##### Evaluation of lateral flow Immunoassay for the diagnosis of Cryptoccocal Meningitis

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**A thesis submitted to School of Public Health, Moi University in partial fulfillment for the degree of Master of Science in Field Epidemiology**

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# Declaration

**Declaration by the candidate**

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# Dedication

This research work is dedicated to my beloved wife Pamelah Kirimi, daughter Blessings Nkatha Kirimi, Son Love Murangiri Kirimi and my parents Julius Gitonga (posthumously) and Sarah Wanja Gitonga for their encouragement and support throughout the study period.

# Abstract

**Background.** Cryptococcal meningitisis the most common opportunistic infection and the second leading cause of death in HIV infected persons in Africa. Many fatalities from cryptococcal meningitis can be averted by early diagnosis and treatment. The aim of this study was to evaluate and compare Lateral Flow ImmunoAssay (LFA) to latex agglutination (LA), India ink microscopy and culture using various sample types.

**Methods.** Ahospital based cross-sectional study was conducted at Mbagathi hospital, Nairobi, Kenya, from April to July 2017. The participants were enrolled through systematic random sampling. HIV patients suspected of cryptococcal meningitis who were scheduled for lumber puncture and routine blood sample collection were enrolled into the study prospectively. Capillary blood, serum and cerebral spinal fluid (CSF) samples were collected from the participants. LFA (IMMY, Norman, Ok) test was performed on capillary blood, serum and CSF and the test results compared with LA (IMMY, Norman, Ok) test performed on serum and CSF, india ink microscopy and culture on CSF. Sensitivity, specificity and predictive values were calculated and test agreement levels estimated using kappa (k) coefficients.

**Results.** Of the 124 capillary blood and serum, and 99 CSF samples, the agreement between LFA and LA on serum was 94.4% and kappa of 0.88 with sensitivity and specificity of 100% and 91%. LA on CSF was 97.9% and kappa of 0.96 with sensitivity and specificity of 100% and 96%. The agreement between LFA and india ink (microscopy) was 96.9% and kappa of 0.94 with sensitivity and specificity of 100% and 94.1%. On CSF culture, the agreement was 72.7% and kappa of 0.43 with sensitivity and specificity of 100% and 64%. The agreement level of LFA on capillary blood, serum and CSF was 100% and kappa of 1.00 with sensitivity and specificity of 100%

**Conclusion.** The high agreement between LFA, LA and india ink on different sample type shows that LFA is a reliable diagnostic test. The evidence of test agreement between LFA in capillary blood, serum and CSF coupled with the fact that it is easy and rapid to perform with accurate results forms the basis for LFA as a choice for point of care (POC) test for Cryptococcal meningitis.

**Key words: Culture, Cryptococcal meningitis, India ink, Latex Agglutination and Lateral Flow Immunoassay**

[Declaration i](#_Toc494375748)

[Dedication ii](#_Toc494375749)

[Abstract iii](#_Toc494375750)

Table of Contents

[List of Tables vii](#_Toc526863012)

[List of Appendices viii](#_Toc526863013)

[Abbreviations ix](#_Toc526863014)

[Acknowledgement x](#_Toc526863015)

[CHAPTER ONE: INTRODUCTION 1](#_Toc526863016)

[1.1 Background 1](#_Toc526863017)

[1.2 Problem statement 1](#_Toc526863018)

[1.3 Justification 2](#_Toc526863019)

[1.4 Research questions 3](#_Toc526863020)

[1.5 Objectives 4](#_Toc526863021)

[1.5.1 Overall objective 4](#_Toc526863022)

[1.5.2 Specific objectives 4](#_Toc526863023)

[CHAPTER TWO: LITERATURE REVIEW 5](#_Toc526863024)

[2.1 Cryptococcal meningitis 5](#_Toc526863025)

[2.2 Cryptococcal meningitis diagnosis 7](#_Toc526863026)

[2.3 Conceptual frame work 10](#_Toc526863027)

[CHAPTER THREE: MATERIALS AND METHODS 11](#_Toc526863028)

[3.1 Study site 11](#_Toc526863029)

[3.2 Study Population 12](#_Toc526863030)

[3.3 Study Design 12](#_Toc526863031)

[3.4 Sample Size Determination 12](#_Toc526863032)

[3.5 Sampling procedure 13](#_Toc526863033)

[3.5.1 Inclusion Criteria 13](#_Toc526863034)

[3.5.2 Exclusion Criteria 14](#_Toc526863035)

[3.6 Sample preparation and Laboratory procedure 14](#_Toc526863036)

[3.6.1 Sample collection and preparation 14](#_Toc526863037)

[3.6.2 LFA testing procedure 15](#_Toc526863038)

[3.6.3 LA testing procedure 15](#_Toc526863039)

[3.6.4 Culture Procedure 16](#_Toc526863040)

[3.7 Data management 16](#_Toc526863041)

[3.7.1 Data collection 16](#_Toc526863042)

[3.7.2 Data analysis 16](#_Toc526863043)

[3.8 Ethical considerations 17](#_Toc526863044)

[CHAPTER FOUR: RESULTS 18](#_Toc526863045)

[4.1 Characteristics of the study participants 18](#_Toc526863046)

[4.2 Comparison of Lateral flow Immuno Assay to Latex agglutination using sera. 18](#_Toc526863047)

[4.3 Comparison of Lateral flow Immuno Assay to Latex agglutination using CSF. 19](#_Toc526863048)

[4.4 Comparison of Lateral flow Immuno Assay to India ink Microscopy using CSF. 19](#_Toc526863049)

[4.5 Comparison of Lateral flow Immuno Assay to culture using CSF. 20](#_Toc526863050)

[4.6 Comparison of CSF culture to LA and india ink using CSF 21](#_Toc526863051)

[4.7 Comparison of the Lateral flow ImmunoAssay using capillary blood to Lateral flow ImmunoAssay using sera. 22](#_Toc526863052)

[4.8 Comparison of the Lateral flow ImmunoAssay using capillary blood to Lateral flow ImmunoAssay using CSF. 23](#_Toc526863053)

[CHAPTER FIVE: DISCUSSION 24](#_Toc526863054)

[CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS 27](#_Toc526863055)

[6.1 Conclusions 27](#_Toc526863056)

[6.2 Recommendations 27](#_Toc526863057)

[REFERENCES 28](#_Toc526863058)

[APPENDICES 33](#_Toc526863059)

# List of Tables

**Table 3.1:** Test method and the samples used in evaluation………………………….13

**Table 4.1:** Comparison of Lateral flow ImmunoAssay to Latex agglutination using sera …………………………………..………………………………………………….…18

**Table 4.2:** Comparison of Lateral flow ImmunoAssay to Latex agglutination using CSF………………………………………………………………………….…………19

**Table 4.3:** Comparison of Lateral flow ImmunoAssay to India Ink microscopy using CSF…………………………………………………………………………….….…...19

**Table 4.4:** Comparison of Lateral flow ImmunoAssay to culture using CSF…………20

**Table 4.5:** Comparison of culture to Latex agglutination and India Ink using CSF…..21

**Table 4.6:** Comparison of Lateral flow ImmunoAssay using capillary blood to Lateral flow ImmunoAssay using sera ………………………………………………………………22

**Table 4.7:** Comparison of Lateral flow ImmunoAssay using capillary blood to Lateral flow ImmunoAssay using CSF………………………………………………………………23

# List of Appendices

Appendix I: Adult Consent Form……………………………………………..33

Appendix 2: Evaluation of LFA Algorithm………………………………….42

Appendix 3: Sample Collection and LFA Results Log……………………….43

Appendix 4: Lateral Flow Assay Testing and Interpretation…………………45

Appendix 5: Interpretation of Cohen’s kappa………………………………...46

Appendix 6: Ethics certificate………………………………………………...47

Appendix 7: Institutional Research and Ethics Committee Approval………..48

Appendix 8: Research Authorization by Mbagathi Hospital…………………49

# Abbreviations

|  |  |
| --- | --- |
| AE | Adverse Event |
| AIDS | Acquired immune deficiency syndrome |
| CCC | Comprehensive Care Clinics |
| CMRL | Central Microbiology Reference Laboratory |
| CFR | Case fatality rate |
| CSF | Cerebral Spinal Fluid |
| EIA | Enzyme Immunoassay |
| HCW | Health Care Worker |
| HIV | Human Immunodeficiency Virus |
| ID | Identification |
| IREC | Institutional Research and Ethics Committee |
| LA | Latex Agglutination |
| LFA | Lateral Flow ImmunoAssay |
| NPHLS | National Public Health Laboratory Services |
| MOH | Ministry of Health |
| NPV | Negative predictive value |
| POC | point-of-care |
| PPV | Positive predictive value |
| RPM | Revolutions per minute |
| US FDA | US Food and Drug Administration |
| WHO | World Health Organization |
|  |  |

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# CHAPTER ONE: INTRODUCTION

## 1.1 Background

Cryptococcal meningitis is a fungal infection caused by *Cryptococcus neoformans* affecting the membranes covering the spinal cord or brain. Soil contaminated with pigeon extracts and chicken droppings provide the environmental source of the fungi (Fletcher, 2018). The fungus is usually inhaled and is believed to remain dormant in the lungs for many years. In healthy people with normal functioning immune systems, infection is usually self-limiting, and the infected person notices no symptoms. Reactivation, which occurs primarily among immunosuppressed individuals such as persons with Human Immunodeficiency Virus (HIV)/ AIDS, leads to infection, the most common of which is meningitis (Park *et al*., 2009). Cryptococcal meningitis is a life-threatening opportunistic infection and a leading cause of morbidity and mortality amongst HIV-infected persons especially if treatment is not initiated promptly(Kanji *et al.*, 2011; Mwaba *et al*., 2001)*.* In Africa, it is the second leading cause of death in HIV–infected persons with a case fatality rate (CFR) of up to 38% amongst outpatients and 81-100% amongst inpatients. In Kenya, up to 33% of people with AIDS develop Cryptococcal meningitis, inpatients CFR of up to 36% among in patients( Kendi *et al.*, 2010; Mdodo *et al*, 2013; Park *et al.*, 2009).

