



DREAMM

Driving Reduced AIDS-associated Meningo-encephalitis Mortality



Cryptococcal Antigen (CrAg) Lateral Flow Assay (LFA) Test:

Treating Cryptococcal Meningitis





Workshop Learning Objective

- To perform the IMMY[®] cryptococcal antigen (CrAg) lateral flow (LFA) dipstick test and outline characteristics of the test.
- To perform the Biosynex[®] cryptococcal antigen (CrAg) lateral flow (LFA) cassette test.
- For more information see online Cryptococcal Meningitis module section: *Diagnosing HIV-associated Cryptococcal Meningitis*



Workshop Preparation

- A room with projection facilities and computer able to display this presentation.
- Pens and paper to allow participants to record notes and discussion points.
- The practical aspect of the workshop will require:
 - A clean (decontaminated) workbench with space for participants to perform the practical procedure. Fume hood not required.
 - An IMMY CrAg LFA test pack which includes CrAg Lateral Flow Strips, LF Specimen Diluent, LF Titration Diluent and CrAg Positive Control.
 - Biosynex CryptoPS test pack including test cassette, diluent and positive control
 - You will also need a P200 Pipette, single use pipette tips, timer/stopwatch and 1.5ml Eppendorf tubes or 3ml glass tubes to hold the test strips.
- Note: Do not use any test kits exceeding their expiry date or if the kit seal is broken or test strip is damaged. The test kit and all reagents should be stored at room temperature 20 – 25 c
- Gloves, safety glasses and lab coats should always be worn when handling specimens and test kits. Aseptic technique should be observed at all times. This test can be performed on a lab bench (no need to be performed under a hood).



IMMY[®] CrAg LFA

- FDA approved Point-of-care and rapid diagnostic test (RDT) lateral flow assay (LFA).
- Detects capsular polysaccharide antigens (CrAg) of *Cryptococcus neoformans* which causes Cryptococcal Meningitis.
- Test is an immunochromatographic dipstick assay for qualitative and semi-quantitative detection of CrAg.
- 10 minute test time, 1 minute technician time. No specimen pre-treatment required.
- 99% sensitive, 99% specific.



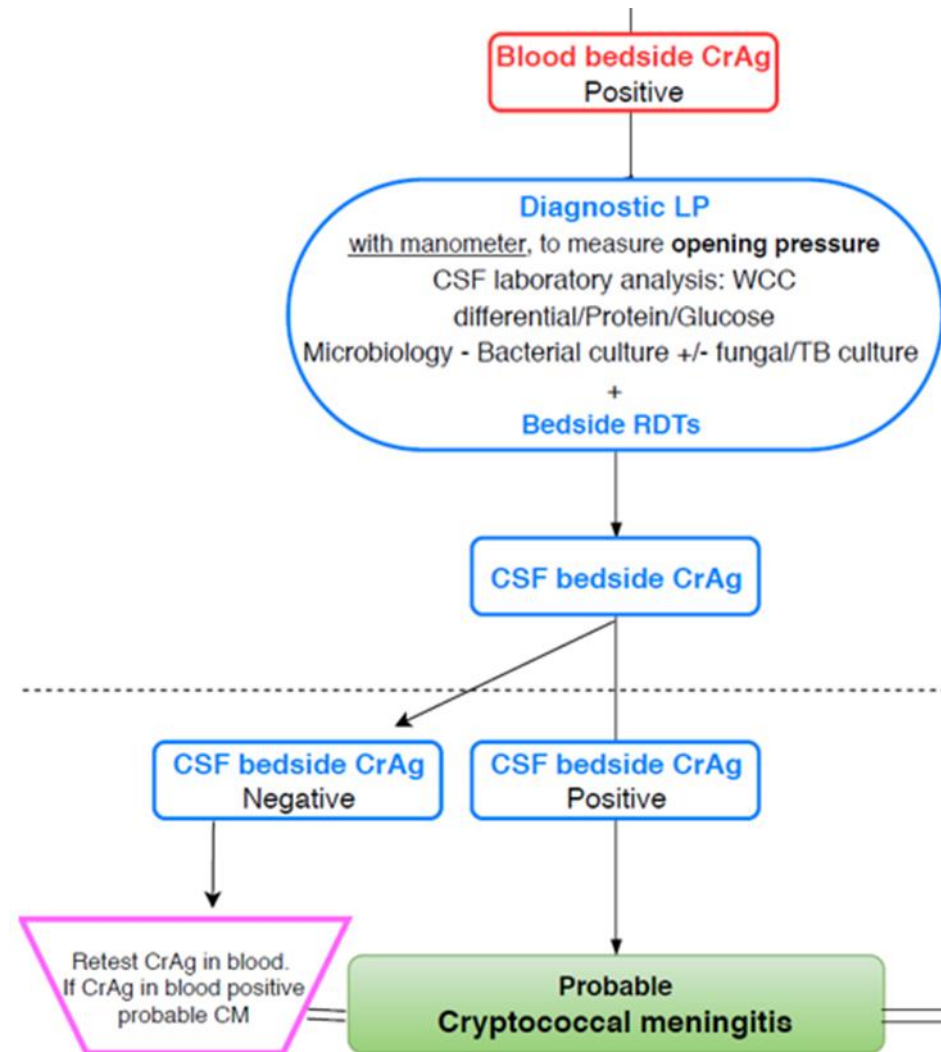
When should the test be used?

