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CMPT Clinical Bacteriology Program

Innovation, Education, Quality Assessment, Continual Improvement

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Challenge M233-4

November 2023

CSF: *Cryptococcus neoformans*

HISTORY

A simulated CSF sample collected from a 40 year old male newly diagnosed HIV positive patient was sent to category A laboratories.

Participants were expected to isolate and report *Cryptococcus neoformans*

CMPT QA/QC/STATISTICS

All simulated CSF samples are produced at CMPT according to CMPT internal protocols. The sample contained a pure culture of *Cryptococcus neoformans.*

The samples are assessed for homogeneity and stability using in-house quality control methods and random selection of samples before and during production, and post sample delivery. The number of random samples selected is 15% of the total production batch.

The challenge sample lot was believed to be homogeneous but was later found to contain 2 different organisms: *Cryptococcus neoformans* and *Candida glabrata.*

Organism identification was confirmed by a reference laboratory.

All challenge components have in-house assigned values based on the most clinically appropriate result; the most clinically appropriate result is determined by expert committee evaluation. No further statistical analysis is performed on the results beyond that described under "Suitability for grading."

SURVEY RESULTS

Reference laboratories: 11/12 labs reported *Cryptococcus neoformans* (4 labs reported *C. neoformans* only, 7 labs reported both *C. neoformans* and *C. glabrata*), 1 lab reported *C. glabrata* and one lab does not normally process this type of sample.

Participants: 17/49 participants reported *Cryptococcus* at least to the genus level. 20/49 labs reported *Cryptococcus* and *Candida glabrata/* species (Table 1)

MAIN EDUCATIONAL POINTS from M233-4

1. *Cryptococcus neoformans* is an important cause of meningitis in immunosuppressed patients in the community.
2. When a CSF sample contains more than a single morphotype of colony it is important to rule out a mixed infection.

Suitability for Grading

A challenge is considered suitable for grading if agreement is reached by 80 percent of selected reference group and at least 50 percent of the participants.

The intended organism was detected and identified by >80% of reference labs and >50% of laboratories. Although the sample was confirmed contaminated from the production at CMPT, it was still graded as it met these criteria. As the *C. glabrata* was a contaminant of the survey sample, the isolation of it did not reach 80% amongst the reference laboratories and its addition to the sample was not standardized, isolation of it was not graded.

Grading

Maximum grade: 4

Reporting *Cryptococcus neoformans*/species was graded 4

Table 1. Identification results

Reported	Total	Grade
Cryptococcus neoformans/±gattii ± refer	13	4
Cryptococcus species, presumptive, refer	4	4
Cryptococcus neoformans/gattii, Candida species	1	4
Cryptococcus neoformans, Candida glabrata/ Nakaseomyces glabrata ± presumptive ± refer	18	4
Levures (possibilité de *Cryptococcus neoformans*), Levures autre que *Candida albicans*	1	4
2+/moderate Yeast, not *Candida albicans*, ± not *C. krusei*, refer	3	3
Yeast/Yeast species, refer	4	3
3+ *Candida krusei* (presumptive), refer	1	0
Candida glabrata	3	ungraded
Yeast species, gram negative bacillus	1	0
no report	2	0
sample not normally processed	3	ungraded
Total	**54**	

COMMENTS ON RESULTS

Despite the presence of the *Candida (Nakaseomyces) glabrata*, the majority of laboratories isolated the *Cryptococcus neoformans.* Laboratories that isolated yeast and referred the sample were graded 3 as this is a critical samples and they should have been able to at least suggest *Cryptococcus*. Laboratories that only isolated the *C. glabrata* were ungraded. Laboratories that misidentified (either) yeast or isolated additional organisms not in the original sample were graded 0.

ISOLATION AND IDENTIFICATION

Cryptococcus neoformans grows as a white or creamy colony, which becomes mucoid and eventually tan-colored.[1] All cryptococci grow well under aerobic conditions on routine agar culture media (including blood agar and chocolate agar), however, they are inhibited by media containing cycloheximide.

On primary isolation media, such as Sabouraud dextrose agar, colonies are smooth, and mucoid, especially in glucose rich media where the capsule formation is enhanced.[1,2]

All members of the genus produce urease, utilize various carbohydrates, and are non-fermentative.[3]

On Cornmeal-Tween 80 agar at 25°C for 72 hours, cells (4-8 um diameter) are round, dark walled, and single budding with a narrow neck between parent and daughter cell. Cells are characterized by the presence of a polysaccharide capsule.