## 1.2 Problem statement

Cryptococcal meningitis is a life-threatening opportunistic infection in HIV /AIDS patients and the leading cause of morbidity and mortality amongst HIV-infected persons. The conventional methods of Cryptococcal meningitis diagnosis which include culture, microscopy and latex agglutination are expensive, complicated, inaccessible, and laborious, requires heavy infrastructure and expertise leading to long waiting hours, delay in diagnosis and patient management. Culture method is considered to be the gold standard diagnostic method for Cryptococcal meningitis. However, it has poor sensitivity, requires a large quantity of specimen, technical expertise and laboratory infrastructure including electricity (Boulware *et al.*, 2014; Lindsley *et al.*, 2011). Latex agglutination (LA) test has sensitivity and specificity of >99%, less labor and time intensive than culture(Gade *et al.*, 1991; S Sekhon *et al.*, 1993). However, testing for Cryptoccocal meningitis requires heavy laboratory infrastructure such as refrigeration, a cold chain for specimen transport, and technical expertise which are not available in resource constrained settings thus limiting their clinical utility ( Kozel and Sean, 2013). Delay in diagnosis ofCryptoccocal meningitis leads to delay in treatment occurring only when meningitis is at an advanced stage and treatment is less effective resulting to high CFR of Cryptoccocal meningitis (Derek and Victoria., 2014; Kanji *et al*., 2011; Tenforde, *et al*, 2016). Therefore a need for a rapid point of care diagnosis of Cryptoccocal meningitis.

## 1.3 Justification

Early diagnosis of Cryptoccocal meningitisallows for the prompt initiation of treatment before the manifestation of the signs and symptoms which leads to reduced Cryptoccocal meningitis *-* related deaths. Therefore due to the urgency of patient management, there is need for a test that is simple to perform, point of care, low cost and has short turnaround time.

LFA is a rapid qualitative point-of-care (POC) test for the detection of capsular polysaccharide antigens of Cryptococcus species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*). The LFA test can use whole blood, sera, CSF. It is stable at room temperature, has a rapid turnaround time of <15 minutes, is simple to perform and can be interpreted by personnel with no or minimal laboratory training. It does not require cold chain or advanced laboratory equipment ( Kozel and Sean, 2013). Majority of the studies on evaluation of LFA are based on sera and CSF (Binnicker *et al*., 2012; Boulware *et al*., 2014; Huang *et al.*, 2015; Rioki *et al*., 2015; Lindsley *et al*., 2011). There is little information on the use of LFA on capillary blood (Williams *et al.*, 2015). In Kenya there is limited documentation on the evaluation of LFA as a diagnostic procedure for diagnosis of Cryptococcal meningitis. Despite versatility of LFA test as a convenient diagnostic method for Cryptoccocal meningitisthere was need to evaluate the test to determine test performance and also compare against other conventional diagnostic methods

## 1.4 Research questions

1. What is the agreement level of LFA to LA on sera and CSF?
2. What is the agreement level of LFA to CSF microscopy?
3. What is the agreement level of LFA to CSF culture?
4. What is the agreement level between LFA on capillary blood to LFA on sera and CSF?

## 1.5 Objectives

### 1.5.1 Overall objective

To evaluate and compare Lateral Flow Immuno Assay (LFA) to latex agglutination (LA), microscopy and culture using various sample types.

### 1.5.2 Specific objectives

1. To evaluate the agreement level of LFA and LA on sera and CSF.
2. To evaluate the agreement level of LFA and microscopy on CSF.
3. To evaluate the agreement level of LFA and culture on CSF.
4. To evaluate the agreement level of LFA on capillary blood to LFA on sera and CSF.

# CHAPTER TWO: LITERATURE REVIEW

## 2.1 Cryptococcal meningitis

Cryptococcus is a type of fungus that is found in the soil contaminated with bird droppings. It causes cryptococcosis disease which infects both immunocompetent and immunocompromised persons (Bicanic *et al*., 2009). The major species of cryptococcus that causes illness in human is *Cryptococcus neoformans*. Cryptococcus neoformans is an encapsulated yeast first isolated from a tibia of 31 year old woman and described by a pathologist Busse to the Greifswald Medical Society in 1894 (Maziarz *et al*., 2016).

There are four serotypes of *C. neoformans* divided into two groups: *C. neoformans* var. *neoformans* (serotypes A and D) and *C. neoformans* var. *gatti* (serotypes B and C). Infection with *C. neoformans* var. *neoformans* is serious and life threatening. Infections with *C. neoformans* var. *gatti* have been shown to occur in healthy persons without compromised immune system (Del Poeta & Casadevall, 2012).

Cryptococcal infection occurs after a person inhales the fungus from the environment. In the lungs, the infection causes shortness of breath, cough, and fever, and in some people, causes no symptoms. Cryptococcal meningitis specifically occurs after he spread of the Cryptococcus from the lungs to the brain. In healthy people with normal functioning immune systems, infection is usually self-limiting, and the infected person notices no symptoms. Reactivation of the infection which occurs primarily among immunosuppressed individuals such as persons with Human Immunodeficiency Virus/ AIDS, leads to infection, the most common of which is meningitis (Bicanic *et al*., 2009).

Meningitis is an infection and inflammation of the membranes that cover the brain and spinal cord (meninges). Meningitis can be due to bacteria, fungi, and viruses. Viruses cause most cases of meningitis. Cryptococcal meningitisis one of the exceptions. Persons infected with Cryptococcal meningitis may develop the following symptoms: headache, nausea, vomiting, neck pain, confusion, hallucinations, lethargy, and sensitivity to light. In some cases, the infected person may experience a stiff neck and fever. If Cryptococcalmeningitis is left untreated, it may lead to more serious complications, such as: brain damage, coma, hearing loss and hydrocephalus. Cryptococcal meningitis is a life-threatening opportunistic infection and a leading cause of morbidity and mortality amongst HIV-infected persons especially if treatment is not initiated promptly (Mwaba *et al*., 2001).

Globally, there are about one million new cases of cryptococcal meningitis each year resulting in 625,000 deaths with the highest burden being in developing countries (Park *et al*., 2009). Infections and deaths due to HIV-related Cryptococcal meningitis are high thus making Cryptococcal meningitis the second leading cause of death in HIV–infected persons in Africa after tuberculosis with a case fatality rate of 38% amongst outpatients and 81-100% among in- patients (French *et al*., 2002; Kendi., *et al*, 2010; Park *et al*., 2009).

More than a third of patients with Cryptococcal meningitis die in low resource settings, such as sub-Saharan Africa. The death rate is so high in these areas due to the tendency to seek healthcare when the disease is too advanced for treatment. The cost of cryptococcal meningitis treatment is expensive, unavailable and in accessible to treatment in resource limited settings (Sloan *et al*., 2014).

## 2.2 Cryptococcal meningitis diagnosis

Early diagnosis of Cryptococcal meningitisis the gateway to effective management of cryptococcal meningitis by providing pre-emptive treatment before symptoms of meningitis develop. Early detection and treatment of asymptomatic or latent cryptococcal infection allows for early prompt treatment which reduces Cryptococcal meningitis related deaths. Treatment of Cryptococcal meningitis with first-line therapy drugs are less expensive and highly available(Derek and Victoria, 2014).

The techniques in Cryptococcal meningitis diagnosis include direct examination of the *C. neoformans* fungus in body fluids (CSF and serum) with India ink examination, histopathology of infected tissue with specific stains to identify capsule (mucicarmine and alcian blue) or presence of melanin (Fontana-Masson), serology from body fluids for antigen detection using latex agglutination (LA), enzyme immunoassay (EIA) and latex flow immunoassay (LFA) and culture of fluids and/or tissues(Boulware *et al*., 2014; Temstet *et al*, 1992).