- Can be used to diagnose symptomatic patients and for screening asymptomatic patients – HIV+ with a low CD4 count.
- Semi-quantitative (titration) may be used after a positive screening result.
- Can be used on serum, plasma, whole blood and CSF.
- Less specific in urine specimens therefore testing of CrAg in urine using IMMY assay is not recommended.



Use of IMMY[®] CrAg in DREAMM

- All DREAMM patients will be tested for CrAg in blood and nearly all in CSF.

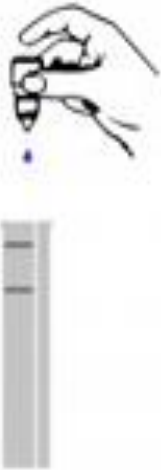




How to perform an IMMY® CrAg test



1



Add 1 drop of
specimen diluent

2



Add 40 µL
of specimen

3



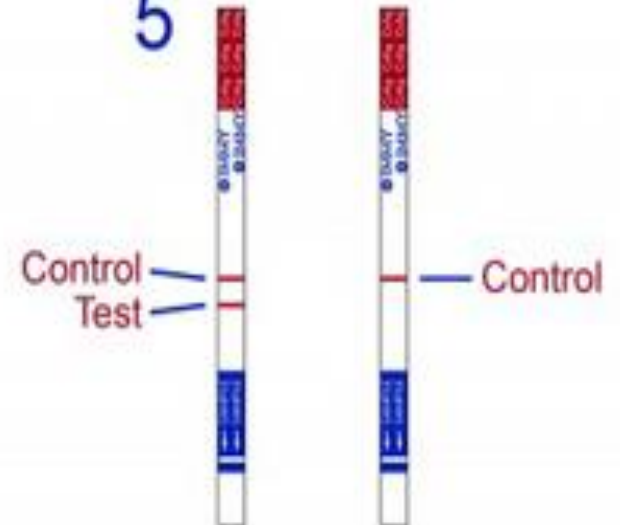
Insert strip

4



Incubate
10 min

5

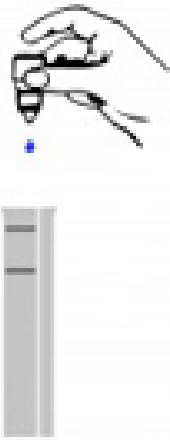


1 line = negative
2 lines = positive



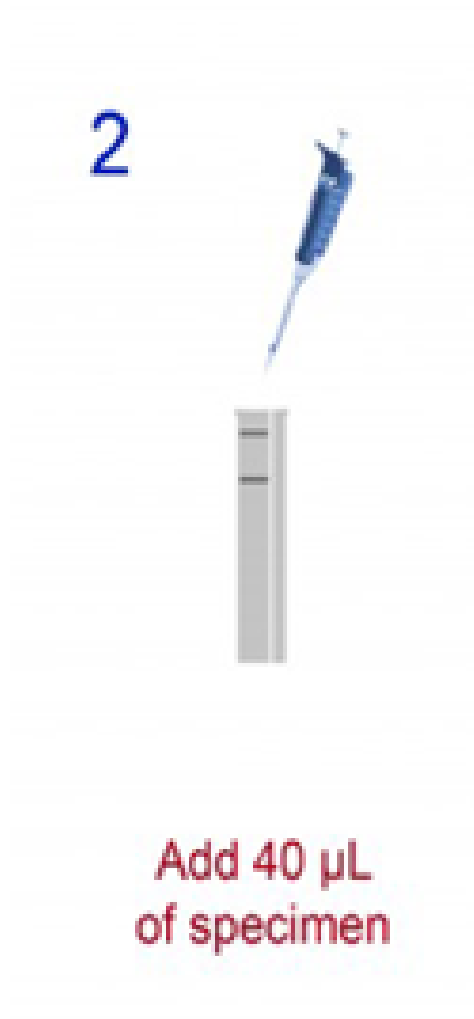
Step 1

1



Add 1 drop of
specimen diluent

- Add one drop or pipette 40 μ l of LF (lateral flow) Specimen Diluent to an appropriate reservoir (Eppendorf tube, disposable micro-centrifuge tube, test tubes, 3ml glass tube etc.).



