

# **CrAg Lateral Flow Assay**

## **Standard Operating Procedure**

### **1.0. Purpose**

The purpose of this standard operating procedure (SOP) is to detail the steps for correctly performing, interpreting, and documenting valid results for CrAg Lateral Flow Assay (LFA). CrAg LFA is a test which can aid in the diagnosis of cryptococcosis which is one of the most common opportunistic infection in AIDS patients.

### **2.0. Scope**

The procedure applies to all facilities performing CrAg Lateral flow Assay for the detection of Cryptococcus Antigen in the patients or clients whose CD4 count is less than 200cells/ $\mu$ L in their first visit. It is not intended for patients already on ARVs treatment.

### **3.0. Definitions**

3.1 CrAg – Cryptococcal Antigen

### **4.0. Responsibility and Authorization**

The persons responsible for performing this test are Laboratory Technologist and trained non-laboratory personnel (e.g Nurses, HTC counselors).

## **5.0. Materials**

- 5.1. CrAg Lateral Flow Assay kit**
  - 5.1.1. LF Specimen diluent (2.5 mL)
  - 5.1.2. LF Titration diluents (6.0.mL)
  - 5.1.3. CrAg LF test Strips
  - 5.1.4. CrAg Positive Control (1mL)
- 5.2. Materials required but not provided in the kit**
  - 5.2.1. Cryo racks
  - 5.2.2. Timer
  - 5.2.3. Gloves
  - 5.2.4. Pipettor 40µL
  - 5.2.5. Cryovial tubes (1.8mL-2.0mL)
  - 5.2.6. 40µL tips
  - 5.2.7. Sharps discard containers
  - 5.2.8. Pen and sharp permanent marker
  - 5.2.9. Biohazard disposable bags

## **6.0. Safety, Health & Environment**

Treat all specimens as potentially infectious and follow basic universal precautions. Wear protective clothing (coat/apron and gloves) when handling the specimens.

## **7.0. Principle**

The CrAg Lateral Flow is a dipstick sandwich immunochromatographic assay. Specimens and specimen diluents are added into appropriate reservoir, such as a test tube, and the lateral flow device is placed into the reservoir. The test uses specimen wicking to capture Gold conjugated, anti-CrAg monoclonal antibodies deposited on the test membrane. If CrAg is present in the specimen, then it binds to the gold conjugated, anti-CrAg antibodies. The gold labeled antibody-antigen complex continues to wick up the membrane where it will interact with the test line, which has immobilized anti-CrAg monoclonal antibodies. The gold labeled antibody-antigen complex forms a sandwich at the test line causing a visible line to form. With proper flow and reagent reactivity, the wicking of any specimen, positive or negative, will cause a gold –conjugated control antibody to move the control line. Immobilized antibodies at the control line will bind to gold-conjugated control antibody and form a visible control line. Positive test results form two lines (test and control). Negative test results form only one line (control). If a control line fails to develop then the test is not valid.

## **8.0. Specimen collection and processing**

Use non-hemolyzed plasma from EDTA anti-coagulated tubes. Plasma can be obtained by allowing blood tube/s containing freshly collected whole blood to stand up right for at least 20 minutes at room temperature.

If specimen processing is delayed, store the specimen at 2-8°C for up to 72 hours. Specimen stored at 2-8°C should be brought to the room temperature 10 to 15 minutes before tested.

### Specimen processing

For patients whose CD4 was analyzed with PIMA, obtain venipuncture specimen

- Use EDTA blood collecting tube and refer to venipuncture SOP or Job Aid.
- Label the specimen with patient identity
- Allow the collected blood to stand up right until sedimentation has occurred and clear plasma is visible (at least 20minutes standing upright). Once plasma is ready start testing as the procedure below.

## 8.1 Reagent storage and preparation

All reagents included in the kit should be stored at room temperature (22-25°C) until the expiration dates listed on the reagent labels. Do not use expired reagents. The entire kit should be at room temperature (22-25°C) before and during use.

## 8.2 Test Procedure

**8.2.1** Collect and organize all above listed materials before starting any testing. See figure 1.



Figure 1: Organize testing materials

- 8.2.2 Label the cryovial tube identical with EDTA tube containing the patient blood to be analyzed. See figure 2.



Figure 2: Label cryovial tube

- 8.2.3 Add one drop of LF specimen diluents into the labeled cryovial tube. See figure 3.

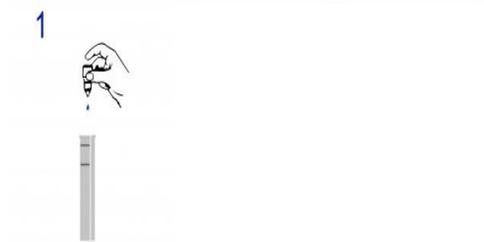


Figure 3: Add 1 drop of specimen diluent

- 8.2.4 Add 40µl of specimen into the cryovial tube containing LF specimen diluents and mix. See figure 4.