The culturing method is considered the gold standard diagnostic method. However, it has poor sensitivity, requires a large quantity of specimen and laboratory infrastructure including electricity (Lindsley *et al*., 2011; Vanselow *et al.*, 2012). Latex agglutination (LA) assays have a much higher sensitivity and specificity than the India ink test (especially in patients with low CSF fungal burden), and are easier to perform and less dependent on technician skill than the India ink test (Dominic *et al*., 2009). However, false-positive results can occur with LA if interfering substances such as rheumatoid factor or other undefined proteins are present (Bennett and Bailey, 1971). Despite the high sensitivity and specificity, less labor- and time-intensity of LA and EIA than culture, EIA has several potential advantages over LA, including having no need for enzymatic pretreatment of specimens, clear discrimination of positive from negative results, ability to provide quantitative information without end-titer dilution and higher sensitivity (Binnicker *et al*., 2012). However, both these methods require laboratory infrastructure such as refrigeration, a cold chain for specimen transport, and technical expertise.

A Lateral Flow ImmunoAssay (LFA) for the detection of cryptococcal antigen developed by IMMY (Immuno- Mycologics) is a potential point-of-care (POC) test. The LFA is a qualitative and semi quantitative test system for the detection of capsular polysaccharide antigens of cryptococcus species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*). LFA test meets the WHO assured criteria for POC diagnostic tests in resource poor settings. It is affordable, more sensitive and specific to the conventional tests, user friendly, rapid, does not require complicated equipments, is portable and requires minimal training to perform (World Health Organization (WHO), 2011). The LFA test is stable at room temperature, has a rapid turnaround time, are simple to perform and interpret by personnel with no or minimal laboratory training, and therefore may be the POC test of choice for the diagnosis of cryptococcosis.

Conventionally, cryptococcal meningitis has been diagnosed using fungal culture, direct microscopy of clinical samples. For accurate identification of the Cryptococcus, are subjective, requires several days, expertise and infrastructure (Binnicker *et al.*, 2012). A variety of different more sensitive and specific immunoassays that include latex agglutination (LA)-based tests, antigen capture sandwich enzyme immunoassays (EIAs), and a lateral flow immunochromatographic assay (LFA) for the diagnosis of cryptococcal meningitis are cleared by the U.S. Food and Drug Administration (FDA) (LFA) (Binnicker *et al*., 2012; Jarvis *et al.*, 2011; Williams *et al.*, 2015). The conventional methods and the immunoassays for the diagnosis of cryptococcal meningitis have shown comparable performance. Antigen tests such as LA or EIA performed on cerebral spinal fluid or serum are highly sensitive and specific diagnostic options that are less labor- and time-intensive than culture. However they require infrastructure, technical expertise performed only in reference laboratories (Lindsley *et al.*, 2011). On comparison of LFA and EIA on serum, the sensitivity was 100% will high agreement level (Lindsley *et al*., 2011). The comparison of LFA performance with LA, EIA in serum and CSF has demonstrated high sensitivity >99%, high specificity >99% and high agreement levels of >97.7% making LFA an equally good alternative method for cryptococcal diagnosis (Binnicker *et al*., 2012; Hansen *et* *al*., 2013). Evaluations on LFA in whole blood, serum/ plasma and CSF conducted in Uganda demonstrated a 100% agreement of LFA in serum/ plasma, CSF and whole blood (Williams *et al.*, 2015). LFA test is a potential POC test for the diagnosis of cryptococcal meningitis; is easy to perform, rapid and can use serum, plasma, CSF and whole blood giving accurate consistent results.

## 2.3 Conceptual frame work



# CHAPTER THREE: MATERIALS AND METHODS

## 3.1 Study site

(Source-Author)

The study was conducted at Mbagathi hospital. Mbagathi hospital is the Nairobi county referral facility which predominantly serves the urban population. The facility has a high volume in routine lumbar punctures performed, and has the availability of a laboratory with the capacity to perform cryptococcal diagnosis.

Nairobi is the largest cosmopolitan city in the Kenya covering 695.1 SQ. KM. It is densely populated with an estimated population of 3,138,369 and a Population density of 4,515 per square kilometer in 2014 (Kenya National Bureau of Statistics, 2014). The main economic activities include; Trading, Tourism, Professional Business, Industrial production of various goods in small, medium and large-scale, Services and Commercial Enterprises.

## 3.2 Study Population

The study population was HIV patients’ 18 years and over who were suspected to have Cryptococcal meningitis and were scheduled for lumber puncture and routine blood sample collection. Samples for this evaluation were collected as part of routine testing as requested by the health care provider. Only remnant sera and CSF from eligible patients was used in the evaluation after the routine diagnostic assays were carried out. Additionally, patients recruited were asked to give a non-routine capillary blood sample from a finger prick.

## 3.3 Study Design

We conducted a hospital based cross-sectional study to evaluate the performance of LFA test for the diagnosis of Cryptococcal meningitis in sera, CSF and capillary blood and compared to LA on sera and CSF, CSF microscopy and CSF culture.

## 3.4 Sample Size Determination

Fisher’s formulae(Cochran, 1977).

Based on the normal approximation the required sample size was given by

**n=Z2P (1−P)**

**d2**

Assumptions: Based on the assumptions in a study by Hansen *et al,* 97.7% (P) was taken as the agreement level between LFA test and the other existing methodologies with the precision of 2.6% at 95% confidence interval (Hansen *et al*., 2013). This gave a sample size of 128.

Each patient gave three samples – CSF, sera and capillary blood and all these sample types were tested by the different diagnostic methods at central microbiology reference laboratory (CMRL) as shown in figure 3.1

**Table 3.1:** Test method and the samples used in evaluation

|  |  |  |  |
| --- | --- | --- | --- |
| LFA | LA | India Ink | Culture |
| CSF | CSF | CSF | CSF |
| Sera | Sera |  |  |
| Capillary blood |  |  |  |

## 

## 3.5 Sampling procedure

We reviewed records for six months at Mbagathi Hospital and got an average of 95 cases of suspected Cryptococcal meningitis in a month. The estimated sampling frame for the study period of three months was 285. Every second HIV patient ≥18 years suspected of Cryptococcal meningitis and scheduled for routine lumber puncture and blood collection for diagnosis who consented to get an extra routine capillary blood and use of the remnant CSF and sera for the evaluation of LFA was enrolled for the study.

### 3.5.1 Inclusion Criteria

Participants who lumbar puncture was requested by health care provider and those who agreed to the blood sample collection procedures.

### 

### 3.5.2 Exclusion Criteria

Any participant/participant with a guardian who was deemed mentally unstable or unable to provide informed consent and participants who were already on antifungal treatment.

Failure to provide capillary blood was NOT an exclusion criterion. This was because the new assay could still be evaluated on CSF and sera.

## 3.6 Sample preparation and Laboratory procedure

### 3.6.1 Sample collection and preparation

Samples were collected as part of routine testing as requested by the clinician. The attending laboratory technologist collected blood samples by venipuncture aseptically from patients who consented, blood was allowed to clot for 10 minutes at room temperature or centrifuged at 1000g for 5 minutes for serum separation in preparation for LA and LFA. CSF samples were collected by lumbar puncture (LP) aseptically into a sterile sealable tubes by the clinician as part of the routine clinical care. CSF was centrifuged at 3000g for 5 minutes and the supernatant used for LFA and LA assays and the pellet for culture and microscopy. For clinical management of the patient as requested by the clinician, sera and CSF (where available) were tested by LA, culture and microscopy (for CSF samples). Left over sera and CSF specimens were used for the laboratory evaluation as shown in appendix 2. A minimum of 500µl of the remaining sample was aliquoted into 1.8ml cryovials. The samples were stored at 4°C for a maximum of 72hrs or at -20°C awaiting transportation to the CMRL. Additionally, a non-routine finger-stick capillary blood sample was requested from all enrolled patients. Using standardized lancets and micro capillary tubes, 1-2 drops (~50 µl) of blood was transferred into a micro centrifuge tubes containing LFA specimen diluent and the test performed at the sample collection site as per the manufacturer’s kit instructions and the results directly submitted to CMRL without being disclosed to the patients for comparison to the other methods.

### 3.6.2 LFA testing procedure

At CMRL, sera and CSF samples were thawed. Forty (40 µl) microliters of patient sera or CSF or 1 drop of capillary blood was mixed with 1 drop of diluents in a micro tube. The LFA strip (IMMY Lateral Flow Immunoassay, Immunomycologics, Norman, OK) was then placed in the specimen-diluent cocktail and incubated at room temperature. Results were read after two incubation times: 10 minutes according to the manufacturer’s instructions and a prolonged incubation time of 15 minutes. The presence of 2 bands (control band and test band) in the test zone of the LFA strip was interpreted as a positive result and a single band in the test zone (control band) was interpreted as a negative result (Appendix 4). These procedures are as per the manufacturer’s instructions.

### 3.6.3 LA testing procedure

LA assays were performed on serum and CSF (where available) according to the manufacturer’s instructions (IMMY Immuno-mycologics, Norman, OK). The assay was performed both at the health-care facility for clinical management of the patients and on the remnant samples sent to CMRL. Results obtained at the health facility were disclosed to the patients for clinical management. The same assay was repeated at CMRL and was used as the reference method for the study. Sera and CSF (where available), either freshly obtained or thawed, were digested with pronase for 15 min at 56°C (for sera) or incubated for 5min at 100°C (for CSF) before the assay, as recommended by the manufacturer. The LA test was then performed as per the manufacturer's instructions.

