Challenge M241-2

May 2024

Vitreous fluid: *Candida albicans*

HISTORY

A simulated vitreous fluid aspirate sample collected from a 35 year old male, in-patient, with leukemia was sent to category A laboratories. Participants were expected to isolate and report *Candida albicans*.

CMPT QA/QC/STATISTICS

All simulated fluid samples are produced at CMPT according to CMPT internal protocols. The sample contained a pure culture of *Candida albicans*.

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 confirmed to be homogeneous and stable for 16 days. 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: 12/12 (100%) labs reported Candida albicans, 1 lab reported it does not normally process this type of sample

Participants: 40/47 (85%) processing labs reported Candida albicans. Two participants reported Candida species and 5 reported Yeast, refer (Table 1)

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.

MAIN EDUCATIONAL POINTS from M241-2

1. Endophthalmitis is often caused by infectious agents and is a serious sight threatening condition.
2. Microscopy of vitreous specimens can be useful but experience is needed to avoid false positives resulting from misidentification of pigment particles.
3. Cultures are important to enable guidance for appropriate treatment as soon as possible.
4. Speciation of *Candida* species is helpful to suggest the likely susceptibility pending susceptibility testing results.

Organism identification was correctly performed by at least 80 percent of reference laboratories and greater than 50 percent of all laboratories and was thus, determined to be suitable for grading.

Grading

Maximum grade: 4

Reporting *Candida albicans* was graded 4.

COMMENTS ON RESULTS

Identification of *Candida albicans* was graded 4. The responses "yeast" and "*Candida* species" was graded 4 also as referral would allow further speciation and testing. The response "*Candida* species" without referral was graded 1 as the speciation can be useful to determine empiric treatment, and no referral was indicated.

Table 1. Identification results

Reported	Total	Grade
Candida albicans	40	4
Candida species, refer	1	4
Yeast, refer	5	4
Candida species	1	1
refer, sample not normally processed	4	ungraded
Total	**51**	

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ISOLATION AND IDENTIFICATION

Intra-ocular specimens include aqueous humor from the anterior part of the eye, and vitreous humor from the part of the eye posterior to the lens and associated structures. Vitreous samples are obtained in most cases, and may be aspirates or vitrectomy specimens. Aspirates are easier to obtain but less sensitive for detection of infectious agents. Vitrectomy is a more invasive procedure that requires the removal of a portion of the intra-ocular gel. Direct microscopy is a rapid and cost-effective means of diagnosing ocular fungal infections. Pigment particles may be seen and may be confused with gram positive cocci. Pigment tends to be refractile, brownish, less round than cocci, and smaller than yeast.[21] Culture is more sensitive than microscopy and important to allow identification and susceptibility to antifungal agents. The sensitivity of vitrectomy culture has been reported to be 38-90%, for vitreous aspirates 25-70%, and 40% for aqueous aspirates.[20,21,22]

Identification to the species level is important as some *Candida* species have high rates of resistance to azoles, which are the antifungals that are often used for treatment. Polymerase chain reaction (PCR) has been used in the diagnosis of ocular fungal infections and appears to be more sensitive than culture. [21,22]

On solid media *Candida albicans* rapidly matures in 1 to 3 days as white or cream, smooth and glistening colonies. *C. albicans* grows in the presence of cycloheximide usually present in selective media, whereas most other yeasts, including *Cryptococcus neoformans*, are inhibited.[3]

When grown on enriched agar (blood and chocolate) small extensions ('feet') may be seen around the border of the colony.

Microscopic morphology and Identification

A wet preparation examination of a colony grown on routine primary media (e.g., FSA, Sabouraud) reveals round to oval budding yeast-like cells or blastoconidia, measuring 3.5-7 by 4-8 µm.[2] On Gram staining the yeast cells retain crystal violet.

On cornmeal-Tween 80 agar incubated at 25°C for 72 hours, pseudohyphae (some true hyphae may be present) with clusters of round blastoconidia at the septa may be easily found. *Candida albicans*, together with *Candida dubliniensis*, are the two *Candida* species that produce large thick-walled asexual spores called chlamydoconidia (chlamydospores). In *C. albicans* these terminal vesicles are usually single, while isolates of *C. dubliniensis* will produce them in pairs, triplets, and clusters.[4] They are most likely seen near the edge of the coverslip, but are inhibited if the medium is incubated at 30-37°C. *Candida albicans* is readily identified by the MALDI-TOF. The germ tube test is insensitive and does not differentiate *Candida albicans* from *Candida dubliniensis*. Chromagars are available that differentiate *Candida albicans* from other *Candida* species (although possibly not *C. dubliniensis*).

