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The Thistle QA CEU No is: **MT00025**.

Each attendee should claim **THREE** CEU points for completing this Quality Control Journal Club exercise, and retain a copy of the relevant Thistle QA Participation Certificate as proof of registration on a Thistle QA EQA.

Cycle 23 Organism 1:

Cryptococcus neoformans

Species

The genus *Cryptococcus* includes around 37 species. Among these, [Cryptococcus neoformans](#) is the only species that is pathogenic. [Cryptococcus neoformans](#) was generally accepted to include two varieties; var. *gattii* and var. *neoformans*. Until recently and traditionally, serotypes A and D were included in var. *neoformans* while serotypes B and C were included in var. *gattii*. However, in addition to the previously observed phenotypic differences, recent molecular studies have detected significant genetic variations between the two serotypes. Thus, it was recently proposed that a new variety, var. *grubii*, be created to contain serotype A. This leaves serotype D as the sole serotype in var. *neoformans*.

The two varieties, *neoformans* and *gattii* are morphologically similar, except that basidiospores of var. *neoformans* are round and those of var. *gattii* are more elliptical in shape. The definitive identification of the two varieties is possible by biochemical tests, such as resistance to canavanine, use of glycine as the sole carbon and nitrogen source, and resistance of their urease enzymes to EDTA.

Pathogenicity and Clinical Significance

[Cryptococcus neoformans](#) is the causative agent of [cryptococcosis](#). Given the neurotropic nature of the fungus, the most common clinical form of cryptococcosis is meningoencephalitis. The course of the infection is usually subacute or chronic. Cryptococcosis may also involve the skin, lungs, prostate gland, urinary tract, eyes, myocardium, bones, and joints. The most commonly encountered predisposing factor for development of cryptococcosis is AIDS. Less commonly, organ transplant recipients or cancer patients receiving chemotherapeutics or long-term corticosteroid treatment may develop cryptococcosis.

The polysaccharide capsule and phenol oxidase enzyme of [Cryptococcus neoformans](#), as well as its ability to grow at 37°C, are its major virulence factors. Recent data suggest that phospholipase enzymes may also play a role as one of the potential virulence factors. The infection commonly starts following inhalation of the yeasts.

Laboratory Features

Colonies of [Cryptococcus neoformans](#) are fast growing, soft, glistening to dull, smooth, usually mucoid, and cream to slightly pink or yellowish brown in colour. The growth rate is somewhat slower than [Candida](#) and usually takes 48 to 72 h. It grows well at 25°C as well as 37°C. Ability to grow at 37°C is one of the features that differentiates [Cryptococcus neoformans](#) from other *Cryptococcus* spp.

On cornmeal tween 80 agar, [Cryptococcus neoformans](#) produces round, budding yeast cells. No true hyphae are visible. Pseudohyphae are usually absent or rudimentary. The capsule is best visible in India ink preparations. The thickness of the capsule is both strain-related and varies depending on the environmental conditions. Upon growth in 1% peptone solution, production of capsule is enhanced.

In Vitro Susceptibility

Amphotericin B exerts a fungicidal effect on *Cryptococcus neoformans*. As with [Candida spp.](#), the NCCLS method has difficulty distinguishing amphotericin B-resistant isolates from the susceptible ones. The other method under investigation, flow cytometry, in general yields rapid and correlated results with CLSI (NCCLS) reference method and for amphotericin B and fluconazole.

Promisingly, amphotericin B combined with fluconazole, itraconazole, or posaconazole yielded no antagonism in vitro and appeared synergistic for some isolates. In vitro synergy between amphotericin B and flucytosine or rifampin was observed for *Cryptococcus neoformans* strains isolated from patients who failed to respond to amphotericin B therapy.

Azoles, particularly [fluconazole](#) and [itraconazole](#), have been shown to have fungistatic effects against most *Cryptococcus neoformans* isolates. Although most *Cryptococcus neoformans* strains are susceptible to fluconazole, isolates with high MICs have been detected. Alternative susceptibility testing methods may yield results that differ sharply from the NCCLS reference method.

Similarly, most of the *Cryptococcus neoformans* strains are susceptible to itraconazole in vitro. Among the newer azoles, [posaconazole](#) (SCH56592) appears most potent on a weight basis, when compared to [voriconazole](#), fluconazole, itraconazole, and [ketoconazole](#).

Flucytosine has long been used as part of combination therapy of cryptococcal infections. Resistance may develop during monotherapy. Use of yeast nitrogen base as the test medium was reported to be very effective in some investigators' hands in testing flucytosine susceptibility.

[Glucan Synthesis Inhibitors](#) (Echinocandins) have essentially no activity against *Cryptococcus neoformans*. In vitro data suggest that activities of amphotericin B and fluconazole against *Cryptococcus neoformans* may be enhanced by [caspofungin](#).

In Vivo Efficacy

The most commonly used agents for treatment of cryptococcal infections are amphotericin B, flucytosine, and fluconazole, and particularly amphotericin B and flucytosine in combination. Fluconazole prophylaxis is also in common practice in patients who have recovered from cryptococcal infections. However, clinical failure with fluconazole has been reported. Fluconazole combined with flucytosine and triple therapy with amphotericin B, flucytosine, and fluconazole have been reported as effective. Itraconazole is less effective than fluconazole as maintenance therapy.

Questions:

1. Consider the common feature of the conditions under which cryptococcosis is likely to occur.
2. What is the most common point of entry of this yeast into the human body?
3. What is your laboratory's routine antibiotic susceptibility profile for this organism? In your experience, what antibiotics are normally prescribed in your hospital or clinic to treat cryptococcosis?