen yeast isolates were tested. Seven strains of *Cryptococcus neoformans* were tested, including ATCC 14116, 3 CAP proficiency test strains, and 3 clinical isolates, including a *Cryptococcus neoformans* var. *gatti* isolate. The remaining yeast included *Cryptococcus albidus* ATCC 10666, *Cryptococcus laurentii* ATCC 76483, *Candida albicans* ATCC 90028, *Candida tropicalis* ATCC 750, *Saccharomyces cerevisiae* and a *Rhodotorula* spp. Each test strain was cultured on Corn Meal Agar and CHROMagar Candida at 25 - 30°C in an aerobic atmosphere for 72 h prior to testing. After inoculating the disks, all 7 (100%) of the *C. neoformans* strains grown on Corn Meal Agar produced brown pigmentation after 2 h of incubation. None of the *C. neoformans* isolates grown on CHROMagar produced a color change within 4 h. This validates the limitation of testing yeast isolates only from non-dextrose

containing media. None of the other yeast isolates produced an appreciable color change within 4 h.

IX. References

A. Package insert: Hardy Diagnostics, 021209md.

B. Clinical Microbiology Procedures Handbook, 3rd ed. and 2007 update, Vol. 2. Garcia, L.S., editor in chief. ASM Press, Washington, D.C.

X. Document Control

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