### 3.6.4 Culture Procedure

CSF samples were centrifuged at 3000g for 5 minutes and the deposit plated on Sabourauds dextrose slants supplemented with chloramphenicol. Incubation was done at 35°C for a period of 48-72 hours. Culture was standardized using ATCC organisms.

## 3.7 Data management

### 3.7.1 Data collection

Data was recorded in the Sample Collection and LFA Results Log (Appendix 3). The variables collected were: age, sex, specimen types, volumes, dates of collection and test outcomes (In culture, LA and LFA on CSF, Serum and capillary blood).

Results from laboratory testing performed on capillary blood were recorded at the study hospital and the results submitted to CMRL for comparison to the other methods. Completed forms were sent with the samples shipped to CMRL. Data collected from both the Sample Collection and LFA Results Log was entered into an Excel database and validated.

### 3.7.2 Data analysis

Data was entered to Microsoft excel 2007 (Microsoft Seattle WA, USA) for cleaning and analyzed using GraphPad QuickCalcs (GraphPad Software, Inc., La Jolla, CA). Sensitivity, specificity, predictive values, confidence intervals of proportion, agreement levels, and kappa (k) coefficients of India ink, LA, culture and LFA were compared in sera, CSF and capillary blood.

## 

## 3.8 Ethical considerations

A written informed consent was obtained from all participants to allow an additional non-routine capillary blood sample taken as well as having their remnant sample used for evaluating new diagnostics for Cryptococcal meningitis. Samples from each participant were assigned a unique sample ID number at the time of specimen collection with no personal identifiers. Only information related to the remnant sample type and the LFA results from capillary blood were collected in the Hospital. Permission to conduct this evaluation was sought from the Ethical Review Board of Moi University (FAN: IREC 1795, 2017)

# 

# CHAPTER FOUR: RESULTS

### 4.1 Characteristics of the study participants

Out of 128 persons suspected to have Cryptococcal meningitisduring the study period, 124 were enrolled for the study and four declined to participate. Most of the participants (59%) were male. The mean age was 35 ± 8 years. A total of 124 capillary blood and serum samples, and 99 CSF samples were collected and analyzed using LFA, LA, India ink microscopy and culture method. There were 25 participants who were unable to produce CSF sample (CSF dry taps).

### 4.2 Comparison of Lateral flow Immuno Assay to Latex agglutination using sera.

On the comparison of LFA to LA on sera, the sensitivity and specificity were 100% (95% CI 92.3-100) and 91% (95% CI 82.6-95.6) respectively, PPV and NPV at 86.8% and 100% with a total agreement of 94.4% (117) and a kappa- value of 0.88 (95% CI 0.80-0.97). (Table 4.1).

**Table 4.1**  Comparison of Lateral flow Immuno Assay to Latex agglutination using sera.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | LA on sera | | Sensitivity  (%) | Specificity  (%) | kappa value |
| Positve | Negative | 100  ( 92.3-100) | 91  ( 82.6-95.6) | 0.96 |
| LFA on Sera | Positive | 46 | 7 |
| Negative | 0 | 78 |

### 

### 4.3 Comparison of Lateral flow Immuno Assay to Latex agglutination using CSF.

LFA to LA on CSF, the sensitivity and specificity were 100% (95% CI 92.7-100) and 96% (95% CI 86.5-98.9), PPV and NPV at 96.1 and 100 with a total agreement of 98% (97) and a kappa- value of 0.96 (95% CI 0.90-1.00). (Table 4.2)

**Table 4.2**  Comparison of Lateral flow Immuno Assay to Latex agglutination using CSF.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | LA on CSF | | Sensitivity  (%) | Specificity  (%) | kappa value |
| Positive | Negative | 100  (92.7-100) | 96  (86.5-98.9) | 0.96 |
| LFA on CSF | Positive | 49 | 2 |
| Negative | 0 | 48 |

### 4.4 Comparison of Lateral flow Immuno Assay to India ink Microscopy using CSF.

Comparison of LFA to India Ink (Microscopy) using CSF, the sensitivity and specificity were 100% (95% CI 92.6-100) and 94.1% (95% CI 84.1-97.9), PPV and NPV at 94.1% and 100% with a total agreement of 97% (96) and a kappa- value of 0.94 (95% CI 0.87-1.00). (Table 4.3).

**Table 4.3** Comparison of Lateral flow ImmunoAssay to india ink microscopy using CSF.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | India ink-CSF | | Sensitivity  (%) | Specificity  (%) | kappa value |
| Positive | Negative | 100  (92.6-100) | 94.1  (84.1-97.9) | 0.94 |
| LFA on CSF | Positive | 48 | 3 |
| Negative | 0 | 48 |

### 4.5 Comparison of Lateral flow Immuno Assay to culture using CSF.

On comparison of LFA to culture on CSF, the sensitivity and specificity were 100% (95% CI 86.2-100) and 64% (95% CI 52.7-73.9), PPV and NPV at 47.6% and 100% with a total agreement of 72.7% (72) and a kappa- value of 0.46 (95% CI 0.32-0.61). (Table 4.4)

**Table 4.4** Comparison of Lateral flow ImmunoAssay to Culture using CSF.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | Culture on CSF | | Sensitivity  (%) | Specificity  (%) | kappa value |
| Positive | Negative | 100  (86.2-100) | 64  (52.7-73.9) | 0.46 |
| LFA on CSF | Positive | 24 | 27 |
| Negative | 0 | 48 |

### 4.6 Comparison of CSF culture to LA and india ink using CSF

Comparison of Culture to LA using CSF, the sensitivity and specificity were 95.8 (95%CI 79.8-99.3) and 65.3 (95%CI 54.1-75.1) with a total agreement of 72% (72) and a kappa value of 0.45 (95%CI 0.30-0.60). On comparison to india ink, the sensitivity and specificity were 95.9% (95%CI 79.8-99.3) and 66.7% (95%CI 55.4-76.3) with a total agreement of 73% (73) and a kappa value of 0.47 (95%CI 0.31-0.62). (Table 4.5)

**Table 4.5**  Comparison of CSF culture to LA and India Ink using CSF.

|  |  |  |  |
| --- | --- | --- | --- |
|  | | **CSF-Culture** | |
| Test and results | | Positive | Negative | Sensitivity  (%) (95%CI) | Specificity  (%)  (95%CI) | Kappa  Value  (95%CI) |
| **LA-**  **CSF** | Positive | 23 | 26 | 95.8  (79.8-99.3) | 65.3  (54.1-75.1) | 0.45 |
| Negative | 1 | 49 |
|  |  |  |  |  |  |  |
| **india**  **ink-**  **CSF** | Positive | 23 | 25 | 95.8  (79.8-99.3) | 66.7  (55.4-76.3) | 0.47 |
| Negative | 1 | 50 |

### 4.7 Comparison of the Lateral flow ImmunoAssay using capillary blood to Lateral flow ImmunoAssay using sera.

LFA on capillary blood was compared with LFA on sera and CSF. On the comparison of LFA on capillary blood to LFA on sera, the sensitivity and specificity were100%, PPV and NPV at 100% with a total agreement of 100% (124) and a kappa- value of 1.00 (95% CI 1.00-1.00) (Table 4.6).

**Table 4.6**  Comparison of Lateral flow ImmunoAssay using capillary blood against Lateral flow ImmunoAssay using serum.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | LFA on Serum | | Sensitivity  (%) | Specificity  (%) | kappa value |
| Positive | Negative | 100 | 100 | 1.00 |
| LFA on capillary blood | Positive | 53 | 0 |
| Negative | 0 | 71 |

### 4.8 Comparison of the Lateral flow ImmunoAssay using capillary blood to Lateral flow ImmunoAssay using CSF.

LFA on capillary blood was compared to LFA on CSF, the sensitivity, specificity and predictive values were all 100% with a total agreement of 100% (99) and a kappa-value of 1.00 (95% CI 1.00-1.00) (Table 4.7).

**Table 4.7**  Comparison of Lateral flow ImmunoAssay using capillary blood against Lateral flow ImmunoAssay using CSF.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | LFA-Serum | | Sensitivity  (%) | Specificity  (%) | kappa value |
| Positive | Negative | 100 | 100 | 1.00 |
| LFA capillary | Positive | 51 | 0 |
| Negative | 0 | 48 |

# CHAPTER FIVE: DISCUSSION

**5.1 Discussion**

This study, evaluated the performance of LFA test and compared it to the other available commonly used diagnostic methods using multiple specimens; capillary blood, sera and CSF from HIV patients with suspected Cryptococcal meningitis in a hospital in Kenya. The overall agreement of LFA to LA and India ink tests using different sample types was almost perfect (Appendix 5).

