



A Pilot Cryptococcal Antigenemia (CrAg) Screening Program among HIV-Infected Patients Attending Mbabane Government Hospital: Prevalence of Cryptococcal Antigenemia, Clinical Utility, Feasibility and Implications for National Roll Out of a CrAg Screening Program

End of Study Report

June 2015

This study report was prepared by University Research Co., LLC (URC) and authored by Rosanna Jeffries, Samson Haumba, Marianne Calnan, Kelly Clarke, Peter Ehrenkranz, Tony Ao, Sikathele Mazibuko, Charmaine Mlambo and Lydia Mpango. The views expressed in this publication are the sole responsibility of University Research Co., LLC (URC) and do not necessarily reflect the views of the funding organisations.

Acknowledgments

This report was prepared by University Research Co., LLC (URC) with funding from the U.S. President's Emergency Plan for AIDS Relief (PEPFAR) through the U.S. Centers for Disease Control and Prevention (CDC) under the Provision of HIV/AIDS and TB Related Laboratory Support and Technical Assistance project. The project is managed by URC under the terms of Cooperative Agreement Number 1U2G/PS001896. Funding for the study reagents was through the African Society for Laboratory Medicine (ASLM).

The work of the following Principal Investigators (PIs), Co- Investigators, and collaborators must be acknowledged:

- Samson Haumba (PI): URC Swaziland
- Charles Azih (PI): ART Unit, Ministry of Health, Mbabane, Swaziland
- Peter Ehrenkranz (PI): Formerly CDC Swaziland, currently Gates Foundation, USA
- Gugu Maphalala (PI): Swaziland Health Laboratory Systems, Ministry of Health, Swaziland
- Charmaine Mlambo (PI): Formerly URC Swaziland, currently Clinton Health Access Initiative, Swaziland
- Tom Chiller (PI): Mycotic Diseases Branch, CDC Atlanta, USA
- Benjamin Park (PI): Mycotic Diseases Branch, CDC Atlanta, USA
- Rachel Smith (PI): Mycotic Diseases Branch, CDC Atlanta, USA
- Marianne Calnan (Co-Investigator): URC Swaziland
- Rosanna Jeffries (Co-Investigator): URC Swaziland
- Joel Chehab (Co-Investigator): CDC Swaziland
- Dan Gama (Co-Investigator): CDC Swaziland
- Gugulethu Tsabedze (Co-Investigator): Swaziland Health Laboratory Systems, Ministry of Health, Swaziland
- Nomcebo Phungwayo (Co-Investigator): Swaziland Health Laboratory Systems, Ministry of Health, Swaziland
- Velephi Okello (Co-Investigator): Formerly ART Unit, Ministry of Health, Mbabane, Swaziland; currently Ministry of Health Directorate
- Yen Duong (Co-Investigator): Division of Global HIV/AIDS (DGHA), CDC Atlanta, USA
- Sikhathele Mazibuko (Co-Investigator): ART Unit, Ministry of Health, Mbabane, Swaziland
- Lydia Mpango (Co-Investigator): Mbabane Government Hospital, Swaziland
- Janet Ongole (Co-Investigator): URC Swaziland
- Rogers Kisame (Co-Investigator): URC Swaziland
- Trong Ao (collaborator): CDC Swaziland
- Madeline DiLorenzo (collaborator): African Society for Laboratory Medicine, Ethiopia
- Gregory Greene (collaborator): Mycotic Diseases Branch, CDC Atlanta, USA
- Kelly Clarke (collaborator): URC Swaziland intern

Sincere thanks are due to Thokozani Maseko and Mulungie Mwembo for their hard work in enrolling participants and collecting samples, and to Nombuso Ntshalintshali for processing and testing the samples in the laboratory.

We are also grateful to the Mbabane Government Hospital VCT clinic, medical wards and laboratory including their staff and to the Swaziland Ministry of Health for allowing and facilitating the conduct of this study.

Finally, we wish to thank the patients and healthcare workers who consented to participating in this study.

Table of Contents

Acknowledgments.....	1
Abbreviations Table	4
Introduction	5
Aims and objectives	6
Study Methods.....	6
Ethics.....	6
Study design.....	7
Study population.....	7
Quantitative data	7
Qualitative data.....	8
Data collection procedures.....	8
Quantitative Data.....	8
Qualitative data.....	9
Data entry and cleaning.....	10
Quantitative data	10
Qualitative data.....	10
Analysis	10
Study Results and Discussion	11
Descriptive statistics	11
Cryptococcal Antigenemia prevalence in patients with CD4 count ≤ 100 cells/mm ³	14
Cryptococcal Antigenemia prevalence in total study population (patients with CD4 count ≤ 200 cells/mm ³)	14
Factors associated with positive plasma CrAg.....	15
Feasibility of routine CrAg screening	16
Sensitivity of urine CrAg LFA	20
Sensitivity of PIMA versus FACS Calibur for CD4 testing	20
Recommendations and Way Forward	21
Overall.....	21
Proposed approach.....	22
Identified enabling factors.....	23
Next steps	23
Bibliography	25
Appendices.....	26
Appendix A: Patient Informed Consent Form.....	27

Appendix B: FGD and Interview Topic Guides	31
Appendix C: FGD Informed Consent Form.....	33
Appendix D: Interview Informed Consent Form	35
Appendix E: Cause of death reports	37
Appendix F: Symptoms of Patients with Positive Plasma CrAg Results.....	39
Appendix G: Qualitative analysis thematic framework outline	40

Abbreviations Table

AFB	Acid Fast Bacilli
AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral Therapy
CDC	U.S. Centers for Disease Control and Prevention
CD4	Cluster of differentiation 4
CI	Confidence Interval
CM	Cryptococcal Meningitis
CMS	Central Medical Stores
CrAg	Cryptococcal Antigenemia
CrAg® LFA	Cryptococcal Antigen Lateral Flow Assay
CSF	Cerebrospinal Fluid
FGD	Focus Group Discussion
HIV	Human Immunodeficiency Virus
IEC	Information and Educational Communication
IRB	Institutional Review Board
LP	Lumbar Puncture
MoH	Ministry of Health
M&E	Monitoring and Evaluation
NSTS	National sample transport system
PEPFAR	U.S. President's Emergency Plan for AIDS Relief
PI	Principal Investigator
PLHIV	People Living with HIV
RVD	Retro-viral disease
SEC	Scientific and Ethics Committee
SHLS	Swaziland Health Laboratory Services
SNAP	Swaziland National AIDS Programme
SOP	Standard Operating Procedures
URC	University Research Co.; LLC
VCT	Voluntary Counselling and Testing
WHO	World Health Organization
ZN	Ziehl-Neelsen

Introduction

Cryptococcosis, caused by *Cryptococcus neoformans* or *Cryptococcus gattii*, is an invasive and potentially life-threatening fungal infection affecting immuno-compromised individuals. Cryptococcal meningitis (CM) is one type of cryptococcosis affecting the meninges which is associated with significant morbidity and mortality [1]. Globally, it is estimated that approximately one million CM cases occur among people living with HIV (PLHIV) each year, resulting in nearly 625,000 deaths. More than 70% of all HIV-related CM cases occur in Sub-Saharan Africa. Among PLHIV, CM is one of the leading causes of death in this region, accounting for an estimated half a million deaths per year [2].

