UK NEQAS EQA Scheme Development: Cryptococcal Antigen Detection in Clinical Specimens to help in the Diagnosis of Cryptococcosis.

Shila Seaton, Arlene Barcenilla, Sanjiv Rughooputh
UK NEQAS for Microbiology, Public Health England, 61 Colindale Avenue, London NW9 5EQ

Introduction

Cryptococcus neoformans is an opportunistic fungus found in the soil contaminated with bird droppings causing systemic mycoses that exhibit high virulence in severely immunocompromised individuals. Globally, approximately one million cases of cryptococcosis occur annually, causing 1700 death every day.
Transmission is via spore inhalation, with figure 1 illustrating the mechanism of infection.
Capsule polysaccharide's (CPS) main component is called glucuronoxylomannan (GXM) (figure 2), a key virulence factor for *C. neoformans*.

The detection of cryptococcal antigen (CrAg) from serum, cerebrospinal fluids (CSF) and broncho-alveolar lavage (BAL) specimens is the basis of diagnosing cryptococcosis in suspected patients.
Latex agglutination (LAT) and Enzyme immunoassay (EIA) are commonly used in diagnostic and reference laboratories with a sensitivity of over 90%. Lateral flow assays (LFA) are used as point of care testing (POCT) with a sensitivity of over 95%. WHO (World Health Organisation) recommends CrAg detection using LFA and LAT as standard first-line test for cryptococcosis.

AIM: To determine the stability and homogeneity of simulated respiratory, CSF and serum specimens containing different concentrations of cryptococcal antigen. Simulated specimens stored at ambient, +4°C and -20°C storage conditions, were tested for CrAg periodically over 12 months.
To analyse the results returned from three pilots studies from laboratories providing the service.

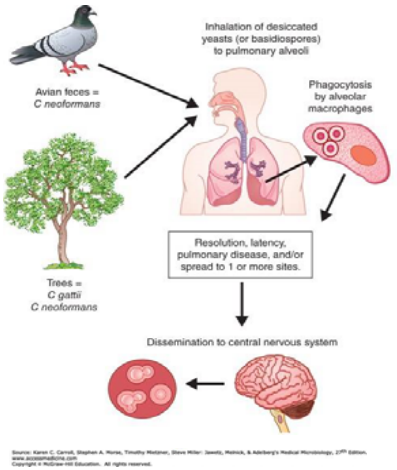


Figure 1: Mechanism of Action of Cryptococcal Infection

Methods

•A questionnaire was initially sent out to 405 laboratories in 2015 to ascertain interest in participating in the pilot studies for the detection of the CrAg in serum.

Specimen preparation

•An NCPF strain; *C. neoformans* was cultured on Sabouraud Dextrose agar (SDA) (figure 3) and incubated at 28°C for 48 hours in aerobic conditions.

- A suspension (1McFarland standard) from the culture plates was prepared and inoculated into 10mL nutrient broth and incubated at 37°C for 24 hours in aerobic conditions in order to mimic the normal host body temperature.
- The nutrient broth was then filtered using 0.2um filter and the suspension was used as neat material for different dilutions.
- Serum (screened negative for CrAg) and simulated CSF (laboratory base composition) were spiked with an inoculum of a filtered suspension at varying concentrations of the *C. neoformans* and stored under three different temperatures to assess stability and homogeneity.
- Samples associated with each set of concentrations were dispensed in 0.5mL volumes into 2mL sarstedt tubes and stored at various conditions including ambient, +4°C and - 20°C.
- Each set of concentrations stored at the specified storage conditions were tested periodically using Lateral Flow Assay and Latex Agglutination test kits (figure 4).

Pilot studies

- The specimens (3718 and 3719) assessed for stability and homogeneity were distributed in the first pilot study.
- Second and third pilot distributed three specimens, two positive for presence of CrAg and one negative serum.

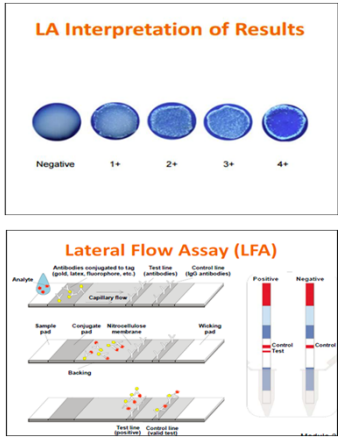
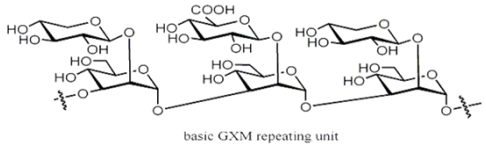


Figure 4. Comparison of reactive results on LAT and LFA.