The results on individual tests show that there was almost perfect agreement between LFA and LA on CSF and sera. The test demonstrated high level of sensitivity (100%) and specificity (94.4%) of LFA compared to LA on sera and CSF. The findings are consistent with similar studies conducted in South Africa and USA that show high sensitivity (100%) and specificity (>99%) using CSF and serum (Binnicker *et al*., 2012; Dhana, 2013). However, there were variations on specificity and PPV of LFA compared to LA on CSF and sera which were lower than sensitivity and NPV. The variations could be due to the LA testing procedure where there is reconstitution and pre heating of CSF and sera with pronase during sample preparation as observed in other studies (Paulo, 2015). Comparable results were reported in a study on multisite validation of Cryptococcal Antigen Lateral Flow Assay using CSF in Uganda and South Africa (Boulware *et al*., 2014). The strong agreement between the LFA and LA tests on sera and CSF is an indicator that LFA test on CSF and sera is equally good as LA test on sera and CSF.

There was a strong agreement between LFA and India ink microscopy using CSF. The findings from comparison of LFA to India ink microscopy using CSF demonstrated high sensitivity (100%), specificity (94.1%), PPV (94%) and NPV (100%). The India ink microscopy requires laboratory infrastructure, dependent on fungal burden and is highly operator dependent rather than the test performance. This is in contrast to the findings from the expert opinion and other studies that documented lower sensitivity (86%) and NPV (80.2%) (Boulware *et al*., 2014; Kozel and Sean, 2013).

On the comparison of LFA to culture using CSF, there was high sensitivity (100%), low specificity (84%), and moderate agreement with a weak kappa value. The findings were consistent with other studies that documented high sensitivity (100%) and low specificity (86%) (Rioki *et al*., 2015; Saha, *et al* 2008). The findings on high sensitivity, low specificity and a weak kappa value were similarly demonstrated when CSF culture was compared to LA and India Ink using CSF. However in contrast to our findings, other studies documented low sensitivity in CSF culture when compared against other diagnostic tests (Boulware *et al*., 2014; Lindsley *et al*., 2011; Kozel and Sean, 2013). The culture method requires large quantity of specimen for the maximum fungal yield, several days required for fungal growth, and requires expertise for accurate identification and laboratory infrastructure which was available at the reference laboratory but a challenge to the routine hospital laboratories.

On comparison of LFA on capillary blood to LFA on sera and CSF, the overall agreement of was excellent (100%) with a perfect kappa value (1.00). The LFA test demonstrated high sensitivity, specificity and predictive values of 100% on capillary blood, sera and CSF, a characteristic that makes LFA test a good diagnostic method of choice for cryptococcal meningitis;that is easy to perform, gives rapid, accurate and precise results using different sample types. The findings were consistent with other studies evaluating LFA using sera, CSF and capillary blood in other countries with sensitivity, specificity and agreement level of 100% (Dhana, 2013; Jarvis *et al*., 2011; Williams *et al*., 2015). The LFA test on capillary blood (finger stick) was easily performed at the bedside using small amount of blood sample giving rapid accurate results. This may form LFA on finger stick a POC test of choice for cryptococcal meningitis diagnosis. The findings were consistent with findings from Thomas and Sean ( Kozel and Sean, 2013).

The limitations of this study included participants not yielding CSF sample due to dry taps thus reducing the CSF samples that were analysed. The difference on CSF samples analysed had no major implications to the overall evaluation since there were more than one sample type used in the evaluation. Only LFA test could use capillary blood. Thus, we could not uniformly use the sample type across other testing procedures.

# CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

## 6.1 Conclusions

This study shows LFA has a high level of agreement with LA and India Ink microscopy on sera, CSF and capillary blood. LFA is a sensitive and specific test for cryptococcal meningitis diagnosis compared to culture. The LFA test has high sensitivity, specificity and an excellent agreement level between different sample types; capillary blood, serum and CSF.

The LFA test is easy to perform, gives rapid, accurate results, less invasive and easy to interpret results, for diagnosis of cryptococcal meningitis.

## 6.2 Recommendations

We recommend use of LFA test in the diagnosis of cryptococcal meningitis; the test is rapid, easy to use, requires minimal training, has a short turnaround time of about 10 minutes and can be used as a POC test. Use of capillary blood which is less invasive and easy to perform in diagnosis of cryptococcal meningitis. Inclusion of LFA test in the HIV care and treatment tool kit for the diagnosis of cryptococcal meningitis. Conduct more studies across different populations to determine the test performance and utility

# REFERENCES

Bennett, J. E. and J. W. Bailey (1971). Control for Rheumatoid Factor in the Latex Test for Cryptococcosis. [*American Journal of Clinical Pathology*, 56(3):](https://www.ncbi.nlm.nih.gov/pubmed/5094497) 360–365.

Bicanic, T., Muzoora, C., Brouwer, A. E., Meintjes, G., Longley, N., Taseera, K., Harrison, T. S. (2009). Independent association between rate of clearance of infection and clinical outcome of HIV-associated cryptococcal meningitis: analysis of a combined cohort of 262 patients. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, *49*(5), 702–9. https://doi.org/10.1086/604716

Binnicker, M. J., Jespersen, D. J., Bestrom, J. E., & Rollins, L. O. (2012). Comparison of four assays for the detection of cryptococcal antigen. *Clinical and Vaccine Immunology*, *19*(12), 1988–1990. https://doi.org/10.1128/CVI.00446-12

Boulware, D. R., Rolfes, M. A., Rajasingham, R., von Hohenberg, M., Qin, Z., Taseera, K., Meya, D. B. (2014). Multisite validation of cryptococcal antigen lateral flow assay and quantification by laser thermal contrast. *Emerging Infectious Diseases*, *20*(1), 45–53. https://doi.org/10.3201/eid2001.130906

Cochran (1977) Sampling Techniques, (3rd edition), John Wiley and Sons.

Del Poeta, M., & Casadevall, A. (2012). Ten Challenges on Cryptococcus and Cryptococcosis. *Mycopathologia*, *173*(0), 303–310. https://doi.org/10.1007/s11046-011-9473-z

Derek J. (2014). Cryptococcal meningitis : Epidemiology and therapeutic options. *Clinical Epidemiology,* 169–182.

Dhana, A. (2013). Diagnosis of cryptococcosis and prevention of cryptococcal meningitis using a novel point-of-care lateral flow assay. *Case Reports in Medicine*, *2013*, 1–5. https://doi.org/10.1155/2013/640216

Dominic RMS, Prashanth HV, Shenoy S, and B. S. (2009). Diagnostic Value of Latex Agglutination in Cryptococcal. *Journal of Lab Physicians.*, *1(2)*(Jul-Dec;), 67–68.

Fletcher, J. (2018). Fletcher, J. (2018, February 26). “Cryptococcal meningitis: Symptoms, risk factors, and complications.” Medical News Today. Retrieved from https://www.medicalnewstoday.com/articles/321031.php. *Current Tropical Medicine Reports*. https://doi.org/10.1007/s40475-015-0046-y

French, N., Gray, K., Watera, C., Nakiyingi, J., Lugada, E., Moore, M., Gilks, C. F. (2002). Cryptococcal infection in a cohort of HIV-1-infected Ugandan adults. *Aids journal*, *16*(January), 1031–1038. https://doi.org/doi: 10.1097/00002030-200205030-00009

Gade, W., Hinnefeld, S. W., Babcock, L. S., Gilligan, P., Kelly, W., Wait, K., Carolina, N. (1991). Comparison of the PREMIER Cryptococcal Antigen Enzyme Immunoassay and the Latex Agglutination Assay for Detection of Cryptococcal Antigens. [*Journal of Clinical* *Microbiology.*](https://www.ncbi.nlm.nih.gov/pubmed/1761681) (11), 1616–1619.

Hansen, J., Slechta, E. S., Gates-Hollingsworth, M. A., Neary, B., Barker, A. P., Bauman, S., Hanson, K. E. (2013). Large-scale evaluation of the immuno-mycologics lateral flow and enzyme-linked immunoassays for detection of cryptococcal antigen in serum and cerebrospinal fluid. *Clinical and Vaccine Immunology*, *20*(1), 52–55. https://doi.org/10.1128/CVI.00536-12

Huang, H., Fan, L., Rajbanshi, B., & Xu, J. (2015). Evaluation of a New Cryptococcal Antigen Lateral Flow Immunoassay in Serum , Cerebrospinal Fluid and Urine for the Diagnosis of Cryptococcosis : *A Meta-Analysis and Systematic Review*, *787*, 1–10. https://doi.org/10.1371/journal.pone.0127117

Jarvis, J. N., Percival, A., Bauman, S., Pelfrey, J., Meintjes, G., Williams, G. N., Kozel, T. R. (2011). Evaluation of a novel point-of-care cryptococcal antigen test on serum, plasma, and urine from patients with HIV-associated cryptococcal meningitis. *Clinical Infectious Diseases*, *53*(10), 1019–1023. https://doi.org/10.1093/cid/cir613

Rioki, J.N., Wamachi, A., Christine Bii. (2015). Evaluation of rapid diagnostic methods for the diagnosis of cryptococcal meningitis in HIV positive patients in a health facility , Nairobi-Kenya. *East African Journal of Pathology,* *2*, 18–22.