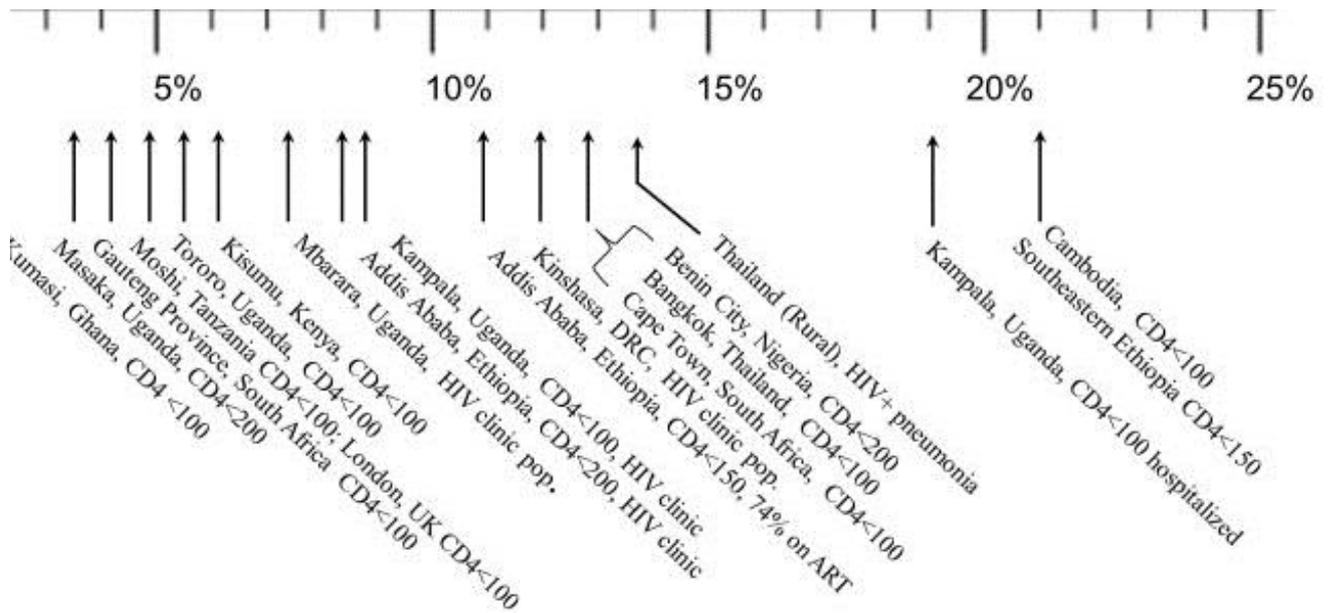
The World Health Organization (WHO)'s rapid advice on cryptococcal prevention, diagnosis, and management incorporates the findings of many investigations and advises targeted screening of HIV patients to enable early detection of cryptococcal infection, with the goal of reducing CM-related mortality through prompt interventions [3]. Studies have shown that the Cryptococcal Antigen (CrAg) can be detected in the blood (whole blood, serum or plasma) around three weeks prior to the development of CM symptoms, and that pre-emptive anti-fungal therapy in CrAg-positive Antiretroviral therapy (ART)-naïve patients can prevent CM-related mortality and morbidity [4].

The CrAg[®] LFA (Cryptococcal Antigen Lateral Flow Assay, IMMY, Oklahoma, USA) is a newly developed point of care assay that detects CrAg in blood and cerebral spinal fluid (CSF). It allows patients to be screened for presence of Cryptococcal antigenemia using a blood sample. If CrAg is detected in the blood, the LFA test can be used to test the patient's CSF sample. This allows healthcare workers to differentiate between cryptococcal antigenemia and CM, thereby informing treatment decisions.

There is growing evidence in support of the utility and cost-effectiveness of the CrAg LFA's use in screening using blood samples, especially in countries with a high burden of cryptococcal disease [5-7]. One study from Vietnam indicates that the total cost per life year gained through screening was approximately \$190 (US dollars) in a population with CrAg prevalence of 2% if treating isolated serum CrAg-positive patients with a full year of fluconazole. This reduces to \$121 if treating with a limited 10-week course of fluconazole (assuming fluconazole needs to be purchased and is not donated) [8]. Although this study was done using cost data that likely differs from those in Swaziland, another cost-effectiveness study was conducted in South Africa with similar conclusions: CrAg screening followed by high-dose fluconazole pre-emptive antifungal therapy for all CrAg positive patients (without further CSF testing) was the most cost effective strategy, from a prevalence of as low as 0.6%. However, following up with a LP and CSF CrAg test to assess for CM and treating those with confirmed CM was more clinically effective and this model, which is the model piloted in our study, was found to be cost-effective with an antigen prevalence as low as 2.5% [9]. Both screening strategies were more cost effective than the standard of care at the time (no screening or pre-emptive therapy).

The prevalence of CrAg varies by country and population group, as shown in Figure 1. In Swaziland the prevalence is currently unknown, however it is expected to be high due to the country's high HIV prevalence of 31-32% among adults aged 18-49 [10]. Despite the high HIV burden, CrAg screening is not currently included or recommended in any of Swaziland's national health guidelines, and its clinical utility, feasibility and acceptability in Swaziland is unknown. This study aimed to fill this knowledge gap, through determining the need for and feasibility of national routine CrAg screening among PLHIV.

Figure 1 CrAg prevalence according to regional studies [11]



Aims and objectives

Overall, the study aimed to provide information necessary to provide lessons and contribute to the tools and planning for a national rollout of a CrAg screening programme. Specifically, the study had the following primary objectives:

1. Determine the prevalence of CrAg in plasma among PLHIV with CD4 count ≤ 200 cells cell/ mm^3 attending Mbabane Government Hospital
2. Understand the factors associated with positive plasma CrAg and CSF CrAg among PLHIV with HIV (CD4 count ≤ 200 cells cell/ mm^3) attending Mbabane Government Hospital
3. Evaluate the feasibility and barriers to routine CrAg screening and pre-emptive therapy among PLHIV (CD4 count ≤ 200 cells cell/ mm^3)
4. Share lessons learned and the implications for a national roll out of a CrAg screen and treat program

Given that the sensitivity of utilizing a urine sample for CrAg testing was unknown, we also included the following secondary objective:

1. Determine the sensitivity of urine CrAg LFA compared with CrAg plasma in the study population.

Study Methods

Ethics

The study protocol was reviewed and approved by the Swaziland Scientific and Ethics Committee (SEC), as well as the CDC Institutional Review Board (IRB CGH HSR Tracking number 2014-139).

Study design

We undertook a cross sectional study, with participants enrolled at Mbabane Government Hospital in Swaziland, from both the Voluntary Counselling and Testing (VCT) clinic and the general medical wards. Initially, participant enrolment was planned to take place over a period of four months. However, this was extended to a period of 8 months due to a slower enrolment than predicted (August 2014-March 2015).

In addition, we conducted a qualitative study to address the following objectives:

- Assess the acceptability of a CrAg screening programme among health care workers
- Establish the feasibility of a CrAg screening programme at a facility and national level
- Identify recommendations for scaling up the CrAg screening programme.

Study population

Quantitative data

The study populations at the Mbabane Government hospital comprised:

- ART treatment naïve patients attending Mbabane VCT (the largest HIV Treatment and Care Centre in Swaziland)
- ART treatment naïve patients admitted to Mbabane Government Hospital general medical wards

Enrolment into the study had two stages, primary and secondary.

Primary enrolment: all ART treatment naïve patients presenting at the study sites were referred to the nurse research assistant, who checked their eligibility for primary enrolment.

Patient eligibility criteria for study participation differed according to whether the patient was a ward or VCT clinic patient.

For patients at the VCT clinic, the inclusion criteria were:

1. Adult patients (≥ 18 years)
1. HIV positive patients with CD4 count ≤ 350 cells/mm³ (according to the Alere PIMA™ CD4 point of care system)
2. Consenting patients who had never been treated for cryptococcal infection or disease

We excluded the following VCT clinic patients:

1. Patients who had been previously diagnosed or treated for CM (recurrent cases)
2. Patients who had ever received fluconazole for more than 5 days prior to enrolment in the study
3. Patients who did not provide consent for the study
4. HIV positive patients who have a PIMA CD4 count > 350 cells/mm³ (according to the Alere PiMA™ CD4 point of care system)
5. Patients who are HIV negative
6. Patients aged <18 years old
7. Pregnant women

For patients from the wards, CD4 counts are not routinely collected and therefore the same inclusion and exclusion criteria applied, with the exception of the CD4 cell counts criteria as there were no PIMA analysers in the medical wards.

Patients were enrolled as study participants if they met the above stated eligibility criteria.

Secondary enrolment: this was a reflexive process at the laboratory whereby participants' eligibility for CrAg screening was determined by FACS Calibur (gold standard) CD4 cell count results. All patients with a CD4 count of ≤ 200 cells/mm³ were eligible for 'secondary enrolment' and therefore their samples were screened for CrAg. Samples collected from those who were not eligible were not screened and were discarded.

