Semi-quantitative cryptococcal antigen rapid test (CryptPS, Bioxyn®) for cryptococcal meningitis in patients living with HIV in Sub-Saharan Africa: prospective multicenter diagnostic accuracy study (DREAMM)

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Poster session 5, September 23, 2022, 12:30 PM – 1:30 PM

Background: Cryptococcal meningitis (CM) remains a leading cause of HIV-related meningoencephalitis in African low- and middle-income countries (LMICs), causing 15%-20% of HIV-related deaths. Rapid Diagnostic Tests (RDTs) are powerful tools and key to speeding-up the diagnosis at the bedside, allowing for rapid and targeted treatment, especially in LMICs. For the past 10 years, Cryptococcal Antigen (CrAg) RDTs have a major role in CM management.

Diving Reduced AIDS Meningo-Encephalitis Mortality (DREAMM) was a multicenter implementation science study and a capstone building project to reduce the mortality of HIV-related central nervous system infections (CNS). One of the main DREAMM approaches was to improve the diagnosis of CNS infections at the bedside and in parallel in local laboratories. Within DREAMM, HIV-infected, adult people living with HIV (>18 years old) with suspected CNS infections were recruited in five hospitals sites in Cameroon, Malawi, and Tanzania.

Objectives: Our objective was to evaluate the implementation of CrAg CryptoPS (Bioxyn®; Béziers, Hérault, France), a new semi-quantitative RDT, in routine case settings in Sub-Saharan Africa.

Methods: All CrAg CryptoPS performed were compared to the reference CrAg lateral flow assay (Immuno®). The evaluation was done by the local research teams in four DREAMM laboratories sites. CrAg CryptoPS implementation was evaluated in 301 plasma samples and 258 cerebrospinal fluid (CSF) samples from 520 participants (patients diagnosed with cerebral toxoplasmosis did not have a lumbar puncture). In this analysis, the results will be considered in a binary way (positive/negative).

Results: Between January 2018 and March 2021, 516 participants were prospectively enrolled with suspected HIV-related CNS infections, including CM, tuberculosis meningitis, cerebral toxoplasmosis, and bacterial meningitis cases. Cryptococcal meningitis was the leading cause of CNS infections in Malawi and Tanzania with 64.3% (183/284) and 39.6% (199/505) cases respectively, and the second cause of CNS infections in 40.1% (199/505) cases after cerebral toxoplasmosis.

In plasma, CryptoPS sensitivity was 99.23% (95% CI, 0.98-1.00) and specificity was 94.15% (95% CI, 0.89-0.96); positive and negative predictive values were 92.8% and 99.4%, respectively. In CSF, the sensitivity and specificity of CryptoPS were 100% (95% CI, 0.00-1.00), and 99.26% (95% CI, 0.98-1.00), respectively, positive and negative predictive values were both 100%.

Conclusion: CryptoPS was evaluated in a context of hospitalized patients within a project including all causes of HIV-related CNS infection, not only CM. The sensitivity and specificity of CryptoPS calculated in these preliminary results are promising. Semi-quantitative CryptoPS has the potential to be used to tailor antifungal therapy but further optimizations need to be done prior to large-scale implementation in African LMICs. In addition, future work to determine CrAg antigen titres is planned, in the perspective to optimize treatment of CrAg positive cases who decline lumbar puncture.

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Estimation of the detection limit of extracted Candida DNA from spiked EDTA blood sample

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Poster session 5, September 23, 2022, 12:30 PM – 1:30 PM

Background: Candidiasis caused by species Candida, are opportunistic pathogens with life-threatening infection in immuno-compromised individuals with a mortality rate being 40%-64% in patients. Blood culture is not a reliable and rapid method for the diagnosis of candidemia as its sensitivity is as low as 50%. Molecular diagnosis is an alternative to conventional culture for early detection of candidemia. An appropriate DNA isolation technique is needed to obtain high purity DNA from blood specimen and improve the sensitivity of the polymerase chain reaction (PCR). In this study, two different methods were used to extract Candida DNA from spiked EDTA blood specimens, the In-house phenol-chloroform and potassium acetate methods.

Objectives: DNA extraction from the spiked blood samples

Comparison of the Candida DNA extracted from the spiked sample

The extracted Candida DNA extracted by phenol-chloroform and potassium acetate method

Methods: A total of 5 ml of EDTA blood samples from healthy volunteers were spiked with 104-106 Candida albicans cells (ATCC 90028) to determine the detection limit of our extraction method. DNA was extracted from whole blood using phenol-chloroform and the potassium acetate methods which involve precipitation with ammonium, isopropanol and buffer B. DNA was used as a template in PCR using primers specific to Candida DNA.

Results: The spiked Candida DNA was extracted from 104-108 yeast cells. The extracted DNA using the phenol-chloroform extraction method was ranging from 18.29 to 51.44 ng/µl respectively. Whereas the total extracted DNA from potassium acetate extraction method ranged from 35.58 to 32.50 ng/µl respectively. The presence of a 535 bp base pair (bp) product was considered positive (Figure 1 and 2). The lower detection limit of PCR has been determined as 186±104 Candida cells for our spiked samples.

Conclusion: In our study, both the extraction methods of DNA was found to be higher by phenol-chloroform method as compared to the potassium acetate method. Further optimizations are required to obtain conclusive data.