Kanji, S. S., Kakai, R., & Onyango, R. O. (2011). Cryptococcal meningitis among human immunodeficiency virus patients attending major hospitals in Kisumu, Western Kenya. *Archives of Clinical Microbiology*, *2*(1), 1–6. https://doi.org/10:3823/220

Kendi C., J. Penner, J. Koech, M. Nyonda, E. Bukusi, C. Cohen, H. Mutai, A. M. (2010). Case fatality due to cryptococcal meningitis in a retrospective cohort in Kenya. In *In AIDS 2010 - XVIII International AIDS Conference: Abstract no. MOPE0115.*

Kenya National Bureau of Statistics, (2014). *Nairobi County population statistics.* *https://www.knbs.or.ke/county-statistics*

Lindsley, M. D., Mekha, N., Baggett, H. C., Surinthong, Y., Autthateinchai, R., Sawatwong, P., Poonwan, N. (2011). Evaluation of a newly developed lateral flow immunoassay for the diagnosis of cryptococcosis. *Clinical Infectious Diseases*, *53*(4), 321–325. https://doi.org/10.1093/cid/cir379

Maziarz, E. K., Perfect, J. R., & Health, I. (2016). Cryptococcosis. *Infectious Disease Clinics of NorthAmerica*, *30*(1), 179–206. https://doi.org/10.1016/j.idc.2015.10.006. *Cryptococcosis*

Mdodo.R., Brown, K., Omonge, E., Jaoko, W., Badddley, J. (2013). Outcome Associated with Cryptococcal meningitis. *East Africa Medical Journal.* *87*(12), 481–487.

Mwaba, P., Mwansa, J., Chintu, C., Pobee, J., Scarborough, M., Portsmouth, S., & Zumla, a. (2001). Clinical presentation, natural history, and cumulative death rates of 230 adults with primary cryptococcal meningitis in Zambian AIDS patients treated under local conditions. *Postgraduate Medical Journal*, *77*(914), 769–773. https://doi.org/10.1136/pmj.77.914.769

Park, B. J., Wannemuehler, K. A., Marston, B. J., Govender, N., Pappas, P. G., & Chiller, T. M. (2009). Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. *Aids*, *23*(4), 525–530. https://doi.org/10.1097/QAD.0b013e328322ffac

Paulo, S. (2015). Lateral Flow Assay for Cryptococcal antigen : An Important Advance to Improve the Continuum of HIV care and reduce cryptococcal meningitis-related mortality. *Journal of the* São Paulo *Institute of Tropical Medicine*, *57*(1), 38–45.

Sekhon, S. A., K Garg, A., Kaufman, L., S Kobayashi, G., Hamir, Z., Jalbert, M., & Moledina, N. (1993). Evaluation of a commercial enzyme immunoassay for the detection of cryptococcal antigen. *Mycoses* (Vol. 36). https://doi.org/10.1111/j.1439-0507.1993.tb00684.x

Saha, D. C., Xess, I., & Jain, N. (2008). Evaluation of conventional & serological methods for rapid diagnosis of cryptococcosis. *Indian Journal of Medical Research*, *127*(5), 483–488.

Sloan, D. J., Dedicoat, M. J., & Lalloo, D. G. (2014). Europe PMC Funders Group Treatment of Cryptococcal Meningitis in Resource Limited Settings Disease Burden in Resource Limited Settings. *Current Opinion in Infectious Diseases*, *22*(5), 455–463. https://doi.org/10.1097/QCO.0b013e32832fa214.Treatment

Kozel Thomas and S, Bauman. (2013). CrAg Lateral Flow Assay for Cryptococcosis. *Expert Opinion on Medical Diagnostics*, *6*(3), 775–784. https://doi.org/10.1517/17530059.2012.681300.CrAg

Temstet, A., Roux, P., Poirot, J., Ronin, O., & Dromer, F. (1992). Evaluation of a Monoclonal Antibody-Based Latex Agglutination Test for Diagnosis of Cryptococcosis : Comparison with Two Tests Using Polyclonal Antibodies. *Journal of Clinical Microbiology,* *30*(10), 2544–2550.

Tenforde, M. W., Wake, R., Leeme, T., & Jarvis, J. N. (2016). HIV-Associated Cryptococcal Meningitis: Bridging the Gap Between Developed and Resource-Limited Settings. *Current Clinical Microbiology Reports*, *3*(2), 92–102. https://doi.org/10.1007/s40588-016-0035-5

Vanselow, M., E.E. Brandt, and B. J. P. (2012). Diagnosis and management of cryptococcal disease in resourse-limited settings. [*Current Fungal Infection Reports*](https://link.springer.com/journal/12281)*.*, 6:35-40.

Williams, D. A., Kiiza, T., Kwizera, R., Kiggundu, R., Velamakanni, S., Meya, D. B., Boulware, D. R. (2015). Evaluation of Fingerstick Cryptococcal Antigen Lateral Flow Assay in HIV-Infected Persons: A Diagnostic Accuracy Study. *Clinical Infectious Diseases*, *61*(3), 464–467. https://doi.org/10.1093/cid/civ263

World Health Organization (WHO). (2011). *Rapid Advice: Diagnosis , Prevention and Management of Cryptococcal disease in HIV- infected adults, adolescent and children*. Geneva: WHO.

APPENDICES

**Appendix I: Adult Consent Form**

Consent to participate in the Cryptococcal Lateral Flow Assay Evaluation

INTRODUCTION AND PURPOSE

We hereby request your participation in an evaluation of a new method to diagnose cryptoccoal meningitis. Cryptococcal meningitis is an organism that causes meningitis, a disease that affects the brain. People who are infected with HIV have a high chance of suffering from Cryptococcal meningitis. Once infected, the person has to be on long-term treatment. There is a new method that detects the organism even before you start feeling sick. This helps your doctor to give you medicine before the disease affects your body. The government of Kenya may start using the new method in some health facilities after the study is completed.

PROCEDURES

The normal process for your clinic visit is to have about one teaspoon of blood drawn from the vein in your arm and the same amount of fluid from your spinal cord. Your health provider usually sends these samples to the laboratory that your health facility uses now. The lab will then return the results to your health care provider. Your health care provider will return them to you. Nobody from the study will change the usual process of your health care. Any leftover blood and spinal fluid from the usual tests will be used to test the new method.

If you choose to participate, you will be asked to give additional drops of blood from a prick of the finger. This is optional and you may choose to give or not to give. The drops of blood will be used to test a faster way of diagnosing the disease. However, your leftover samples will still be used to test the new method. The finger prick will only take a few minutes. We will collect 2-3 drops of blood from your finger prick using a thin tube and place directly into a small tube. The laboratory will test the blood for Cryptococcal meningitisusing the new method. We will not write your name or anything else that identifies you on this sample. Any extra sample will not be stored and will be discarded by the MOH staff.

RISKS OR DISCOMFORTS

You are already having spinal fluid and blood taken from you as requested by your health care provider. A small extra amount of blood will be taken from your finger.

You might feel brief pain while taking blood from a vein in the arm or a prick in the finger, bruising, or dizziness. It is possible but not likely for infections to occur where the needle enters the arm or the finger, but this is not likely to happen. No more than three attempts will be taken to draw blood from the finger. You may interrupt the blood collection at any time.

BENEFITS

There is no immediate benefit or compensation to you for participating in this evaluation. You may benefit in the future if the new method is approved for use in Kenya. The benefit would be having another way to diagnosing Cryptococcal meningitis at an early stage before sickness sets in. The new method is easier and faster than the one your health provider uses now.

CONFIDENTIALITY

Your name will not be written on any form for this study or on your samples. We will identify you by a number assigned by this study. Your name and other identifying information will not be kept with this study. The study details will be kept in a locked file; only the researchers for this study will have access to the records.

COSTS TO PARTICIPANT

There is no cost to you if you participate in this study.

RIGHT TO REFUSE OR WITHDRAW

Participation in this study is voluntary. It is your choice to be in this study. You can stop participating in the study at any time without giving a reason. You will receive the same health care if you participate or if you do not participate in the study.

QUESTIONS

Do you have any questions?

PERSONS TO CONTACT

You and/or your next of kin will be given a blank copy of this consent form. If you have questions about the study or believe you have been harmed by being in the study, you may contact:

Lawrence Gitonga,

Field Laboratory and Training Program

Tel: 0721479241

For questions about your rights as a participant in this evaluation, please contact ethical review committee (Moi University):

Secretary or Chairman of Moi University ERB

Moi University, Main Campus,

P.O Box 3900-30100

Eldoret, Kenya

CONSENT STATEMENT (Interviewer: choose one statement)

□ I have read the information in this form. OR

□ The information in this form was read to me in the presence of a witness. OR

□ I am the next of kin to the participant and I have read / been read to the information on this form

I have talked about what this form says with the interviewer. I had a chance to ask questions and my questions were answered. I understand the information in this form.

