Challenge MY2208-1

August 2022

CSF: *Cryptococcus neoformans*

HISTORY

This challenge was sent as a simulated cerebrospinal fluid (CSF) culture sample from a 40 year old HIV+ patient. Laboratories were expected to isolate and identify *Cryptococcus neoformans* and perform susceptibility testing to their lab ability.

CMPT QC/QA/Statistics

All Mycology samples are produced at CMPT according to CMPT internal protocols.

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 sample was verified by a reference laboratory. *Cryptococcus neoformans* was isolated as a pure culture.

The challenge sample lot was confirmed to be homogeneous and stable for 49 days.

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.

SURVEY RESULTS

10/10 (100%) processing laboratories correctly reported *Cryptococcus neoformans* (Table 1). Three laboratories performed susceptibility testing (see Table 2 for results) and 7 indicated they would refer the sample for testing.

Table 1. Identification results

Reported	Labs	Grade
Cryptococcus neoformans	10	Acceptable
Sample not normally processed	1	Ungraded
Total	**11**	

Table 2. Susceptibility results reported

Amphotericin B	Fluconazole	5-Fluorocytosine
Susceptible (1ug/mL)		
*INE (2) MIC: 0.25ug/mL	*INE (2) MIC: 4ug/mL	*INE (2) MIC: 8ug/mL

INE: interpretation not established

One laboratory commented: "This susceptibility method has been designated for "Research Only" purposes and has been internally validated for diagnostic use in the Microbiology Laboratory. Standardized interpretive criteria or clinical breakpoints are not available for all organism-drug combinations; in these cases an interpretation is not provided."

Reference lab reported: AmB MIC = 0.25ug/mL; Fluconazole MIC = 4ug/mL; and 5-Fluorocytosine MIC = 4ug/mL

COMMENTS ON RESULTS

The susceptibility testing results reported are appropriate. CLSI does not have interpretive breakpoints for *Cryptococcus neoformans*, so reporting an MIC with no interpretation is appropriate.

EUCAST has an interpretive breakpoint for amphotericin B and *Cryptococcus neoformans*, so it would be appropriate to report the organism as susceptible to amphotericin B so long as that laboratory is following the EUCAST yeast susceptibility testing method.

IDENTIFICATION

The optimal CSF volume for mycological analysis is 3 to 5ml. CSF sent for fungal cultures should be centrifuged when the volume exceeds 2ml.The supernatant can be used for antigen detection tests while the sediment is used for culture and direct examination.

Inoculation of more than 0.5ml of CSF onto an agar slant is usually counterproductive; *Cryptococcus neoformans* will not grow on agar covered with liquid CSF.[1]

Grading

Reporting *Cryptococcus neoformans* was graded Acceptable.

Primary Culture Media

Specimens must be inoculated onto media that ensure the growth of the etiologic agents of fungal meningitis: *Cryptococcus neoformans*, *Candida* species and more rarely *Histoplasma capsulatum* and *Blastomyces dermatitidis* and *Coccidioides* species.[2]

Cycloheximide-containing media should be avoided since it is known to inhibit the growth of *Cryptococcus neoformans* and some *Candida* species.[1,3]

It is also recommended that enriched media, without antimicrobial agents, are used to ensure the growth of aerobic actinomycetes and fastidious thermally dimorphic fungi.[3]

Direct examination

Presumptive laboratory diagnosis is often based on the observation of the characteristic extracellular polysaccharide capsule by means of the India ink preparation method.

The capsules vary from 2-10mm but they tend to be smaller in specimens from immunocompromised patients.

Sediment examination of CSF is usually positive in approximately 40% in patients without AIDS and in 80% of patients with severe immunodepression. False positives are usually due to confusion with lymphocytes.[4]

Antigen detection

The cryptococcal polysaccharide capsular antigen can be detected via latex agglutination (LA) or lateral flow methods.[5]

In a comparison of commercial methods for cryptococcal antigen testing, LA methods demonstrated to have a sensitivity of 83-100% with specificities of 95-100%.[6]

Positive serum cryptococcal antigen results have been reported in 88–91% of solid organ transplant recipients with cryptococcal meningitis.[7]

Blood cultures should be obtained routinely in AIDS patients because they are often positive and have prognostic value.[4]

The search for specific antibodies to *Cryptococcus* is not a useful procedure for diagnosing cryptococcosis as these have been detected in healthy individuals also.[4]

Colony morphology

Cryptococcus neoformans grows as a white or creamy colony, which becomes mucoid and eventually tan-colored.[3]

All cryptococci grow well under aerobic conditions in routine agar culture media, 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.[3,4] Colonial colour is white-to-cream cream at first, later becoming tannish.[3]

All members of the genus produce urease, utilize various carbohydrates, and are non-fermentative. [8]

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 PAS or methanamine silver.

Culture on blood agar or chocolate agar, incubated at 37°C with 10% carbon dioxide, increases the development of the capsule.[4]

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.[9]

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*.[10] Considering the availability and facility of use of CGB agar, more laboratories might consider having plates available to advance identification.

ANTIFUNGAL SUSCEPTIBILITY

CLSI currently does not have interpretive breakpoints for *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 ECVs are available for *Cryptococcus neoformans* VN1 and several different 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 you are confident that VN1 is the most common circulating molecular genotype in your region.

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). Prior to reporting yeast susceptibility results with EUCAST breakpoints, the laboratory should ensure that the EUCAST method is followed.

CLINICAL RELEVANCE

The genus *Cryptococcus* contains two pathogenic species, *C. neoformans* and *C. gattii*. The former has a universal geographic distribution and is found especially in bird droppings and tree bark whereas *C. gattii* is prevalent in tropical and subtropical areas and humans primarily contract infections through the infected leaves, bark, and fruits of trees.[1,4]

Cryptococcosis is contracted by inhalation of desiccated yeasts or spores; most infections compromise the airway, are benign, and self-limited. If the host becomes immunocompromised, the infection can emerge and disseminate; the organism shows particular tropism for the central nervous system causing meningitis.[12]

Progressive forms of cryptococcosis may be linked—or not—to factors that modify host defense mechanisms.

Once a rare disease (in the 1950s fewer than 300 cases of cryptococcosis were reported worldwide),[13] disseminated cryptococcosis has been associated to more than 600,000 deaths at the peak of the HIV pandemic.1[4] 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.[4] This figure has been reduced by the impact of effective antiretroviral therapies.

Predisposing factors to cryptococosis in non-AIDS patients are: aggressive immunosuppressive treatments in organ transplant patients, high doses of corticosteroids, sarcoidosis, and lymphoma.[15] Cryptococcosis linked to *C. gattii* often occurs in a healthy host and presents more often with lung involvement. Outbreaks have been identified in Vancouver, British Columbia and the Northwest USA.

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