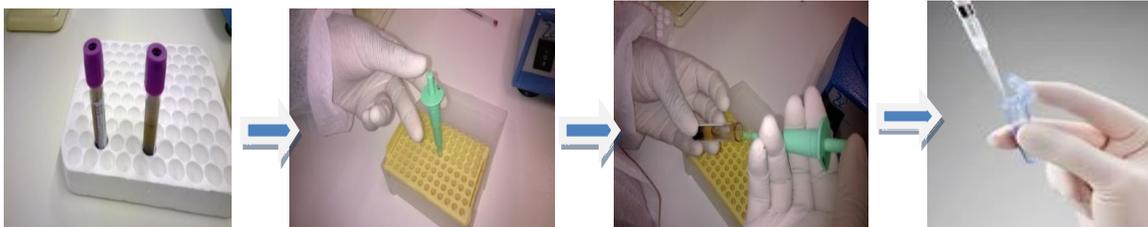


Figure 4: Add sample into the cryovial tube

- 8.2.5 Take one test strip from LF test strip vial and insert into the mixture, recap LF test strip vial firmly with desiccant cap immediately. See figure 5.

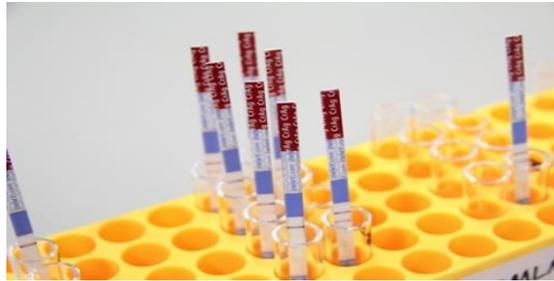


Figure 5: Insert one LF test strip in to the mixture

8.2.6 Wait 10 minutes before reading the test results. See figure 5



Figure 5: Read results at 10 minutes

### 8.3 Quality control testing

Conduct Quality control for CrAg test weekly, before you test the first specimen to be analyzed during that particular week. In the case where there are no specimens to be test for CrAg, Quality Control may not be Evaluated in that week. Record quality control results in the CrAg result log book.

8.3.1 Follow the following procedure to evaluate CrAg quality control:

#### CrAg positive control

- First label the cryovial tube with CrAg Positive control
- Add 1 drop of LF specimen Diluent (REF GLF025) followed by 1 drop of CrAg positive control (REF CB0020) in the labeled cryovial tube and mix.
- Take one test strip from LF test strip vial and insert into the mixture and read results after 10 minutes

## CrAg Negative control

- Label the cryovial tube with CrAg negative control
- Add 2 drops of specimen diluents in the labeled cryovial tube
- Insert LF test strip into the cryovial tube and read results after 10 minutes

Two lines (test and control) indicate a positive control has past while one line (control) indicates a Negative control past. Refer to results interpretation below for CrAg test Positive and Negative control results appearance.

**NB: Do not processed with testing if either positive or Negative control fail (giving unexpected test results)**

### 8.4 Results interpretation

#### 8.4.1 Invalid Test result

Identify the validity of each test by the absence of control line even if there is line in the test zone. See figure 6. The test with no control line is interpreted as invalid test and treats it as follows:

- Record it in the CrAg results log book
- Repeat testing with a new LF test strip, if invalid results continued contact your district Quality officer for assistance.

#### 8.4.2 Positive test result

The presence of two lines (a control and a line in the test zone) indicates positive results. See figure 6.

#### 8.4.3 Negative test result

The presence of one line (control line only) indicate negative result, the specimen currently not contain no or undetectable antigen. See figure 6.

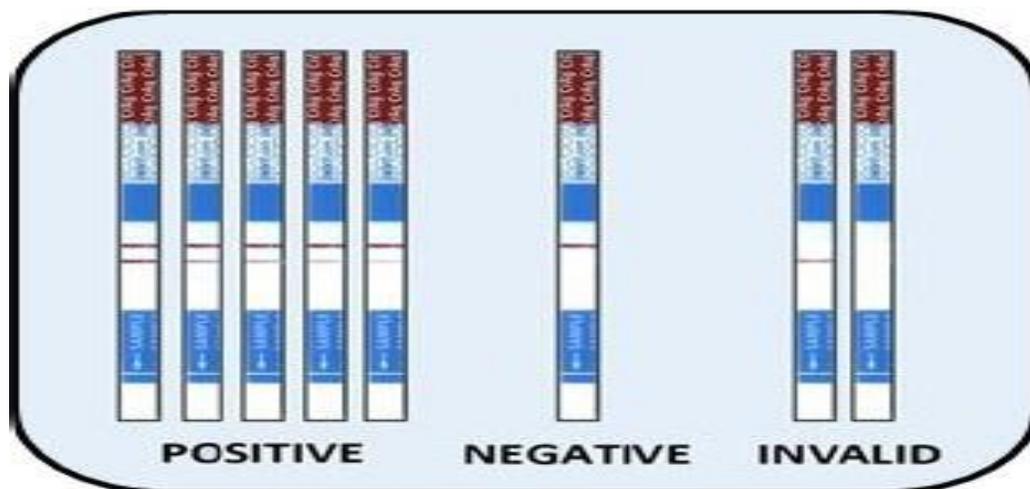


Figure 6: Three expected CrAg test results

Figure 4: Three expected results outcomes (Positive, Negative and Invalid)

## **9 REFERENCES**

CrAg Lateral Flow Assay-For the detection of Cryptococcal Antigen- REF CR2003-IMMY  
Leaflet