ANTIMICROBIAL SUSCEPTIBILITY

Fungal infections of the eye are treated as an emergency to prevent serious consequences.[5] However, the management of fungal eye infections is challenged by the availability of effective antifungal agents and poor eye penetration. [6,7] Susceptibility testing of intra-ocular yeast isolates should always be done, and should include fluconazole, voriconazole and amphotericin. Echinocandins do not penetrate the eye well, and are not useful for treatment of intraocular infection.[22]

Broth macro-dilution and micro-dilution reference methods are now available for susceptibility testing of yeasts (CLSI document M27-A3:2008 – Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts).[8] A more recent revision of an international standards microbroth dilution document for yeast fungi (ISO 16256:2021)[9] has been published with updated quality control and performance criteria. Laboratories should ensure they are using the most up-to -date documentation for test performance. A disk diffusion method for testing *Candida* species against fluconazole and this standard is used by manufacturers to quality control their microdilution panels. voriconazole has been also developed (CLSI document M44-A – Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts.[10] Because of increasing incidence of *Candida* infections in hospitals, laboratories in large tertiary care facilities should be able to perform antifungal testing against primary agents used to treat these infections, or refer to reference laboratories that can provide rapid turn-around of results.

Fluconazole still tends to be quite active against most isolates of *Candida albicans* (1.2% of isolates reported as resistant)[11] and this rate has remained stable despite the continued widespread use of fluconazole both for therapy and for prevention.

Voriconazole has better penetration into the eye than fluconazole and can be used for some isolates resistant to fluconazole.[22] Liposomal amphotericin B can used for isolates with resistance to the azoles (and may be used in combination with flucytosine). Amphotericin is also sometimes injected into the eye at the time of vitrectomy.

CLINICAL RELEVANCE

Fungal eye infections affect more than one million people annually.[5] Keratitis (infection of the cornea) is the most frequent form, but the orbit, eyelids, lacrimal apparatus, conjunctiva, sclera, and intraocular structures (endophthalmitis) can also be affected.[13] Fungal eye infections are less common compared to bacterial or viral infections, but they are usually serious and can lead to vision loss.[1]

Fungal keratitis may develop as a result of the traumatic inoculation of plant material contaminated by fungi thus, filamentous and saprophytic species constitute the dominant group of pathogens in these cases.[16,17] It may also be the result of contamination of contact lenses in contact with defects in the cornea.

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The most common fungal etiologic agents in eye infections are the genera *Aspergillus*, *Candida*, and *Fusarium*, followed by other genera such as *Blastomyces, Cryptococcus*, and *Sporothrix*.[14,15]

Endophthalmitis is a condition where there is inflammation of the internal ocular structures, including the vitreous or aqueous humors. Most cases are caused by infectious agents. Exogenous endophthalmitis occurs when the infection spreads to the eye from the outside, for example, after surgery, or because of keratitis. [17] Mold infections predominate in the fungal causes of exogenous endophthalmitis. Endogenous endophthalmitis occurs when the eye is infected via the blood stream from an infectious focus elsewhere in the body. [16,18] Molds predominate in exogenous fungal endophthalmitis, but *Candida albicans* is the most common cause of endogenous fungal endophthalmitis. [19] Risk factors for this infection include immunosuppression combined with a source of fungemia, such as central lines, prolonged broad spectrum antimicrobial use, or granulocytopenia.

Fungal endophthalmitis tends to have a less inflammatory presentation than bacterial endophthalmitis. Symptoms may take days to develop. *Candida* endophthalmitis may have few symptoms and patients may be too ill to convey symptoms to caregivers. Recommendations for screening for ocular infection in patients with candidemia have been made by the Infectious Disease Society of America. Debate about screening continues with the American Academy of Ophthalmology and may be particularly useful in cases where *C.albicans* is the cause of bacteremia and the patient is receiving TPN. [23] Treatment that is prompt and effective can minimize damage and preserve vision.

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