Qualitative data

The study population for the qualitative data comprised:

- Health care providers (nurses and medical officers) at Mbabane Government Hospital VCT clinic
- Laboratory staff at the Mbabane Government Laboratory;
- The senior pharmacist at Swaziland's Central Medical Stores

These personnel were purposively sampled on the basis of their role in the study and/or knowledge of relevant systems.

Data collection procedures

Quantitative Data

All patients found to be eligible, were given a purpose-developed Information and Educational Communication (IEC) leaflet, providing basic information on Cryptococcus and HIV infection as well as the study. They were also taken through the informed consent form (see Appendix A) and given an opportunity to ask questions about the study.

Following the consent procedure, patients were asked to provide a urine specimen. Plasma CrAg screening was conducted on blood samples collected for routine CD4 testing, and therefore additional venepuncture was not required. The laboratory blood tests were CD4 cell count by BD FACS Calibur™ flow cytometry (BD, San Jose, USA) which is considered the 'gold standard' test to verify CD4 results.

Blood and urine samples were labelled with the same information as on the laboratory request form and packaged in plastic sample packaging bags together with the laboratory request form. Samples were then taken to the laboratory research assistant based at the Mbabane Government Hospital laboratory. Upon receipt of samples, the laboratory research assistant took them for processing in the CD4 department where the PIMA CD4 cell count result was confirmed by FACS Calibur. Results from this FACS Calibur CD4 count confirmed eligibility for patients' secondary enrolment and, therefore, CrAg testing. Urine and blood samples for all patients found to have CD4 count of ≤ 200 cell/mm³ using FACS Calibur were automatically tested for CrAg using the CrAg dipstick LFA test and those found to have a CD4 count >200 were discarded. Patients were informed, in the consenting process, that testing for CrAg would be determined by their CD4 count.

All FACS Calibur, plasma and urine CrAg results were sent back to the nurse research assistant for recording purposes and to share with the doctors. Ambulatory patients with positive plasma CrAg (with or without signs of CM) were telephoned by study nurses and asked to return to the health facility as soon as possible following receipt of results. Plasma CrAg-positive patients then underwent a symptom screen for CM (information collected in the data collection form) and were requested to undergo lumbar puncture (LP) to obtain cerebrospinal fluid (CSF) as part of a routine procedure for diagnosing CM [11, 12]. Collected CSF samples were sent back to the laboratory research assistant to be tested for CrAg, again using the CrAg LFA test, in addition to culture of CSF for Cryptococcus and

Ziehl-Neelsen (ZN) staining for the detection of acid fast bacilli (AFB) as per current national standard tests.

Patients diagnosed with CM were treated according to the standard WHO guidelines: Amphotericin B (0.7mg/kg/day) and fluconazole (400mg) for 2 weeks, followed by fluconazole 400 mg for 8 weeks, then fluconazole 200mg daily until CD4 cell count > 200 cells/ mm³ for at least 6 months on ART [3]. On the other hand, for patients with positive plasma CrAg and negative CSF CrAg results, ART was delayed for two weeks and they were prescribed pre-emptive antifungal therapy for Cryptococcus using fluconazole oral treatment, to prevent development of meningeal infection. Pre-emptive antifungal therapy followed the WHO guideline recommendations: 800 mg/day of fluconazole for the first two weeks, followed by eight weeks of 400mg of fluconazole daily, and finally 200 mg/day fluconazole maintenance dosage [3] which, in this study, was until CD4 count was greater than 200 cells/mm³ twice over six months. Patients refusing LP but with positive plasma CrAg results and no CM signs and symptoms were also offered fluconazole pre-emptive therapy. Urine CrAg positive results were recorded for study purposes alone, but not utilized for diagnosis or treatment initiation decisions. All patients starting treatment or pre-emptive therapy were followed up at three months or earlier by the nurse research assistant and treating medical officer, to evaluate adherence to as well as side effects of fluconazole therapy.

The laboratory research assistant conducted daily quality control testing for the CrAg LFA reagents. Positive and negative control LFA testing as well as lot-to-lot testing were performed to ensure the quality of the reagents. All positive and negative controls yielded the expected results, therefore reagents were not discarded.

In addition to data collected from laboratory results (urine CrAg, plasma CrAg, CSF CrAg, CD4 count) the nurse research assistant collected the following patient-level variables, either through abstraction from patient file or patient interview:

- Key demographic variables: age, sex, marital status, household location (rural/urban), level of education
- Key clinical variables: WHO clinical stages of HIV infection (I & II, III, and IV); Clinical signs and symptoms

Qualitative data

Data comprised one semi-structured interview and two focus group discussions (FGD) with a total of 9 participants at from Mbabane Government Hospital VCT clinic and 12 from the national laboratory.

We conducted both FGDs at the Mbabane Government Hospital. For the first FGD we purposively sampled health care workers, including doctors and nurses, at the VCT clinic who had been directly involved in the CrAg screening pilot programme. Through this discussion we aimed to: (i) understand healthcare workers experiences of the pilot CrAg screening programme; understand how a facility-based and national screening programme could be organised; and (iii) identify any potential challenges to the screening programme. Through the second FGD, we aimed to: (i) understand laboratory personnel's experiences of the CrAg screening pilot programme; (ii) identify potential barriers to implementing a national screening programme; (iii) establish how the screening programme could be integrated into the existing laboratory structure. We purposively sampled laboratory staff at the Mbabane Hospital Laboratory who had been involved in the screening pilot. URC and CDC Atlanta staff members¹ collaboratively developed FGD topic guides to address the

¹ Rosanna Jeffries (URC), Kelly Clarke (URC), Greg Greene (CDC Atlanta)

qualitative study aims, through discussion and reflection on the quantitative study findings. The finalized FGD topic guides are included in Appendix B. URC staff members² facilitated the FGDs, which were conducted in English.

We conducted a semi-structured interview with the Senior Pharmacist at the Swaziland Central Medical Stores (CMS) in order to determine the feasibility of rolling out a national CrAg screening programme from a pharmacy perspective. Due to limited availability and time constraints we only interviewed one pharmacist, however this pharmacist is solely responsible for drug forecasting and procurement at a national level and is therefore most knowledgeable about the feasibility of a national CrAg screening programme from a drug procurement perspective. URC and CDC staff members collaboratively³ developed a topic guide to address the study objectives (see Appendix B) URC staff members⁴ conducted the interview at the CMS.

Prior to the FGDs and interviews, we provided participants with a written overview of the study, including aims, objectives and outcomes, as well as their role as participants. They were asked to sign a consent form included in Appendix C and D. We will disseminate the final report to all qualitative study participants.

Data entry and cleaning

Quantitative data

URC study nurses⁵ entered all data into a purpose-designed data collection tool and stored these in study-numbered personal participant files with the participants' informed consent forms. These files were kept in a locked filing cabinet at VCT clinic. The paper-based forms were then used by the study nurses to enter data into an electronic database using Epidata data entry software, entering only the participant study code, with no personal identifiers. Upon completion of data entry, data files were exported from Epidata software to Excel.

The laboratory research assistant⁶ additionally entered laboratory results data into a separate Excel spreadsheet such that these data were double entered.

This allowed URC staff⁷ to compare the two data sets, identify and resolve any errors, combine the data from the two sites and clean the data, producing one single cleaned raw data set in Excel.

Qualitative data

Interviews and FGDs were transcribed verbatim into Word by a URC data clerk⁸.

Analysis

Upon completion of data cleaning, the quantitative data were exported from Excel to STATA 12 (STATA Corp, Texas) for analysis by URC and CDC Swaziland staff⁹.

² Rosanna Jeffries (URC) and Kelly Clarke (URC)

³ Rosanna Jeffries (URC), Kelly Clarke (URC), Greg Greene (CDC Atlanta)

⁴ Rosanna Jeffries (URC) and Kelly Clarke (URC)

⁵ Thokozani Maseko and Mulungie Mwembo (URC)

⁶ Nombuso Ntshalinthsali (URC)

⁷ Rosanna Jeffries (URC)

⁸ Nontsikelelo Simelane (URC)

⁹ Rosanna Jeffries (URC) and Tony Ao (CDC Swaziland)

Interview and FGD transcripts were used for qualitative analysis which was done manually by URC staff¹⁰ using Excel and Word.