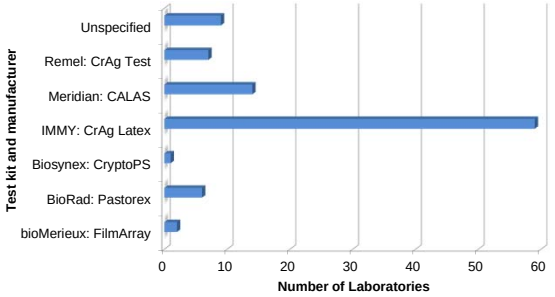
Results

Figure 2. Molecular structure of GXM



Results from the questionnaire sent out in 2015, showed 117 of laboratories provided cryptococcal testing service, 206 laboratories did not and 121 expressed interest in participating in the pilot studies. All laboratories stated they used either the qualitative and semi-quantitative latex agglutination test or the lateral flow test, (figure 5).

Figure 5: Test Kits used by Laboratories in Pilots



Specimen 3718 contained a level of cryptococcal antigen determined to be 1/16 titre from the neat (tested in triplicate) and specimen 3719 contained a titre of 1/4 titre of antigen from the neat suspension.
Results obtained for specimens 3718 and 3719 tested with the LFA kit, detected the CrAg over a period of 12 months, stored at the three atmospheric conditions, (table 1).
The LAT kit however, did not detect the CrAg in both specimens after six months of storage and levels were undetectable in the ninth month, at ambient temperature.
The first pilot study (3718 and 3719) showed excellent concordance with intended results 100% (46/46) and 97.8% (45/46) respectively, were achieved from the 46 laboratories returning results.
Results from the second pilot and third pilot distributions are shown in table 2, demonstrating excellent concordance from participants testing for the presence of the antigen, further supporting the stability and homogeneity of the simulated specimens.

Table 1: The qualitative results of Crag in specimens distributed in the 1st pilot, stored at different temperatures.

Date of Testing	IMMY CrAg LFA			Remel LAT		
14/10/2016	RT	4°C	-20°C	RT	4°C	-20°C
3718	+	+	+	+	+	+
3719	+	+	+	+	+	+
11/11/2016 (1 month)						
3718	+	+	+	+	+	+
3719	+	+	+	+	+	+
11/01/2017 (4 months						
3718	+	+	+	+	+	+
3719	+	+	+	+	+	+
13/04/201 (7 months)						
3718	+	+	+	+/-	+	+
3719	+	+	+	+/-	+/-	+
09/06/2017 (9 months)						
3718	+	+	+	-	+	+
3719	+	+	+	-	+/-	+
20/10/2017 (12 months)						
3718	+	+	+	-	+	+
3719	+	+	+	-	+/-	+/-

Table 2: Results attained f rom second and third pilot studies

CrAg content	July 2017 distribution4263	October 2017 Distribution 4268
	% concordance (n)	
CrAg positive	100 (78/78)	91.9 (79/86)
CrAg negative	96.2 (75/78)	100 (82/82
CrAg positive	88.5 (69/78)	100 (83/83)

Figure 3: *Cryptococcus neoformans* colonies on SDA at 5 days

Conclusion

The result of this study has established that cryptococcal antigen exhibits excellent stability and homogeneity spiked in negative serum over a period of time stored at 4°C and -20°C. The longest period of time specimens can be prepared pre- distribution of the specimens is 4 months, stored at -20°C for both latex agglutination (CrAg) and LFT tests to detect the presence of the antigen. These factors are fundamental in the development an EQA scheme for distributing to laboratories worldwide.
The results from the three pilot studies have demonstrated excellent concordance with intended results from each study.
The results of stability and homogeneity and results from the pilot studies have supported the delivery of an EQA for the presence or absence of the CrAg for April 2018.

References

1. Patterson T and Andriole V, 1989. Current Concepts in Cryptococcosis. European Journal of Clinical Microbiology and Infectious Diseases 8,457-465
2. Jawetz, Melnick , & Adelberg's Medical Microbiology, 27E. (Lange) 27th ed. by Karen C. Carroll, Janet Butel

Acknowledgements

We would like to thank all colleagues, past and present in UK NEQAS for Microbiology