The capsules are best demonstrated with an India ink preparation. Production of capsular material may be increased by growth in 1% peptone solution.[3,8] Unfortunately high-quality India ink is difficult to find, expensive and must be filtered often, so most laboratories rely on Gram smear (the cells may appear mottled, but the capsule can be observed), or histological stains such as Periodic Acid Schiff, mucicarmine, alcian blue or methenamine silver. Capsule deficient isolates can be detected using the Fontana-Masson stain.

Culture on blood agar or chocolate agar, incubated at 37°C with 10% carbon dioxide, increases the development of the capsule.[2]

There are two predominant species that are pathogenic to humans: *C. neoformans* and *C. gattii*. Differentiation between these two species is usually difficult for regular laboratories and specialized agar and/or molecular methods must be utilized. MALDI-Tof is currently the method of choice to differentiate the two species.[4]

A solid agar medium containing canavanine, glycine and bromothymol blue (CGB agar) is the medium of choice to differentiate *C. gattii* from *C. neoformans*. This test is based on the ability of *C. gattii* isolates to grow in the presence of L-canavanine and to assimilate glycine as a sole carbon source. Growth and a color change to "vivid cobalt blue" after 48 hours incubation indicate *C. gattii*. No growth or minimal growth (medium yellow or green) indicate *C. neoformans*.[5] If *Cryptococcus* spp are isolated periodically, a laboratories might find it useful to have CGB plates available to advance identification.

ANTIMICROBIAL SUSCEPTIBILITY

CLSI currently does not have interpretive breakpoints for susceptibility testing of *Cryptococcus* species. For *Cryptococcus neoformans* it is appropriate to report out MIC's with no interpretation.[11] Another alternative is to use the epidemiologic cutoff values (ECVs) published in the CLSI M59Ed 3 (2020) document which are available for *Cryptococcus neoformans* VN1 and the most commonly used antifungal agents. Most laboratories, however, do not perform molecular genotyping of *Cryptococcus* species, so it is only appropriate to use the ECVs when molecular typing has been completed or if the VN1 is the most common circulating molecular genotype in the region. The ECV can be used to indicate if resistance mechanisms are likely to be present in isolates, if the CLSI method described in CLSI M27Ed4 is used, and may be of help to guide clinical decisions. Reporting the MIC and indicating if the value exceeds (or not) the ECV is appropriate. It is useful to indicate the implications of the ECV (and how it differs from a breakpoint) as many clinicians may not be familiar with this value.

EUCAST has published interpretive breakpoints for Cryptococcus neoformans and amphotericin B (S≤1 ug/mL; R>1 ug/mL). It is appropriate to use the EUCAST interpretive criteria only when following the EUCAST yeast susceptibility testing method (Note: the EUCAST yeast susceptibility testing method differs from the CLSI yeast susceptibility testing method).

CLINICAL RELEVANCE

The genus *Cryptococcus* contains two pathogenic complexes, *C. neoformans* and *C. gattii*. The former has a cosmopolitan distribution and is found especially in bird droppings and trees whereas *C. gattii* is found in trees and decaying wood and is prevalent in tropical, subtropical areas, and south-western British Columbia and the northwestern states in the continental United States. It is found sporadically elsewhere. Humans contract infections from exposure to the fungus in the environment. [2,6]

Cryptococcosis caused by *C. neoformans* is contracted by inhalation of desiccated yeasts or spores; most infections are benign, and self-limited, but may establish a latent or dormant state. If the host becomes immunocompromised, the infection can reactivate and disseminate; the organism shows particular tropism for the central nervous system causing meningitis.[7]

Progressive forms of *C. neoformans* infection are linked to defects in cell mediated immunity in contrast to *C. gattii* infections that often occur in immunocompetent patients.

Once a rare disease (in the 1950s fewer than 300 cases of cryptococcosis were reported worldwide),[13] disseminated cryptococcosis has been associated with more than 600,000 deaths at the peak of the HIV pandemic.[8] Disseminated cryptococcosis presents as a severe infection with multi-organ involvement in severely immunosuppressed HIV-positive patients with a CD4+ cell count of less than 100/µL.[2] This figure has been reduced by the impact of effective antiretroviral therapies.

Predisposing factors to cryptococcosis in non-AIDS patients are: aggressive immunosuppressive treatments in organ transplant patients, high doses of corticosteroids, sarcoidosis, and lymphoma.[9]

Rapid detection of cryptococcal infection is possible using the antigen test, which detects glucuoronoxylomannan in the capsule, and is commercially available in several test formats. The test is sensitive and specific, and can be used on sera and CSF. Testing allows the diagnosis of infection within hours.

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