Analysis was conducted per study objectives as follows:

Primary Objectives:

1. To determine the prevalence of cryptococcal antigenemia among PLHIV with CD4 count less than or equal to 200 cells/mm³ at MGH, we tested plasma samples for CrAg among eligible patients attending the VCT clinic and the medical wards and then calculated estimated prevalence with a 95% confidence intervals (CI).
2. To understand the factors associated with positive plasma CrAg and CSF CrAg among PLHIV attending Mbabane Government Hospital, we conducted bivariate analyses between plasma CrAg positivity and patient demographic and clinical characteristics. To conduct the bivariate analysis, FACS Calibur CD4 counts and age were categorised. For categorical variables, proportions were compared between CrAg-positive and negative groups using Chi-squared test of association. Where association was found ($p \leq 0.05$), logistic regression was done to determine odds ratios. For continuous variables (age and CD4 count), logistic regression was conducted.
3. To evaluate the feasibility and barriers to routine CrAg screening and pre-emptive therapy among PLHIV, we conducted a thematic analysis of the data from FGDs and interviews using an inductive approach. We held debrief sessions after each FGD and interview to discuss emerging themes. We read and re-read the transcripts for themes related to feasibility of rolling out a national CrAg screening programme. We coded the data separately and afterwards compared and revised coding frames. Codes were used to formulate a thematic framework which directly informs our recommendations for a national CrAg screening programme.

Secondary Objective

1. To determine the sensitivity of urine CrAg LFA compared with CrAg plasma in the study population, we evaluated the agreement of the urine test results with the plasma test results by calculating the sensitivity and specificity of the urine sample CrAg results relative to plasma sample CrAg results (considered the gold standard).

In addition to the analysis conducted by objective, we also calculated descriptive statistics. Continuous variables were described as medians and categorical variables were described as proportions. Wilcoxon rank-sum tests were used to evaluate differences in medians.

Furthermore, to determine whether PIMA or the FACS Calibur CD4 readings should be used as a determinant of eligibility for screening, we calculated the sensitivity and specificity of the PIMA results relative to the FACS Calibur gold standard.

Study Results and Discussion

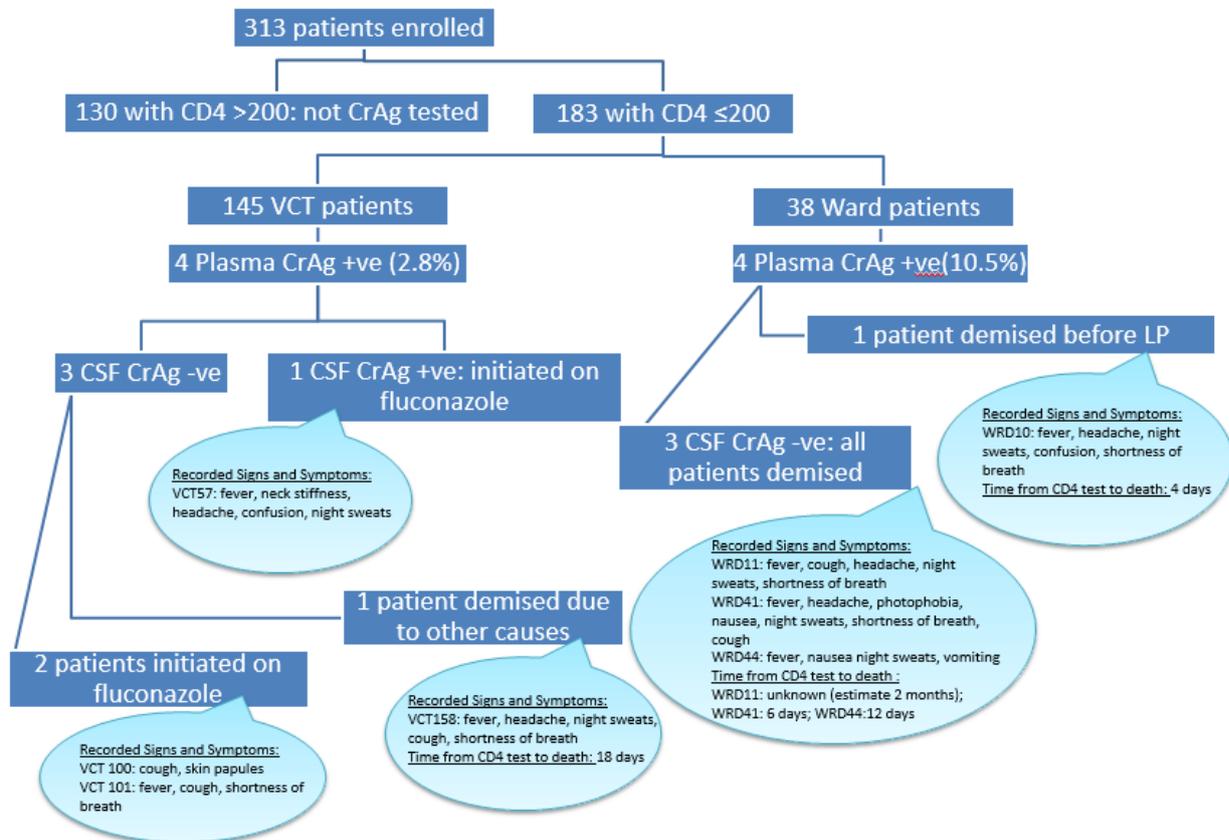
Descriptive statistics

A total of 183 patients with CD4 cell counts of ≤ 200 cells/mm³ were evaluated for plasma CrAg.

¹⁰ Kelly Clarke and Rosanna Jeffries

Figure 2 below provides a visual illustration of how many participants were enrolled at each participating site, plasma CrAg positivity, CSF CrAg results and outcomes. It additionally details the symptoms of plasma CrAg positive patients and, for those that demised, the time (in days) between having their CD4 count done and their death.

Figure 2 Participant results and outcomes



In the ward, one patient demised before an LP could be done so we do not know if that patient was CSF CrAg positive. However, the remaining three with positive plasma CrAg results had negative CSF CrAg results, indicating that they did not have CM. Nevertheless, all three patients demised. Based on this, there is need to discuss whether CrAg screening in medical wards is worthwhile given that patients are very sick in the wards and it may be too late for them to benefit from the screening for CrAg.

By contrast, although the proportion of plasma CrAg positive cases was lower in the VCT clinic, only one of these patients demised from other causes. One patient was identified as having CM through the CrAg CSF testing and was initiated on treatment. Two with positive plasma CrAg results tested negative of CrAg CSF test so were initiated on pre-emptive antifungal therapy. See Appendix E for table of causes of death for those patients who demised during the course of this study. Appendix F provides a table of symptoms for patients with plasma CrAg results. Tables 1 and 2 below show the descriptive statistics for categorical and continuous variables, respectively.

Table 1 Descriptive Statistics (categorical variables)

Categorical Variable	Proportions	Numbers
Sex (n=183)		
Male	58.5%	107
Female	41.5%	76
Marital Status (n=183)		
Single, never married	61.8%	113
Married	33.9%	62
Widowed	3.8%	7
Divorced	0%	0
Separated	0.5%	1
Educational Level (n=183)		
No schooling completed	8.2%	15
Primary school	24.6%	45
Some secondary school	39.3%	72
Secondary school graduate	16.4%	30
Trade/technical/vocational training or diploma	10.4%	19
Tertiary Degree	1.1%	2
Residence Type (n=183)		
Rural	38.3%	71
Urban	61.2%	112
Region (n=183)		
Lubombo	1.6%	3
Manzini	10.4%	19
Shiselweni	2.7%	5
Hhohho	85.3%	156
WHO Stage (n=183)		
Stage I&II	54.7%	100
Stage III	39.9%	73
Stage IV	5.5%	10
Plasma CrAg results (n=183)		
Negative	95.6%	175
Positive	4.4%	8
Urine CrAg results (n=183)		
Negative	71.6%	131
Positive	21.9%	40
Missing	6.6%	12

Table 2 Descriptive Statistics (continuous variables)

Continuous variables	Median	Minimum	Maximum
Age (n=182)	35	19.5	67
FACS CD4 (n=183)	78	1	198
FACS CD4 VCT (n=145)	88	1	176
FACS CD4 Medical wards (n=38)	47.5	3	198
FACS CD4 if Plasma CrAg positive (n=8)	35	6	88
FACS CD4 if Plasma CrAg negative (n=175)	81	1	198

Median CD4 count in our study population was 78 cells/mm³, and was significantly lower in the medical wards compared with the VCT clinic (47.5 vs. 88 cells/mm³, p=0.03). Median CD4 count was

also statistically significantly lower among CrAg-positive patients than CrAg-negative patients (35 vs. 81cells/mm³, p=0.03).

