DETECTION OF CRYPTOCCOCCAL CAPSULAR POLYSACCHARIDE ANTIGEN OF Cryptococcus spp. FROM CULTURE SUSPENSIONS BY IMMUNOASSAY (CRAG® LFA)

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Abstract:

The Cryptococcus neoformans/C. gattii complex is formed by yeast fungi that can cause cryptococcosis, an opportunistic disease that affects immunosuppressed individuals, especially those with human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS). CrAg® LFA is a serologic rapid test for the diagnosis of cryptococcal meningitis, being able to detect cryptococcal antigen (CrAg) in cerebrospinal fluid (CFS), plasma and serum. Thus, we sought to evaluate the accuracy of the assay using cultures of C. neoformans/C. gattii suspended in saline (0.9% sodium chloride solution) in the Laboratório de Micologia (LabMicol). All Cryptococcus isolates were CrAg positive, demonstrating the clinical utility of the test, however a greater number of isolates will be further evaluated. Therefore, we suggest that CrAg® LFA can be adapted for cultures suspensions.

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Introduction:

Cryptococcosis is a fungal disease caused by Cryptococcus neoformans/C. gattii complex with internal morphology similar to the other yeasts with oval or globose blastopores with whitish coloration and mucoid texture, however surrounded by a prominent mucopolysaccharide capsule, composed of glucuronoxylomannan (GXM) and galactoxylomannan (GALXM) which contributes to its virulence (DE JESUS et al., 2010). These fungi are found in soil, accumulated birds droppings, mainly domestic pigeons, decaying wood and hollows of live trees (NWEZE et al., 2015; ANDREADE-SILVA et al., 2018).

Cryptococcus neoformans is the main cause of cryptococcal meningitis in immunocompromised individuals with HIV/AIDS (PARK et al., 2011). The infection occurs through the inhalation of propagules from the environment, mainly affecting the lungs, but can spread through the hematogenic pathway and reach the nervous system (KWON-CHUNG et al., 2014), causing neurocryptococcosis (MITCHELL; PERFECT, 1995), a frequent AIDS-defining disease worldwide that can reach 63% fatality of patients in Brazil, which can be reduced by screening for the cryptococcal antigens in the serum and in the case of positivity, initiate early treatment (VIDAL et al., 2013).

CrAg® LFA is a serologic rapid test on strips for diagnosis of cryptococcal meningitis, reportedly more rapid and sensitive than standard latex agglutination and enzyme-linked immunosassay (VIDAL; BOULWARE, 2015). It is an immunochromatographic lipid assay that allows detection of cryptococcal polysaccharide antigen in plasma, serum and CSF with more than 90% sensitivity (WILLIAMS et al., 2015).

Tests were carried out in order to evaluate the exequibility of CrAg® LFA for cryptococcal capsular antigen detection from culture suspensions of yeast cells (not yet suggested as an option for CrAg® LFA). The capsular antigen, among with other virulence factors, such as thermotolerance, as well as laccase, phospholipase, lipase, protease and urease production are essential for its pathogenicity (CHATTERJEE; TATU, 2017). The presence and integrity of the capsule will determine the intensity of the test result.

We aimed to evaluate the accuracy of the CrAg® LFA assay using stored cells cultures of Cryptococcus neoformans/C. gattii suspended in saline, mainly in cases where culture could be necessary to confirm cases of false negative results.

Metodology:

The study was conducted in the Laboratório de Micologia (LabMicol)/Laboratório de Microbiologia Clínica (LMC) of the Instituto de Ciências Biológicas e da Saúde (ICBS) of the Universidade Federal de Alagoas (UFAL) during December 2017 and February 2018.

Cryptococcus isolates were incubated on Sabouraud dextrose agar (SDA) at 30°C for 48h in order to
evaluate the accuracy of the kit. The tests were done following the manufacturer's instructions: one drop of sample diluent was added to the microtube, then 40μL of the isolates (C. neoformans/C. gattii dissolved in saline) was added to the microtube and mixed. The white end of the test strip was submerged in the sample and after ten minutes the results were read (Figure 1).

