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

Research Engineer Aude Sturny Leclere[1], Emma Beaumont[6], Cecilia Kanyama[2], Sayoki Mfinanga[4], Charles Kouanfack[3], Sokoine Lesikari[4], Saulos Nyirenda[5], Samuel Phiri[5], Timothée Boyer-Chammard[9], Síle Molloy[8], Jérémie F. Cohen[10], Mina Hosseinipour[2], John Bradley[6], Shabbar Jaffar[7], Thomas Harrison[8], Olivier Lortholary[1,11], Angela Loyse[8]

[1] Molecular Mycology Unit, Institut Pasteur, Paris, France
[2] University of North Carolina Project–Malawi, Lilongwe, Malawi
[3] University of Dshang, Dschang, Cameroon
[4] National Institute for Medical Research Muhimbili Medical Research Centre, Dar es Salaam, Tanzania
[5] Zomba Central Hospital, Zomba, Malawi
[6] London School of Hygiene & Tropical Medicine, London, United Kingdom
[7] Liverpool School of Tropical Medicine, Liverpool, United Kingdom
[8] St George's University of London, London, United Kingdom
[9] Department of Infectious Diseases and Tropical Medicine, Centre Hospitalier d'Ajaccio, Ajaccio, France
[10] Service de Pédiatrie Générale et Maladies Infectieuses, Hôpital Necker - Enfants malades, Paris, France
[11] National Reference Center for Invasive Mycoses and Antifungals, Institut Pasteur, France

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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.

Driving Reduced AIDS Meningo-Encephalitis Mortality (DREAMM) was a multicenter implementation science study and a capacity-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 hospital sites in Cameroon, Malawi, and Tanzania.

Objectives: Our objective was to evaluate the implementation of CrAg CryptoPS (Biosynex, Illkirch Graffenstaden, France), a new semi-quantitative RDT, in routine care settings in Sub-Saharan Africa.

Methods: All CrAg CryptoPS performed were compared to the reference CrAg lateral flow assay (Immy®). The evaluation was done by the local research teams in four DREAMM laboratories sites. CrAg CryptoPS's implementation was evaluated in 301 plasma samples and 258 cerebrospinal fluid (CSF) samples from 320 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, 356 participants were prospectively enrolled with suspected HIV-related CNS infections, including CM, tuberculous meningitis, cerebral toxoplasmosis, and bacterial meningitis cases. Cryptococcal meningitis was the leading cause of CNS infections in Malawi and Tanzania with 66.3% (53/80) and 59.6% (59/99) cases respectively, and the second cause in Cameroon with 40.0% (39/90) cases after cerebral toxoplasmosis.

In plasma, CryptoPS's sensitivity was 99.23% (95% CI, 0.98-1.01) and specificity was 94.15% (95% CI, 0.91-0.98); positive and negative predictive values were 92.8% and 99.4%, respectively. In CSF, the sensitivity and specificity of CryptoPS were 100% (95% CI, 0.0-0.0), and 99.26% (95% CI, 0.98-1.01), respectively; positive and negative predictive values were both 100%. A low number of false-positives were observed (<4% in plasma and <0.5% in CSF).

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

Anitha Subramanian, Uthra Balakumar, Anupma Jyoti Kindo
Sri Ramachandra Institute of Higher Education and Research, Chennai, India

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Background: Candidemia caused by genus *Candida*, are opportunistic pathogens with life-threatening infection in immunocompromised individuals with a mortality rate being 40%–54% 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 specimens and improves 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 method.

Objectives:

DNA extraction from the spiked blood samples

Quantification of the *Candida* DNA extracted from the spiked sample

Comparison of the extracted DNA by phenol-chloroform and potassium acetate method

Methods: A total of 5 ml of EDTA blood samples from healthy volunteers were spiked with 104-108 *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 involves pretreatment with erythrocytes, leukocytes and fungal lysis buffer. DNA from C. *albicans* were amplified using ITS1 and ITS4 based primers. PCR products were visualized by agarose gel electrophoresis.

Results: The spiked *Candida* cells taken were from 104-108 yeast cells. The total extracted DNA by phenol chloroform extraction method ranged from 18.29 to 51.64 ng/μl respectively. Whereas the total extracted DNA from potassium acetate extraction method ranged from 15.58 to 32.30 ng/μl respectively. The presence of a 535 base pair (bp) product was considered positive (Figs. 1 and 2). The lowest detection limit of PCR has been determined as 106-108 *Candida* cells for our spiked samples.

Conclusion: In our study, till date, the extracted quantity of DNA was found to be higher by phenol-chloroform method as compared to the potassium acetate method. Greater numbers and processing are required to obtain conclusive data.