Cryptococcal Antigenemia prevalence in patients with CD4 count ≤ 100 cells/mm³

From a total of 183 patients enrolled, 103 (56%) had a CD4 cell count below 100 cells/mm³ and all CrAg positive cases in our study sample fell within this group. There were no plasma CrAg positive cases in participants with CD4 cell counts between 100-200 cells/mm³. Thus, before presenting results from the 200 cells/mm³ cut-off, it is more interesting to present the prevalence at the WHO-recommended 100 cells/mm³ cut-off for screening. Although the confidence intervals are wide (see Table 3), this calculation provides prevalence estimates of 7.8% overall, comparable with those found in the region as discussed in the introduction.

Table 3 Plasma CrAg prevalence by enrolment site (CD4 ≤ 100)

	Prevalence	95% Confidence Interval	
Overall	7.8% (8/103)	3.4%	14.7%
Ward	15.4% (4/26)	4.4%	34.9%
VCT	5.2% (4/77)	1.4%	12.8%

It is notable that the prevalence of CrAg is considerably higher among patients in the medical wards than in the VCT clinic. This is not surprising given that patients presenting at the medical wards are usually very sick whereas this is not the case in the VCT clinic. However, despite the higher prevalence in the medical wards, for reasons discussed above, there is need for further consideration of whether CrAg screening in this population group is worthwhile.

However, among the VCT clinic patients the case is different. Although CrAg prevalence is lower, the outcomes for patients screening positive were more favourable in this population than in the medical wards, indicating that it may be worthwhile in this group of patients.

Although this study did not include cost-effectiveness analysis, South African evidence presented in the introduction suggests this study's model of CrAg screening (screen and treat with LP) may be cost effective at 2.5% CrAg prevalence whilst screen and treat without confirmation with LP may be cost effective at a 0.6% CrAg prevalence [9].

Cryptococcal Antigenemia prevalence in total study population (patients with CD4 count ≤ 200 cells/mm³)

No plasma CrAg positive cases were found in participants with a CD4 cell counts between 101-200 cells/mm³, so the prevalence in this population group is 0%. Despite this, given that this study set out to measure cryptococcal antigenemia prevalence among participants with a CD4 cell count ≤ 200 cells/mm³, this information is nevertheless presented below in Table 4. However it is important to note, for programmatic purposes, that the prevalence results for participants with a CD4 cell count ≤ 100 cells/mm³ (as presented in Table 3 above) is more relevant.

Overall, the plasma CrAg prevalence in our study population (CD4 ≤ 200) was lower than at the CD4 ≤ 100 cells/mm³ cut-off because the numerator (number of plasma CrAg positive results) remains the same but the denominator (number of participants) is larger. The overall prevalence in this population was 4.4% with variation between the medical wards and the VCT clinic. A total of eight plasma CrAg-positive samples were identified through this study; four from the medical wards (10.5%, 95% CI 3-25%) and four from the VCT clinic (3%, 95% CI 1-7%), as shown in Table 4.

Table 4 Plasma CrAg prevalence by enrolment site (CD4 \leq 200)

	Prevalence	95% Confidence Interval	
Overall	4.4% (8/183)	1.9%	8.4%
Ward	10.5% (4/38)	2.9%	24.8%
VCT	2.7% (4/145)	0.8%	6.9%

Whilst the overall CrAg prevalence appears to be relatively low in the study population compared with findings in other settings, this is partly linked to the CD4 cell cut-off of ≤ 200 cells/mm³ as explained above. It is also conceivable that the results would have been higher had we included treatment defaulters in the study sample. This path was taken in an Ethiopian study where 50% of enrolled treatment defaulters had Cryptococcal Antigenemia (3/6) versus 14% of ART naïve patients (18/127)[11].

Factors associated with positive plasma CrAg

Results from the chi-squared tests of association for categorical variables, and logistic regression for those with statistically significant associations, are presented in Table 5 along with logistic regression results for continuous variables. Associations were found between plasma CrAg positivity and recruitment site with the odds of patients from medical wards being plasma CrAg positive approximately four times the odds of a VCT patient. WHO stage of disease was also found to be associated with plasma CrAg positivity with patients recorded as WHO stage III having a nearly 9-fold odds of plasma CrAg positivity than those recorded as WHO stage I&II. Finally, a 0.02 decrease in the odds of a patient being plasma CrAg positive was found with each increased each unit change in CD4 count. However, due to the low number of CrAg positive identified in the sample, bivariate analyses between the outcome and demographic and clinical factors should be interpreted with caution.

Table 5 Bivariate results table

Variable	Chi-squared value	P-value
Sex	0.25	0.62
Categorical CD4 cell count (FACS)	6.88	0.07
Categorical Age	8.67	0.28
Type of patient(VCT vs medical ward)	4.35	0.04*
Marital status	0.80	0.85
Education level	2.26	0.81
Residence type	1.98	0.16
Region	2.19	0.53
WHO Stage	6.06	0.05*
Variable	Unadjusted Odds Ratio	P-value
Site (VCT=reference)	4.15	0.05*
WHO Stage I&II(reference)		
III	8.86	0.05*
IV	11.00	0.1
Variable	Unadjusted Odds Ratio	P-value
Age (continuous)	1.02	0.48
CD4 cell count (FACS)	0.98	0.05*

*denotes statistically significant results

Feasibility of routine CrAg screening

Qualitative methods were used to analyse data on feasibility of CrAg testing. Upon completion and reviewing of identified codes, we grouped these to formulate a total of five themes relating to the feasibility of routine CrAg screening in Swaziland, each with associated codes and their respective components. Some information collected through FGDs and interviews was in the form of direct recommendations or proposed solutions to identified challenges, from the participants themselves. This information was grouped as such and the outline framework is presented in Appendix G.

The five identified themes were:

1. Screening is valued
2. Patient acceptability is multi-faceted
3. Provider-driven model is preferable
4. Roll out of the screening programme may burden existing resources
5. Lab-facility communication barriers compromise patient care

Screening is valued

Under the first theme 'screening is valued', there is evidence from both FGD and interview participants that CM is considered to be an issue worth addressing, and that CrAg screening is therefore worthwhile. Laboratory staff participating in the FGD told us that their colleagues were requesting the reagents, saying "*we want that CrAg*". Participants at the VCT clinic wanted to ensure that a national

CrAg screening programme would be equally accessible to patients across the country, and would include ART defaulters. This suggests that they perceived CrAg screening as beneficial for patients.

Participants in the laboratory and VCT clinic FGDs assessed the value of CrAg screening by weighing up the potential benefits to patient health versus necessary resource inputs. On the whole, laboratory staff felt the inputs were minimal. They noted the simplicity and low resource requirements of the CrAg LFA test compared to other Cryptococcal laboratory tests. Some VCT clinic participants felt the value of CrAg screening was dependent on prevalence (which was unknown to participants). For example, one doctor at the VCT clinic felt that a CrAg prevalence of 10% justified a screening programme, describing the difficulties in diagnosing CM:

"[...] when you have been in the HIV field for a long time, you learn that cryptococcal meningitis does not come with classic symptoms. You will not necessarily suspect cryptococcal meningitis but through screening we were able to check for it in time before the patient even complains about it and also ensure that it has not affected the meninges. We have cases that were diagnosed through the study, people who did not have symptoms but were screened to check infection." VCT clinic doctor

VCT and laboratory participants felt that CrAg screening was worthwhile because it minimized invasive procedures. Both VCT clinic and laboratory FGD participants raised concerns about the LP procedure, with particular emphasis on children from the laboratory respondents: *"for me [LP] is one of the most painful procedures for children, so using [CrAg] as a screening test is quite good and it is safe"*(laboratory medical technologist). Although under this study's model, screened patients with positive plasma CrAg results were offered the option to undergo LPs for confirmation of CM diagnosis, participants' responses indicate that this was perceived as better than the current practice: conducting LPs on all suspect CM cases. Along the same vein, the value of urine as a minimally invasive and easy to collect sample was mentioned by VCT respondents, although the need to validate urine first was recognized and using the same blood sample as collected for PIMA testing was seen as preferable where feasible.