![Figure 1: Cryptococcal antigen lateral flow assay (CrAg® LFA). Source: http://www.immy.com](image)

The test uses gold-conjugated, anti-CrAg monoclonal antibodies and gold-conjugated control antibodies impregnated on the test membrane. If CrAg is present in the specimen or cell suspensions, it links to the gold-conjugated, anti-CrAg antibodies. The gold-labeled antibody-antigen complex migrates up the test membrane by capillary action and will interact with immobilized anti-CrAg monoclonal antibodies, forming a sandwich and creating a visible red line (test). Independent of the assay being positive or not, the gold-conjugated control antibody rises through the membrane and reacts with the immobilized antibodies, which forms a second red visible line (control). In summary, positive test results creates two lines and negative results only one line (IMMY, 2018).

Results and Discussion:

CrAg® LFA uses monoclonal antibodies which allows for consistent performance. The lateral flow assay uses a combination of two monoclonals, one of them is highly reactive to the cryptococcal antigen (CrAg) of serotypes A (Cryptococcus neoformans var. grubii), B and C (C. gattii), and the second one is highly reactive to CrAg of serotypes A and D (C. neoformans var. grubii and C. neoformans var. neoformans, respectively). Used together, the antibodies are highly reactive across the range of cryptococcal serotypes, an advantage when compared with CrAg-latex or EIA (VIDAL; BOULDAWARE, 2015).

Four samples of C. neoformans and one of C. gattii were tested (sample selected in Figure 2). The test line varied in color intensity, but all of them had a positive result (100% sensitivity) (Figure 3).

![Figure 2: Cryptococcus neoformans on Sabouraud dextrose agar (SDA) after incubation at 30°C for 48h (right) and positive CrAg result (left).](image)
In general, the band is intense when testing fresh biological samples with considerable presence of the antigen, however, in this study the cells were preserved in SDA culture medium, contributing to the loss of capsular material, and consequently reduced band (line) intensities. Sensitivity of CrAg® LFA was reported high by Huang et al. (2015) for serum (97.6% of the samples), CSF (98.9%), and urine (85%). The same behavior was shown by Vidal et al. (2018) for whole blood (95%), and 88% in saliva of symptomatic patients (KWIZERA et al., 2014).

After undertaking a literature search we could not find records of the use of yeast cells suspensions for CrAg detection. Using cells derived from fungal culture may be helpful in cases where specimens were discarded or as suggested by Perfect; Bicanic (2015), in case of false-negatives due to prozone phenomenon or low fungal load, especially with poorly encapsulated yeasts or other organisms with similar macromorphological appearance, such as Candida spp. In these cases, the sample from cultures could confirm the identity of Cryptococcus sp. Additionally, there are records of false-negative results by CrAg latex agglutination assay from the CSF because of capsule-deficient yeasts, failed to be detected many times on CSF, due to the susceptibility of these yeasts to be caught by macrophages on its way to the lumbar CSF space (SUGIURA et al., 2005).

These results implement the range of specimens that can be evaluated by the CrAg® LFA, contributing to optimize diagnosis time and resources, avoiding new invasive procedures for the patient when recollection is needed in order to confirm the identity of the yeast. In addition, we can point some advantages of the test: only ten minutes assay time, does not require specimen or culture pretreatment, qualitative results and no external control requirements (IMMY, 2018).

Conclusions:

We suggest that CrAg® LFA could be adapted for cryptococcal cell cultures to confirm results obtained from the biological specimens (plasma, serum or CSF) that were discarded, avoiding new recollections, as well in cases of false-negatives.

Bibliographic references:


Figure 3: Test strips with positive results (first red line) independent of its intensity (Source: http://www.immy.com/products/lateral-flow-assays/crag-lfa/).