Step 2

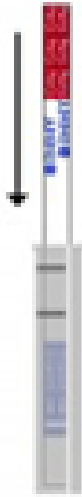
- Add 40µl of specimen to the reservoir and mix.



Step 3

- Submerge the white end of the Cryptococcal Antigen Lateral Flow Strip into the specimen.

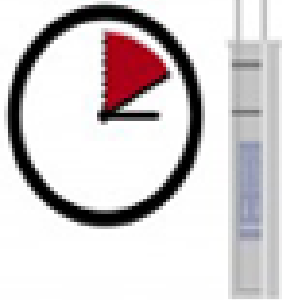
3



Insert strip



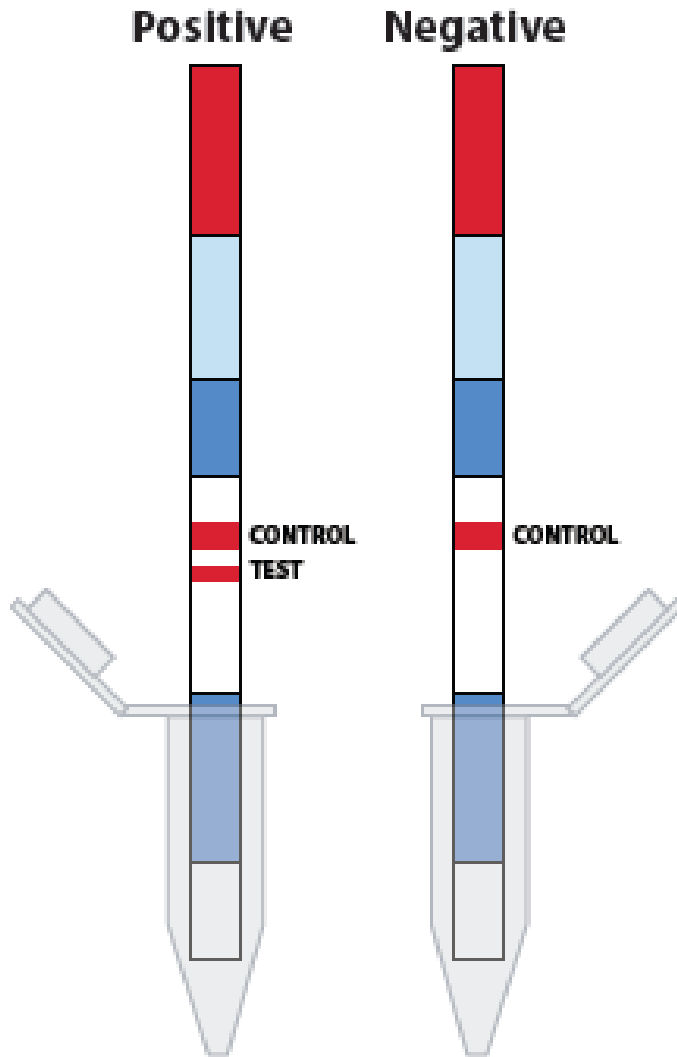
4



Incubate
10 min

Step 4

- Wait 10 minutes



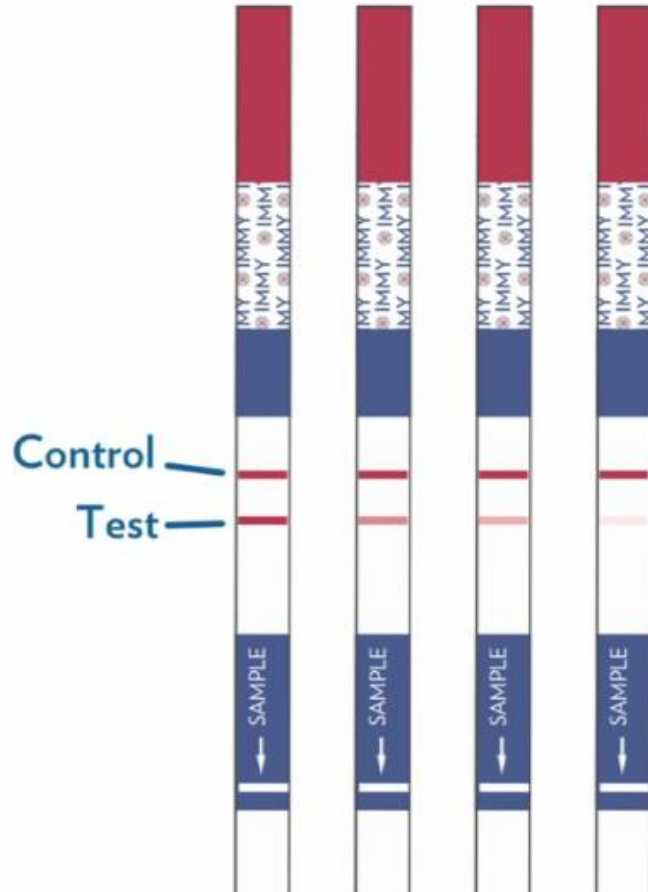
Step 5

- Read and record the test results.
- 2 lines shows a positive result.
- 1 line shows a negative result.
- If no control line is seen, or only the test line is seen, the test should be repeated as the result is invalid.



Interpreting Results

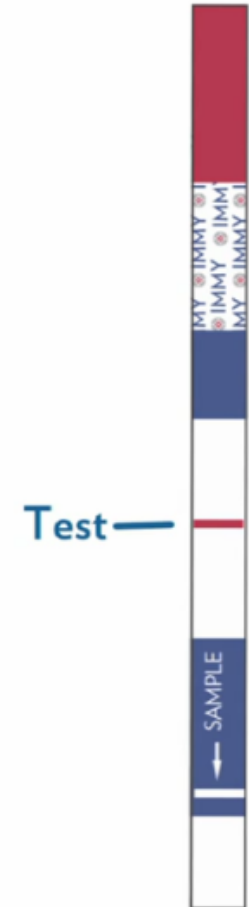
Positive



Negative



Invalid



Biosynex® CryptoPS