Although screening was perceived as valuable, this was largely discussed in terms shortcomings of the alternative option: in this case, no screening. Laboratory and VCT participants' actual understanding of the CrAg screening mechanism and purpose was not uniform, with some misconceptions indicative of a knowledge gap. Participants from the VCT mentioned the need for comprehensive healthcare worker sensitization before any roll-out of a CrAg screening programme: *"In the case of rolling out training will be essential because our staff needs to have the right information and have everything in place"* (VCT nurse).

Patient acceptability is multi-faceted

Although VCT clinic participants felt that patients found CrAg screening acceptable, they outlined a number of factors that influenced acceptability. First, participants mentioned that patients were less likely to consent to screening if it delayed them, and emphasised the need to integrate screening into the existing patient flows:

"If screening is incorporated into routine service then the acceptability was higher because it does not add any extra visits" (VCT nurse)

The VCT clinic nurses and doctors felt that was achievable, as much of the burden from the study was related to additional study-required paperwork such as the consent form.

Participants also noted that patient acceptability increased with improved understanding of CM and its consequences. Patients knew about “*the headache that kills*”¹¹, however they were not familiar with the medical term for the condition. VCT clinic participants felt that patient education and sensitization activities would be necessary for a future CrAg screening programme, using existing VCT clinic ‘morning health talks’ as a potential forum, in order to create patient demand.

The nurse research assistant said that the Information Educational Materials provided for this study were helpful, especially the diagrams, but that some patients had been reluctant to take the materials home with them as their family were unaware of their HIV status. VCT clinic participants also suggested that patient acceptability and uptake of screening was influenced by symptom burden. In the absence of feeling unwell, it can be difficult for patients to understand the value of secondary prevention. Specifically, it was mentioned by the VCT study nurse that “*I had a few cases of patients who were negative about consenting mostly in the case of time and some they opted for ‘oh maybe when I’m ill’*” (nurse research assistant).

Finally, laboratory participants felt that patients were less likely to consent to the screening programme if they thought it would increase their chance of having to undergo a lumbar puncture: “*[The patients] would come when you call them for the lumbar puncture, yet they will be scared. They had the problem of being scared of the process of the lumbar puncture*” (Laboratory technologist)

Provider-driven model is preferable

We presented FGD participants with three potential models for CrAg screening: (1) a provider-initiated model whereby clinical staff order the CrAg test based on PIMA results; (2) a reflexive model involving laboratory staff automatically testing all samples with CD4 cell counts below a standardised cut-off; and (3) a point of care model whereby a phlebotomist or laboratory assistant at the health facility conducts the testing. There was a consensus of opinion that the provider-initiated model of CrAg screening was preferable for patient care. There was a shared laboratory/VCT perception that, if doctors or nurses order the test, they will be more likely to utilize the results because of their direct patient-interaction.

“I am concerned about laboratory driven [reflexive] option. What would be the trigger for health care workers to follow up unless the patient is symptomatic? This is because the results may delay depending on back-log so without an interest from the health care worker there will be no intervention. From a patient-care perspective, facility driven is better because I would have done my PIMA and the CD4 would be less than 200 when the patient comes back I would track the results for CrAg.” (VCT clinic doctor)

Laboratory participants were also concerned about maintaining quality of testing if it were conducted in the clinic rather than the laboratory. Laboratory participants were also concerned that the point of care model would over-burden clinical staff, and that the training and supervision needs would be significant.

“For instance a simple test like an HIV test. I think that is the simplest test but what we have learned from it is that there is a lack of assurance aspect that come with it, it is very simple but because other workers are overburdened by other work when we just talk about quality assurance or quality control they just don’t want to hear because they are already have a huge workload on the other side. So even with this test, it is better to keep it in the laboratory” (laboratory technical advisor).

¹¹ In SiSwati: Inhloko Lebulalanako

Roll out of the screening programme may burden existing resources

The concept of adding additional burden, the fourth identified theme, was seen throughout the discussions. Much as the screening was perceived as valuable, participants from both the laboratory and VCT expressed concerns about resources. In terms of human resources, the loss of focal persons (provided through the study) both in the laboratory and in the VCT was raised as a potential issue. VCT respondents, on the whole, did not appear to suggest that additional staff would be needed to fill this gap as the screening can be streamlined into existing workloads. However, should the screening be rolled-out at a clinic level, respondents noted the necessity of clarifying and training nurses on referral mechanisms and also task-shifting fluconazole prescription writing or refills to nurses: *“nurses cannot prescribe fluconazole not even refills. If we are to roll out, there is a need for an agreement with the pharmacy to give patients the medication if they have nurses’ signatures”* (VCT nurse). At the laboratory, by contrast, discussions suggested that additional human resources may be needed based on anticipated influx of samples. This is dependent on patient volumes per laboratory, but additional support to the serology departments may need to be considered.

In terms on other resources, VCT respondents were concerned about fluconazole procurement as current stocks donated by Pfizer cannot be used for pre-emptive therapy. As one VCT nurse cautioned: *“The availability of drugs is essential and a good start because all our efforts would be futile if we do not have the drugs”*. The CMS pharmacist’s proposed solution to this would be to procure the generic through application to the Essential Medicines Committee. This would require forecasting of quantities and responsibility would fall on the SNAP programme to take the lead in this regard and provide the budget. This sentiment was echoed by the laboratory staff: *“I think SNAP has a big stake in this and a laboratory alone cannot spearhead that kind of intervention”* (laboratory technical advisor).

From the laboratory perspective, the need for continuous supply of reagents was discussed and a recommendation made that the CrAg tests be added to the tender list as soon as possible. To facilitate this, *“you have to quantify it with estimations you will need for that test, bearing in mind the current trends, assumptions on how many percentages it will rise before it makes it to the tender list”* (Chief Laboratory Technologist). The short timeframes were alluded to, with an indication that the tender application should be done by August this year (2015). The VCT staff were also concerned about reagent stock outs, and emphasized the need to ensure that reagents are constantly available to meet patient demands.

Lab-facility communication barriers compromise patient care

This last concern leads into the final theme identified from the data: that laboratory-facility communication barriers can compromise patient care. FGD respondents from both sides raised this theme independently. From the VCT side, frustrations were raised that poor communication of reagent stock-outs can impede clinical healthcare workers professionalism when faced with a patient. Better communication was proposed as a solution by one VCT doctor: *“As long as we work with Lab it is okay, they need to communicate stock out with us in a timely manner so that we can be able to communicate with our patients other than finding out after the fact”*. Similarly, with returning of results: *“We do face the challenge of not having much insights on why we don’t have some results which makes us a bit (...) scared to go educate patients about the test”* (VCT nurse). Both of these issues could undermine the clinical healthcare worker-patient relationship with implications for patient acceptability of CrAg screening.

On the laboratory side, the communication concern was linked to use of results and lack of certainty that the testing service is benefitting the patient: *“Our greatest fear as we introduce any new test [...] is whether or not we are compiling a new pile of results on this test [...] How then are we sure that ever results get back directly to the patient file?”* (Chief Laboratory Technologist). Proposed solutions from the laboratory staff include a forum with the clinicians, as well as sensitizing clinicians on handling result or data management to ensure patient linkages to care.

Overall, the five themes emerging from the qualitative provided rich information to help formulate recommendations. Although the pharmacy perspective does not feature heavily in these themes, the factual information obtained from this interview was of use in the final development of recommendations and next steps.

Sensitivity of urine CrAg LFA

Results from the sensitivity analysis of the urine CrAg LFA relevant to the plasma indicate that the urine CrAg LFA produces a high number of false positives but no false negatives, relative to plasma CrAg as gold standard. Table 6 indicates the total numbers as well as the calculated sensitivity and specificity of the CrAg LFA on a urine sample.

