Cryptococcus Detection by the Biofire® FilmArray® Meningitis/Encephalitis (ME) Panel

TECHNICAL
::: NOTE

1. Introduction

The purpose of this technical note is to examine the use and interpretation of the BioFire ME Panel as an aid to the diagnosis of cryptococcal meningitis. The note provides background information about the organism, the disease, and the relative sensitivity and specificity of various laboratory methods for the detection of *Cryptococcus*.

2. *Cryptococcus* spp.

Cryptococcal disease is caused by two closely related species of yeast: *Cryptococcus neoformans* and *Cryptococcus gattii.* Although both species cause pulmonary and central nervous system (CNS) infections, they differ in their ecology, epidemiology, and pathobiology[1,2]. *C. neoformans* is the most common *Cryptococcus* spp. worldwide and mainly affects immunocompromised hosts (primarily HIV infected persons with low CD4 counts and solid organ transplant patients on immune-suppressive drugs). In contrast, *C. gattii* mainly affects immunocompetent hosts and often forms mass-like lesions called cryptococcomas. Missed or delayed diagnosis of cryptococcal disease can lead to poor clinical outcomes.

3. Diagnosis of Cryptococcal meningitis

The diagnosis of cryptococcal meningitis can be aided by various laboratory techniques. India (China) ink staining of cerebrospinal fluid (CSF) is still a common tool but the sensitivity is generally <86% and is reduced to 42% if the fungal burden is low (1,000 colony forming units (CFU)/mL or fewer), which is common in persons presenting early after symptom onset or those presenting on antiretroviral therapy[3-5]. Culture of CSF is considered the gold standard for diagnosis of cryptococcal meningitis, but has several disadvantages including 1) slow growth (up to 7 days or up to 10 days for accurate quantification) and 2) poor sensitivity when fungal burden is low leading to false negative results[1,2,4]. Nevertheless, fungal culture should be performed on all CSF specimens when cryptococcal infection is suspected. Culture is central in the diagnosis and differentiation of cryptococcal meningitis relapse versus paradoxical immune reconstitution syndrome (IRIS) due to initiation of highly active antiretroviral therapy (HAART)[1,2,4].

The **most sensitive test** for the diagnosis of primary cryptococcal meningitis is the detection of cryptococcal antigen (CrAG - major capsular polysaccharide) in CSF and/or blood serum[1,2,4]. CrAG is shed in large

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amounts in the blood and CSF and can be detected even prior to the onset of clinical symptoms. The initial CrAG titer is also prognostic. False negative CrAG tests can be the result of several factors, including low fungus load, prozone reaction due to high antigen titers (≥1:256), immune complexes preventing antigen shedding, hypocapsular (small levels of capsule) or acapsular (lacking a capsule) strains of *Cryptococcus* [2,6-9].The disadvantage of CrAG is that it can remain positive for months to years after fungal clearance from the CSF and thus cannot be used for a test of cure (requires culture) or for differentiation of fungal relapse from IRIS[1,2,4].

PCR-based diagnosis of cryptococcal meningitis has not been widely developed given the high sensitivity, wide availability, and low cost of CrAG testing. Data on the performance of PCR in comparison to India ink, culture, and CrAG is limited, especially from clinical specimens[3,9-13]. However, there are some data comparing the detection of *Cryptococcus* by CrAG, culture, and the BioFire® FilmArray® Meningitis/Encephalitis (ME) Panel[11,13,14].

Table 1. BioFire ME Panel Cryptococcus Detections

Source	CrAG positive samples	Fungal culture positive samples
BioFire IFU	1/8 (12.5%)	2/3, (66.6%)
Leismen et.al	26/50 (52%)	13/14 (92.9%)
Rhein, et. al.	Unknown	49/51[a] (96%)
Overall		64/68 (94.1%)

[a]defined as >100 cfu/mL

Correlation with CrAG assays - The table above shows that a very high proportion of samples that are positive by CrAG are not detected by the BioFire ME Panel. However, in many of these cases the samples were collected after the initial diagnosis of cryptococcal meningitis and most were already on antifungal therapy or had a previous diagnosis of cryptococcal meningitis (7/8 from the BioFire IFU and ~50% in the Leisman publication). As previously discussed, CrAG will remain positive for months to years after initial infection and even after resolution of the infection.

Correlation with fungal culture – Based on currently available data, the sensitivity of the BioFire ME Panel Cryptococcal assay is approximately 94% as compared to culture and the sensitivity is impacted by fungal burden.

However, there have been reports of negative results by the BioFire ME Panel in patients with newly diagnosed cryptococcal meningitis and positive CrAG and/or culture[10,15]. Additionally, discordant CrAG and BioFire ME Panel results have been observed in persons either on antifungal treatment at the time of testing or with a past history of treated disease in which the CrAG remains positive[11,12,14].

Hence the tests of choice for detection of primary disease remain CrAG with concomitant fungal culture. Fungal culture is also required to determine therapeutic response (fungal clearance from the CSF) and to determine true disease relapse versus IRIS.

Patients with a suspicion of cryptococcal meningitis and a negative cryptococcal PCR result, such as by the BioFire® FilmArray® Meningitis/Encephalitis (ME) Panel, should be tested for CrAG. Additionally, a CrAG test can determine the baseline titer for patients with a positive cryptococcal PCR result.

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