I agree to participate in the study (circle one): Yes No

I agree to give blood from my arm vein for Cryptococcal testing (circle one): Yes No

I agree to give blood from my finger for Cryptococcal testing (circle one): Yes No

Participant name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Participant signature: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_or Mark: \_\_\_\_\_\_\_\_\_\_\_\_\_ Date: \_\_\_/\_\_\_\_/\_\_\_\_

Participant next of kin name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Relation to participant: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Participant next of kin signature: \_\_\_\_\_\_\_\_\_\_\_or Mark: \_\_\_\_\_\_\_\_\_\_\_\_\_ Date: \_\_\_/\_\_\_\_/\_\_\_\_

Witness name:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Witness Signature: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Date: \_\_\_/\_\_\_\_/\_\_\_\_

Name of clinician/laboratorian/HCW taking consent: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Signature of the clinician/laboratorian/HCW taking consent: \_\_\_\_\_\_\_\_\_­­\_Date: \_\_\_/\_\_\_\_/\_\_\_\_

**Kiambatisho I: Fomu ya Idhini kwa watu wazima**

**Ridhaa ya kushiriki katika Tathmini na uchanganuzi wa Cryptococcal Lateral Flow**

**UTANGULIZI NA KUSUDI**

Unaombwa kushiriki katika tathmini ya mbinu mpya ya kutambua Cryptococcal ya uti wa mgongo. Cryptococcal ya uti wa mgongo ni ugonjwa ambao huathiri ubongo. Watu ambao wameambukizwa virusi vya ukimwi wana nafasi ya juu ya kuipata shida hii. Kila anayeambukizwa hulazimishwa kupata matibabu ya muda mrefu. Hata hivyo, kuna mbinu mpya ya kutambua kinachosababisha hali hii hata kabla ya mja kuanza kuhisi ugonjwa. Hii husaidia daktari wako ili kukupa dawa kabla ya ugonjwa kuathiri mwili wako.

Serikali ya Kenya inaweza kuanza kutumia mbinu mpya katika baadhi ya vituo vya afya baada ya kukamilisha uchunguzi huo.

**TARATIBU**

Kwa kawaida, unapotembelea kliniki/ kituo chako cha afya, kijiko kimoja cha damu hutolewa kutoka kwa mshipa ya mkono wako na kiasi hicho cha maji kutoka kwa uti wa mgongo. Mhudumu wako wa afya atapeleka hizo sampuli kwenye maabara. Matokeo kutoka maabara yatapewa mhudumu huyo wa afya atakayeikabidhi kwako. Mchakato wa kutunzwa kwa afya yako haitabadilishwa na yeyote . Damu yoyote na maji ya uti wa mgongo itakayobakia baada ya mchakato huo utatumika kutahini mbinu hii mpya.

Iwapo utashiriki , unaombwa kutoa matone ziada ya damu kutoka chomo ya kidole chako Hii ni hiari na unaweza kuchagua kutoa au kukataa. Matone ya damu zitatumika kutambua ugonjwa huu katika njia ya haraka. Hata hivyo, sampuli zako zilizobaki bado zitatumika na hii mbinu mpya. Sindano ya kidole huchukua dakika chache tu. Matone 2-3 ya damu kutoka kwa chomo chako cha kidole itakusanywa kwa kutumia mpira nyembamba na kuiweka moja kwa moja kwenye bomba dogo. Damu hiyo itapelekwa maabarani ili kubainisha iwapo kuna ugonjwa wa uti wa mgongo. Uchunguzi huu utafanywa kwa kutumia njia hii mpya. Hatutaandikisha jina lako au chochote kitakacho kuhusisha kuwa mwenyeji wa sampuli hii. Sampuli yoyote ya ziada haitahifadhiwa na itatupwa na wafanyakazi wa wizara ya afya.

**HATARI**

Maji ya uti wa mgongo na damu yako inachukuliwa kama ilivyoagizwa na mhudumu wako wa afya. Kiasi kidogo cha damu ya ziada itachukuliwa kutoka kwa ncha ya kidole chako. Mchakato huu inaweza sababisha, maumivu, au majeraha kwenye kidole kilichochomwa -ambayo inapaswa kutatuliwa bila matibabu yoyote. Hatari ya kuchukua damu kutoka mshipa katika mkono au chomo la kidole ni kwamba unaweza kuhisi maumivu ya muda mfupi kutoka chomo , kupata alama kwenye ngozi au kizunguzungu. Ingawaje ni vigumu kupata maambukizi katika sehemu ambayo sindano huingilia mkono au kidole, mara nyingine watu huambukizwa. Hata hivyo, kesi kama hizi ni chache sana. Majaribio ya kutoa damu kutoka kwenye kidole haipaswi kuzidi mara tatu. Unaweza kupinga ukusanyaji wa damu wakati wowote.

**FAIDA**

Hakuna faida ya mara moja au fidia utakayopata kwa kujiunga na tathmini hii. Unaweza kufaidika katika siku zijazo iwapo mbinu hii mpya itapitishwa kwa ajili ya matumizi nchini Kenya. Faida ni kuwa kutakuwa na njia nyingine ya kupima Cryptococcal ya uti wa mgongo itakayotumika kugundua hatua za awali kabla ya ugonjwa kuanza.Vile vile, mbinu hii mpya ni rahisi kutumia zaidi ya inayotumika na mhudumu wako kwa sasa.

**USIRI**

Jina lako halitoandikwa kwenye fomu yoyote ya utafiti au hata kwenye sampuli zako. Tutakutambua kupitia nambari utakayopewa kwa ajili ya utafiti huu.

**GHARAMA YA WASHIRIKI**

Hakuna gharama kwa mshiriki yeyote wa utafiti huu.

**HAKI YA KUKATAA AU KUJIONDOA**

Kushiriki katika utafiti huu ni kwa hiari. Ni uchaguzi wako kuwa katika utafiti huu. Unaweza kuacha kushiriki wakati wowote bila ya kutoa sababu. Utapokea huduma za afya ikiwa utashiriki au ukose kushiriki.

**MASWALI**

Je una maswali yoyote?

**MTU WA KUWASILIANA NAYE**

Wewe na jamaa yako wa karibu mtapewa nakala ya fomu hii ya idhini ambayo haijajazwa. Kama una maswali /shauku kuhusu utafiti huu , unaweza kuwasiliana na : Lawrence Gitonga,

Field Epidemiology and Laboratory training Program

Nambari ya simu : 0721479241

Kwa maswali kuhusu haki zako kama mshiriki katika utafiti huu, tafadhali wasiliana na kamati ya kudhibiti maadili (Chuo kikuu cha Moi):

Katibu au Mwenyekiti wa chuo kikuu cha Moi IREC

**KAULI YA IDHINI ( anayehoji kuchagua kauli moja )**

 Nimesoma habari katika fomu hii AU

 Nimesomewa habari katika fomu hii mbele ya shahidi AU

 Mimi ni jamaa wa karibu wa mshiriki na nimesoma/somewa habari katika fomu hii

Nimeongelea yaliyomo katika fomu hii na mhojaji . Nilikuwa na nafasi ya kuuliza maswali na maswali yangu kujibiwa. Nimeelewa habari katika fomu hii.

Nimekubali kushiriki katika utafiti ( chagua moja ): Ndiyo Hapana

Nakubali kutoa damu kwa mshipa ya mkono wangu kwa ajili ya upimaji wa Cryptococcal

(chagua moja ): Ndiyo Hapana

Nakubali kutoa damu kutoka kidole changu kwa ajili ya upimaji wa Cryptococcal”

(chagua moja ): Ndiyo Hapana

Jina la mshiriki: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Sahihi la mshiriki: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ au alama: \_\_\_\_\_\_\_\_\_\_\_\_\_ Tarehe: \_\_\_ / \_\_\_ / \_\_\_

Jina la jamaa wa karibu wa mshiriki: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Uhusiano na Mshiriki: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Sahihi la jamaa: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ au alama: \_\_\_\_\_\_\_\_\_\_\_\_\_ Tarehe: \_\_\_ / \_\_\_\_ / \_\_\_\_

Jina la Shahidi: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Sahihi la Shahidi: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Tarehe: \_\_\_ / \_\_\_\_ / \_\_\_\_

Jina la msaidizi wa utafiti anayechukua ridhaa: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Sahihi ya msaidizi wa utafiti anayechukua ridhaa: \_\_\_\_\_\_\_\_\_\_\_\_\_Tarehe: \_\_\_ / \_\_\_\_ / \_\_