Table 6 Analysis of urine sample for CrAg LFA testing

True positives (% of total)	7 (4%)
False positives (% of total)	33 (19%)
True negatives (% of total)	131 (82%)
False negatives (% of total)	0
Sensitivity (95% CI)	100% (59%-100%)
Specificity (95% CI)	80% (73%-86%)

It is interesting to note that, with no false negative and an associated 100% sensitivity, the urine CrAg results can be useful in ruling out the disease if the result is negative. However, with an 80% specificity, it would be concerning to use urine samples for ruling-in screening purposes as this would result in a large number of patients undergoing LPs unnecessarily. It could, potentially, be used as pre-screening test with those testing positive going on to be tested for plasma/serum/blood CrAg but this has cost implications and may not be helpful if patients are providing a blood sample anyway. Currently, there is no published explanations or potential causes for this low specificity but it is consistent with findings from the literature with one recently published Tanzanian study reporting sensitivity of 100% and specificity of 73.8% [13] , and the manufacturer is investigating further.

Sensitivity of PIMA versus FACS Calibur for CD4 testing

Comparison of PIMA and FACS Calibur CD4 counts was not an original objective of the study. However, given the relevance to screening logistics, interesting findings emerged which are presented here. Results from the sensitivity analysis of the CD4 count readings from PIMA versus FACS Calibur at a CD4 cell count threshold of ≤ 200 cells/mm³ are presented in Table 7 below, along with explanations of what is meant by true positives, false positives, true negatives and false negatives. For this analysis, we used all participants' for which we had a result for both tests which included only participants from the VCT as ward patients did not have a PIMA result (n=259).

Table 7 PIMA CD4 count sensitivity analysis (CD4 cell count ≤ 200 cells/mm³)

CD4 count ≤ 200 in both tests (True positives)	138
CD4 count ≤ 200 by PIMA but >200 by FACS Calibur (False positives)	36
CD4 count >200 in both tests (True negatives)	78
CD4 count ≤ 200 by FACS Calibur but >200 by PIMA (False negatives)	7
Sensitivity (95% CI)	95% (90%-98%)
Specificity (95% CI)	68% (59%-77%)

The seven false negatives indicate patient who would not have been screened in this study had we only used PIMA for screening eligibility. By contrast, 36 patients who were not CrAg screened in this study because their FACS Calibur CD4 cell count exceeded 200 cells/mm³ would have been screened had we used PIMA CD4 counts for screening eligibility. This indicates that use of PIMA for determination of screening eligibility would result in more patients being screened than vice versa.

Under a hypothetical scenario of only screening those with a CD4 cell count of less than or equal to 100 cells/mm³, results are similar. The number of participants who would have been CrAg screened had we been using PIMA to determine eligibility drops to 19 and the number who would be missed if using PIMA instead of FACS Calibur CD4 counts drop to six (see Table 7).

Table 8 PIMA CD4 count sensitivity analysis (CD4 cell count ≤ 100 cells/mm³)

CD4 count ≤ 100 in both tests (True positives)	71
CD4 count ≤ 100 by PIMA but >100 by FACS Calibur (False positives)	19
CD4 count >100 in both tests (True negatives)	163
CD4 count ≤ 100 by FACS Calibur but >100 by PIMA (False negatives)	6
Sensitivity (95% CI)	92% (84%-97%)
Specificity (95% CI)	90% (84%-94%)

Although in an ideal world, no patients would be missed if using PIMA for eligibility for screening, in reality these cut-offs are arbitrary in nature as CD4 counts fluctuate in a given individual. However, results from this sensitivity analysis do indicate that using PIMA to determine eligibility for CrAg screening is the more 'generous' option. Although this may have cost implications (more CrAg testing being done), given that PIMA is used for initiation CD4 cell count purposes, using FACS Calibur would require an additional CD4 re-test for pre-ART patients. The human resource and reagent costs of this additional CD4 cell count would, no doubt, negate the cost of additional CrAg screening.

Recommendations and Way Forward

Overall

Based on findings from the quantitative and qualitative components of our study, authors of this report recommend that Swaziland seriously considers rolling-out routine targeted CrAg screening among all patients with CD4 cell count of less than or equal to 100 cells/mm³ and, if affordable, offering LPs for those with plasma CrAg positive results to rule in or out CM. This recommendation is based on the study finding of an overall plasma CrAg prevalence of 7.8% in the population with CD4 cells count of less than or equal to 100 cells/mm³, and no cases found among participants with a CD4 cell count above 100 cells/mm³. It is also based on presented South African evidence of cost-effectiveness thresholds of this intervention.

Beyond this, our recommendations are presented in terms of a proposed approach for roll-out of CrAg screening in Swaziland, identified factors that will enable this, and concrete next steps to facilitate the process.

Proposed approach

Based on findings from our qualitative research, we recommend considering a provider-initiated screening model as this was the preferred model by both laboratory and VCT clinic FGD respondents in this study. In this model, the healthcare workers would be responsible for writing a laboratory request form, ordering CrAg testing for any patient identified with CD4 cell count less than or equal to 100 cells/mm³, regardless of symptoms. However, given challenges with the provider initiated model in the Western Cape in South Africa, it may be advisable to simultaneously pilot the provider-initiated approach and the laboratory-reflexive model in two different facilities, to evaluate the proportion of eligible patients who are actually receiving the screening in the different models.

Under a provider-initiated model, we recommend that PIMA CD4 count results be used to determine eligibility for screening at a CD4 cell count threshold of 100 cells/mm³, based on evidence from our study. Overall, our findings suggested that PIMA CD4 cell counts are more conservative than FACS Calibur. Specificity of PIMA relative to FACS Calibur was found to improve at a CD4 threshold of 100 cells/mm³ whereas a higher CD4 count thresholds leaves more potentially to over-screen. Use of PIMA has the following additional advantages:

- i) It does not require an additional laboratory test (which has cost implications)
- ii) It allows the healthcare workers to use a point-of-care results allowing real-time determination of whether a patient needs to be CrAg screened (rather than waiting for a laboratory result as FACS Calibur CD4 count results can take up to 48 hours)

In addition to being provider-initiated, we recommend that the CrAg testing itself be centralized. That is, it should be conducted in the Serology department of health centre or hospital level laboratories, not decentralized to point of care. This is based on findings from discussions with the laboratory staff as presented in the results section in which, although CrAg testing was recognized as simple and requiring little training, concerns were raised around quality control if done at point of care.

Considering evidence from the quantitative results of this study, we further suggest that the screening be rolled-out at VCT clinics and that further discussions are required regarding medical wards patients. The need for further discussion on this matter emerges from the fact that the CrAg prevalence was highest in this group, but that all participants who screened positive for plasma CrAg from the medical ward demised from non-related causes, raising the concern that this population group is 'too sick' to reap the benefits of CrAg screening. In addition to the imperative to prioritize patients likely to benefit from CrAg screening, there are associated complications in conducting LPs on patients who are very weak and frail.

In terms of which sample to use, we recommend that plasma be used for CrAg testing, based on evidence from this study of just 80% specificity for the urine sample relative to plasma samples. Ideally, the same sample as used for PIMA testing should be re-used to minimize patient blood collections. If this is not possible (e.g. finger-prick method is used), we recommend that the sample be drawn at the same time as for other blood tests to ensure that the patient is not pricked multiple times and unnecessarily.