- CE marked Point-of-care and rapid diagnostic test (RDT) lateral flow assay (LFA).
- Detects capsular polysaccharide antigens (CrAg) of *Cryptococcus neoformans* which causes Cryptococcal Meningitis.
- Semi quantitative detection and titration of *C. neoformans*.
- 10 minute test time, 1 minute technician time. No specimen pre-treatment required.

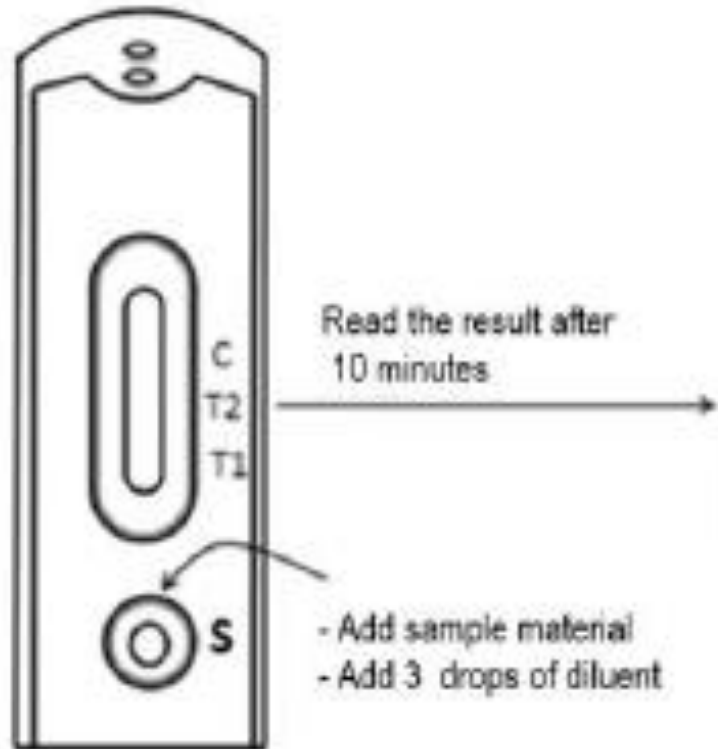


When should the test be used?

- Can be used on serum, plasma, whole blood and CSF samples.
- Can be used to diagnose symptomatic patients and for screening asymptomatic patients – HIV+ with a low CD4 count.
- Semi-quantitative (T2) test line indicates a high CrAg antigen presence.
- Less specific in urine specimens therefore testing of CrAg in urine using CryptoPS assay is not recommended.



