remel

# CAFFEIC ACID DISK

**INTENDED USE**
Remel Caffeic Acid Disk is a reagent-impregnated disk recommended for use in qualitative procedures for rapid, presumptive identification of *Cryptococcus neoformans*.

**SUMMARY AND EXPLANATION**
In 1962, Staib observed that colonies of *C. neoformans* produced brown pigment when grown on media containing an extract of *Guizotia abyssinica* seeds.[1] Strachan et al. demonstrated *C. neoformans* developed brown-pigmented colonies on a similar medium containing caffeic acid which had been extracted from *G. abyssinica* seeds.[2] Shaw determined that phenol oxidase, produced by *C. neoformans*, causes an enzymatic reaction resulting in the production of melanin which is absorbed by the yeast cell wall.[3] In 1975, Hopfer and Groschel incorporated caffeic acid and ferric citrate into a filter paper disk to detect phenol oxidase production by *C. neoformans*.[4]

**PRINCIPLE**
Caffeic acid and ferric citrate serve as substrates for detection of phenol oxidase which is produced by *C. neoformans.* This enzymatic reaction results in the production of melanin which is absorbed by the yeast cell wall resulting in brown-pigmented colonies.

**REAGENTS**
Reactive Ingredient: Caffeic Acid

**PRECAUTIONS**
This product is for *In Vitro* diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Directions should be read and followed carefully.

**STORAGE**
This product is ready for use and no further preparation is necessary. Store product in its original container at 2-8°C until used. Allow product to equilibrate to room temperature before use. Do not incubate prior to use. Protect from light, as the substrate is light sensitive.

**PRODUCT DETERIORATION**
This product should not be used if (1) the color has changed from white, (2) the expiration date has passed, (3) the desiccant has changed from blue to pink, or (4) there are other signs of deterioration.

Protect disks from moisture by removing from the vial only those disks necessary for testing. Promptly replace the cap and return the vial to 2-8°C.

**SPECIMEN COLLECTION, STORAGE, TRANSPORT**
Specimens should be collected and handled following recommended guidelines.[5,6]

**MATERIALS REQUIRED BUT NOT SUPPLIED**
(1) Loop sterilization device, (2) Inoculating loop, swabs, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) Forceps, (7) Filter paper, (8) Petri dish, (9) Microscope slide.

**PROCEDURE**
1. Place Caffeic Acid Disk on the agar surface of a nondextrose containing medium, such as cornmeal agar. Alternatively, place the disk on a microscope slide in a Petri dish with several drops of sterile water.
2. Place a piece of filter paper moistened with water in the lid of the Petri dish to keep the disk moist during incubation.
3. Inoculate the disk with several yeast colonies, yielding a visible inoculum.
4. Incubate aerobically at 35-37°C.
5. Examine the disk for the production of a brown pigment at 30 minute intervals for up to 4 hours.

**INTERPRETATION**
Positive Test - Brown pigmentation on disk within 4 hours

Negative Test - No color development on disk within 4 hours

**QUALITY CONTROL**
All lot numbers of Caffeic Acid Disk have been tested using the following quality control organisms and found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

| CONTROL | INCUBATION | RESULTS |
|---|---|---|
| *Cryptococcus neoformans* ATCC® 66031 | Aerobic, 4 h @ 35-37°C | Positive |
| *Candida albicans* ATCC® 10231 | Aerobic, 4 h @ 35-37°C | Negative |

## LIMITATIONS

1. Colonies for testing should be removed from a medium that does not contain dextrose, as dextrose inhibits phenol oxidase activity.[5]
2. Caffeic Acid Disk is only part of the overall scheme for identification of *C. neoformans*. Additional biochemical testing may be required for definitive identification. Consult appropriate references for further instructions.[5,6]

## BIBLIOGRAPHY


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## PACKAGING

REF R21128, Caffeic Acid Disk.....25 Disks/Vial

### Symbol Legend

| | |
|---|---|
| **REF** | Catalog Number |
| **IVD** | In Vitro Diagnostic Medical Device |
| **LAB** | For Laboratory Use |
|  | Consult Instructions for Use (IFU) |
|  | Temperature Limitation (Storage Temp.) |
| **LOT** | Batch Code (Lot Number) |
|  | Use By (Expiration Date) |



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