**Appendix 2: Evaluation of LFA Algorithm**

##### 

**Assess**

**eligibility**

**Informed**

**Consent**

**-**

**Yes**

**Proceed with normal care**

**Unique Study ID assigned**

**Informed Consent**

**--**

**No**

**Venous blood collection**

**Serum**

**separation**

**Serum**

**/**

**CSF tested**

**at facility as**

**per clinician request**

**Remaining serum**

**/**

**CSF ≥**

**µl**

**500**

**aliquoted**

**into**

**cryovilas**

**Results given for**

**patient management**

**Capillary blood collection**

**Finger stick**

**(**

**)**

**Capillary blood sample**

**-**

**tested using LFA**

**Results recorded in study sample log**

**register and NOT released for patient**

**care management**

**Samples sent to the lab for**

**testing using LFA ,LA and**

**culture (for CSF)**

**LFA capillary blood results**

**sent to CMRL**

**CSF collection (if requested by**

**HCP)**

**Appendix 3: Sample Collection and LFA Results Log**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Site Name: \_\_\_\_Mbagathi District Hospital\_\_\_\_ Site Code \_\_\_01\_\_\_ | | | | | | | | | |  |  |
| **S/N** | **Type of specimens**  **collected**  (check all that apply) | | **Date of**  **collection**  dd/mm/yyyy | **Sample ID**  XX-XXX-X | Consent given to use sample\* | **LFA Batch Number**  \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  Expiry\_\_/\_\_\_/\_\_\_\_  dd/mm/yyyy | | | | **(sample volume sent to CMRL)** | **Collected by /**  **Remarks** |
| **Finger-stick results** | | | |
| Sample 1 | | Sample 2 | |
| 5 min | 15 min | 5 min | 15 min |
| 1 | **√** | Finger-stick blood | 20**/**11**/**12 | 01-001-F | **Yes** No | P | P | P | P |  |  |
| **√** | Serum | 20**/**11**/**12 | 01-001-S |  |  |  |  |  | 1.2ml | Ann  Ochola |
| **√** | CSF | 20**/**11**/**12 | 01-001-C |  |  |  |  |  | 0.5ml | Ann  Ochola |
| 2 |  | Finger-stick blood | \_\_/\_\_\_/\_\_\_ |  | Yes No |  |  |  |  |  |  |
|  | Serum | \_\_/\_\_\_/\_\_\_ |  | Yes No |  |  |  |  |  |  |
|  | CSF | \_\_/\_\_\_/\_\_\_ |  | Yes No |  |  |  |  |  |  |
| 3 |  | Finger-stick blood | \_\_/\_\_\_/\_\_\_ |  | Yes No |  |  |  |  |  |  |
|  | Serum | \_\_/\_\_\_/\_\_\_ |  | Yes No |  |  |  |  |  |  |
|  | CSF | \_\_/\_\_\_/\_\_\_ |  | Yes No |  |  |  |  |  |  |
| 4 |  | Finger-stick blood | \_\_/\_\_\_/\_\_\_ |  | Yes No |  |  |  |  |  |  |
|  | Serum | \_\_/\_\_\_/\_\_\_ |  | Yes No |  |  |  |  |  |  |
|  | CSF | \_\_/\_\_\_/\_\_\_ |  | Yes No |  |  |  |  |  |  |
| 5 |  | Finger-stick blood | \_\_/\_\_\_/\_\_\_ |  | Yes No |  |  |  |  |  |  |
|  | Serum | \_\_/\_\_\_/\_\_\_ |  | Yes No |  |  |  |  |  |  |
|  | CSF | \_\_/\_\_\_/\_\_\_ |  | Yes No |  |  |  |  |  |  |

Submitting officer: Name\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Sign\_\_\_\_\_\_\_\_\_\_\_\_ Date\_\_\_\_\_\_\_\_\_\_\_ Time\_\_\_\_\_\_\_\_\_\_\_\_

Cool Box temperature on submission\_\_\_\_\_\_°C

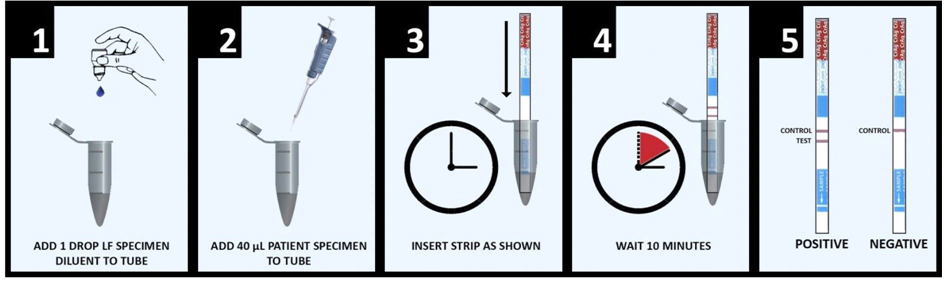
Receiving officer: Name\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Sign \_\_\_\_\_\_\_\_\_\_\_\_Date \_\_\_\_\_\_\_\_\_\_\_ Time\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Cool Box temperature of receipt:\_\_\_\_\_\_\_°C

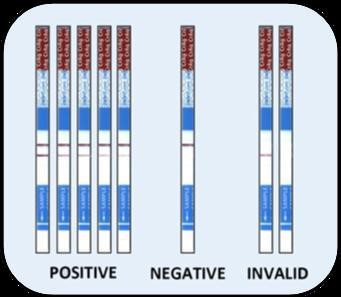
COMMENTS:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Appendix 4: Lateral Flow Assay Testing and Interpretation**

**Testing**



**Interpretation**



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* **POSITIVE:** The presence of two lines (test and control), regardless of the intensity of the test line, indicates a positive result.
* **NEGATIVE:** A single control line indicates a negative result**.**
* **INVALID:** If the control line does not appear, the results are invalid and the test should be repeated.

**Appendix 5: Interpretation of Cohen’s kappa**

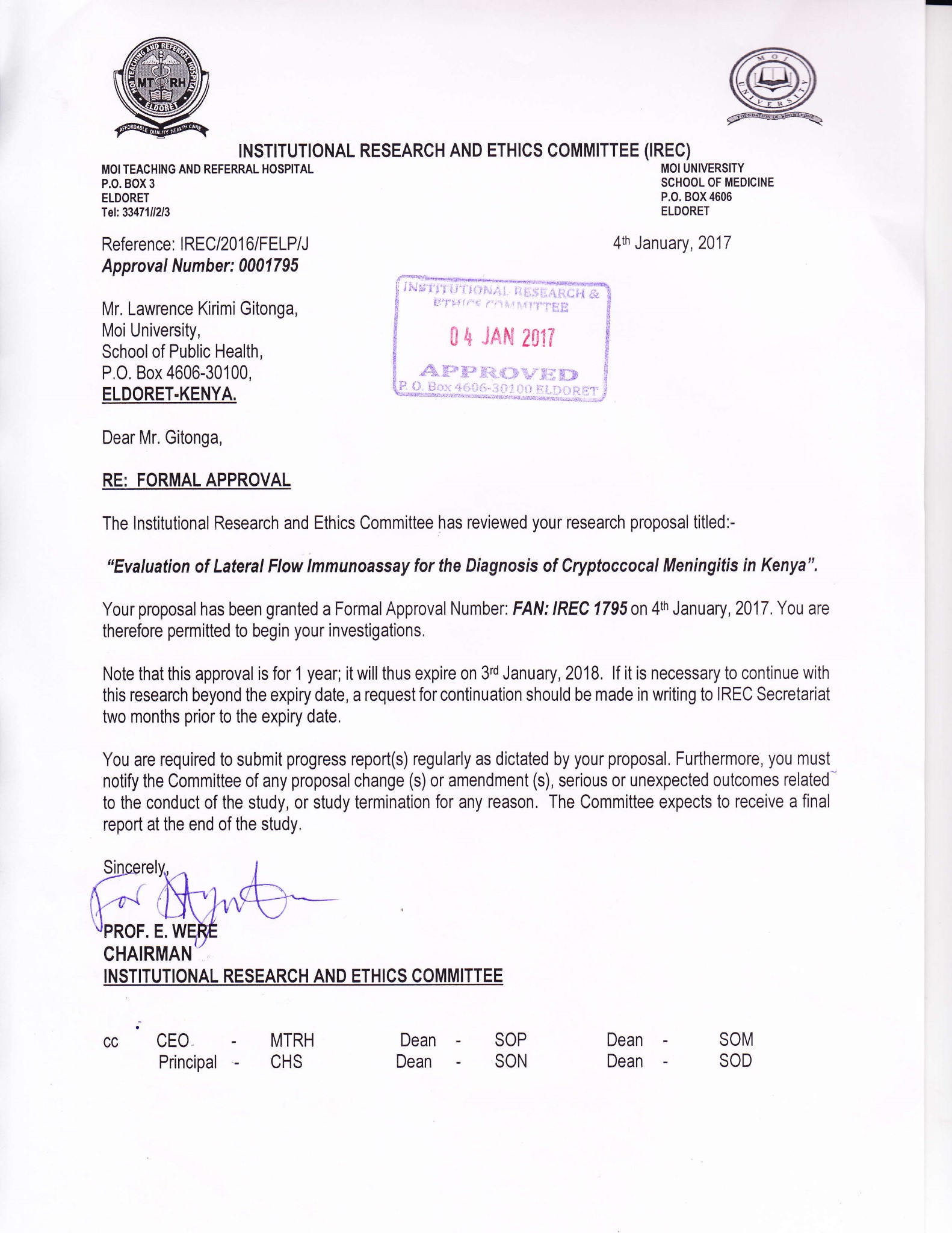
|  |  |  |
| --- | --- | --- |
| **Value of Kappa** | **Level of Agreement** | **% of Data that are Reliable** |
| **0–.20** | None | 0–4% |
| **.21–.39** | Minimal | 4–15% |
| **.40–.59** | Weak | 15–35% |
| **.60–.79** | Moderate | 35–63% |
| **.80–.90** | Strong | 64–81% |
| **Above.90** | Almost Perfect | 82–100% |
|  |  |  |

\* **(**McHugh, M. L. (2012)).

**Appendix 6: Ethics certificate**



**Appendix 7: Institutional Research and Ethics Committee Approval**

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**Appendix 8: Research Authorization by Mbagathi Hospital**