How to perform a Biosynex® CryptoPS test



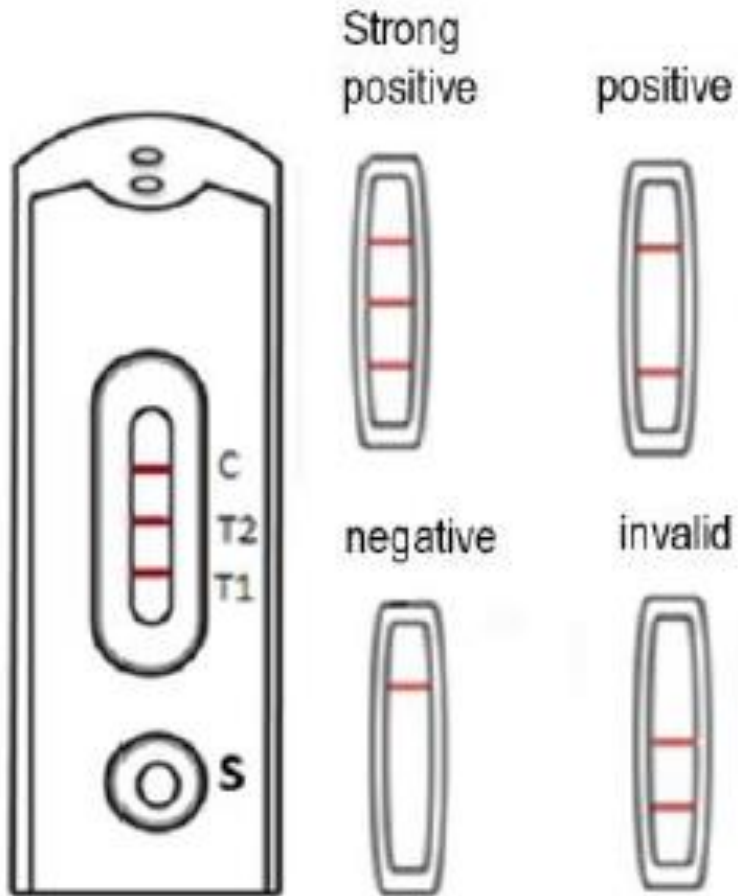
Step 1

- Pipette 20 μ l of the sample into the sample well (S) of the cassette.

Step 2

- Add 3 drops of diluent into the sample well.

Step 3



- Start the timer and read the result after 10 minutes.
- Do not interpret results after 15 minutes.
- Positive – presence of coloured lines in T1 and/or T2 test lines
- Negative – coloured line appears in control line (C)
- Invalid – no presence of control line

Summary



- CrAg LFA tests have revolutionised the diagnosis of cryptococcal meningitis- 99% sensitive 99% specific.
- CrAg LFA testing is the cornerstone of the DREAMM diagnostic and treatment algorithm for HIV-associated meningo-encephalitis.
- Cryptococcal meningitis is the leading cause of HIV-associated meningo-encephalitis in African low-income and middle-income countries (LMICs).
- CrAg LFA can be used to test blood and CSF specimens.



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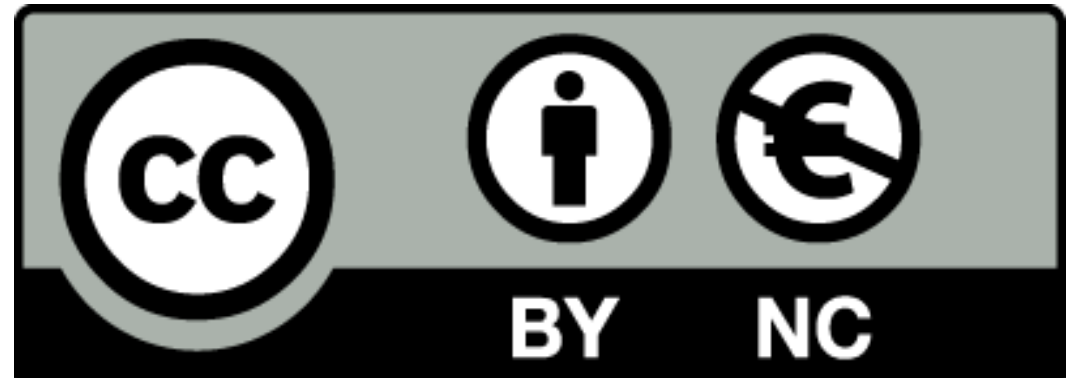
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Suggested Citation:

DREAMM Clinical Training: HIV–associated Cryptococcal Meningitis. DREAMM Project 2018, St George’s University of London, UK. figshare. Available at DOI: 10.24376/rd.sgul.7398596