We also recommend for CrAg screening roll-out to be conducted in a phased approach in one of two ways:

1. We could start phase I with a pilot at a hospital VCT clinic before rolling out to all national hospitals. In phase II we would then pilot at a health center before comprehensive health-center level roll-out and in phase III we would pilot at a clinic before comprehensive rollout at clinic-level. This is based on discussions with both the National laboratory and Mbabane Government Hospital VCT clinic staff, with consideration of both the practicalities of operationalization but also fairness to patients. Focusing on all levels in just one region of Swaziland, for example, introduces inequality in access of services. If it is available at one hospital-level only, but across the country, then the service is theoretically more equally available.
2. An alternative is to pilot at the mother facilities i.e. Hospitals and health centers (Phase I) and use the mother baby relationship to cascade the service down to the baby clinics (Phase II) with support from mother facilities and clinical mentors from the supporting partner organizations at regional level. Phase III could be roll out to selected private facilities (especially if drugs and materials are provided by MoH)

Identified enabling factors

In addition to the above mentioned recommendations, our study revealed certain factors that would help maximize likelihood of success of any national CrAg screening roll-out. These include:

1. Ownership: success of a national CrAg screening will be improved by joint ownership by the Swaziland National AIDS Programme (SNAP) and the Swaziland Health Laboratory Services (SHLS). A joint forum will facilitate operational planning, particularly in synchronizing tendering needs
2. Patient sensitization: raising awareness among patients that CM is preventable through screening will likely influence patient acceptability. Using the forum of morning health talks to sensitize patients on what CM is and the purpose of CrAg screening is likely to be essential to raising patient acceptability
3. Communication: strengthening laboratory-clinician communications will assist both sides when facing stock-outs or other resource constraints. Training laboratory and clinical staff jointly on CrAg screening may help to address this
4. Empowering nurses to prescribe or renew prescriptions for fluconazole: currently, only doctors are able to order fluconazole and this may present challenges with roll-out to clinic level
5. Syncing the national sample transport system (NSTS): with roll-out of CrAg screening to clinic level, there will be need to carefully examine the sample transport networks to ensure that samples are picked up in a timely manner and results returned within 48 hours
6. Working with the Health Promotion Unit in all patient sensitization and educational efforts to help address potential HIV-associated stigma of CM

Next steps

First and foremost, findings and recommendations from this report should be presented to the Ministry of Health (MoH) Directorate. With their agreement, fulfilment of the above recommendations would depend on a number of concrete next steps. Firstly, there is need to identify a champion for the cause at both SNAP and SHLS to lead the planning and implementation of a roll-out of CrAg screening. These individuals would need to work with supporting partners to implement the following activities that this report's authors believe should be prioritized initially:

1. Agree on whether or not to include in-patients and ART-defaulters in targeted screening
2. Agree on whether to conduct screen and treat with confirmatory LP or without
3. Agree on how to phase roll-out
4. Pilot provider-initiated and reflexive screening at hospital-level VCT clinics under non-study conditions and monitor proportion of eligible patients screened under each model
5. Agree on timelines for phased approach
6. Quantify reagent and other laboratory resource needs and costs for the first year (based on anticipated testing demands) and complete application for inclusion in next year's tender list (by August 2015)
7. Quantify fluconazole and flucytosine drug needs and costs for the first year based on study data
8. Review budgetary requirements and identify potential sources of funding for phase I roll-out
9. Identify next National Essential Medicines committee meeting and complete application form for inclusion of fluconazole and flucytosine
10. Develop CrAg screening recording and Monitoring and Evaluation (M&E) tools
11. Develop screening, testing and clinical management algorithms, standard operating procedures (SOPs) and training materials
12. Develop patient educational material (using materials from this study)
13. Develop phase I roll-out plan in collaboration with facility and laboratory staff including training plan
14. Implement phase I roll-out

Bibliography

1. Jarvis, J.N., et al., *High Ongoing Burden of Cryptococcal Disease in Africa despite Antiretroviral Roll out*. AIDS, 2009. **23**(9): p. 1182–1183.
2. Park BJ, et al., *Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS*. Aids, 2009 **23**(4).
3. WHO, *Rapid Advice: Diagnosis, Prevention and Management of Cryptococcal Disease in HIV-Infected Adults, Adolescents and Children*. 2011, WHO: Geneva.
4. Rajasingham, R., et al., *Cryptococcal meningitis treatment strategies in resource-limited settings: a cost-effectiveness analysis*. PLoS Med, 2012. **9**(9): p. e1001316.
5. McMullan, B.J., et al., *Clinical utility of the cryptococcal antigen lateral flow assay in a diagnostic mycology laboratory*. PLoS One, 2012. **7**(11): p. e49541.
6. Meya, D.B., et al., *Cost-effectiveness of serum cryptococcal antigen screening to prevent deaths among HIV-infected persons with a CD4+ cell count < or = 100 cells/microL who start HIV therapy in resource-limited settings*. Clin Infect Dis, 2010. **51**(4): p. 448-55.
7. Micol, R., et al., *Prevalence, determinants of positivity, and clinical utility of cryptococcal antigenemia in Cambodian HIV-infected patients*. J Acquir Immune Defic Syndr, 2007. **45**(5): p. 555-9.
8. Smith, R.M., et al., *Prevalence of cryptococcal antigenemia and cost-effectiveness of a cryptococcal antigen screening program--Vietnam*. PLoS One, 2013. **8**(4): p. e62213.
9. Joseph N. Jarvis, T.S.H., Stephen D. Lawn, Graeme Meintjes, Robin Wood, Susan Cleary, *Cost Effectiveness of Cryptococcal Antigen Screening as a Strategy to Prevent HIV-Associated Cryptococcal Meningitis in South Africa*. . PLoS ONE, 2013. **8**(7).
10. Bicego, G.T., et al., *Recent patterns in population-based HIV prevalence in Swaziland*. PLoS One, 2013. **8**(10): p. e77101.
11. Beyene, T., et al., *Comparison of Cryptococcal Antigenemia between Antiretroviral Naïve and Antiretroviral Experienced HIV Positive Patients at Two Hospitals in Ethiopia*. PLoS ONE, 2013. **8**(10).
12. Rajasingham, R., D.B. Meya, and D.R. Boulware, *Integrating cryptococcal antigen screening and pre-emptive treatment into routine HIV care*. J Acquir Immune Defic Syndr, 2012. **59**(5): p. e85-91.
13. Kinanga A Magambo, S.E.K., Shikha W Kapoor, Jeremiah Seni, Awilly A Chofle, Daniel W Fitzgerald, and Jennifer A Downs, *Utility of urine and serum lateral flow assays to determine the prevalence and predictors of cryptococcal antigenemia in HIV-positive outpatients beginning antiretroviral therapy in Mwanza, Tanzania*. J Int AIDS Soc, 2014. **17**(1).

Appendices

Appendix A: Patient Informed Consent Form

INFORMED CONSENT FOR PARTICIPANTS: A Pilot Cryptococcal Antigenemia (CrAg) Screening Program among HIV-Infected Patients Attending Mbabane Government Hospital: Prevalence of Cryptococcal Antigenemia, Clinical Utility, Feasibility and Implications for National Roll Out of a CrAg Screening Program

This Informed Consent Form has two parts:

- Information Sheet (to share information about the study with you)
- Certificate of Consent (for signatures if you choose to participate)

The informed consent process will be preceded by a counselling session between the participant and the attending health care worker with regard to the implications of the participant's HIV infection status, the low CD4 count result (<350) and the possible positive CrAg result. The patient brochure will also be used to explain screening for and management of cryptococcal disease. The participants will be given a copy of the signed Informed Consent Form.

Study Principal Investigators: Samson Haumba, Gugu Maphalala, Charles Azih, Peter Ehrenkranz, Tom Chiller, Rachel Smith, Benjamin Park, Charmaine Khudzie Mlambo.

Organizations: Swaziland Health Laboratory Services (SHLS); Swaziland National AIDS Programme (SNAP); Mbabane Government Hospital (MGH); University Research Co. LLC (URC); Centers of Disease Prevention and Control (CDC); and African Society for Laboratory Medicine (ASLM).

Part I: Information Sheet

Purpose for the study

The MoH is conducting a study on ways to improve detection of a disease called Cryptococcal infection in people living with HIV (PLHIV) using a new easy to use test which has been recommended by the World Health Organization (WHO). Cryptococcus is a fungus which, when it infects the body, can cause disease in the brain, lungs, skin, and bones, especially in immune-compromised people, such as HIV/AIDS patients. Meningitis (which is an inflammation of the tissue surrounding the brain) is the most common form of cryptococcal disease in HIV/AIDS patients. The test which will be used in the study can identify Cryptococcus infection *before* it causes disease and therefore enables doctors to provide you with medicine to *prevent* the infection from causing disease. The study will take a period of 4 months in total. I will go through this information sheet with you now. There may be words or ideas that you are not familiar with so please interrupt me at any time and ask questions.

Voluntary Participation

As you will be receiving care for HIV and because PLHIV are at a higher risk for cryptococcal infection than the non-HIV infected members of the community, you are being requested to participate in this study. Your participation in this research is entirely voluntary meaning that it is *your choice* whether to participate or not. Whether you choose to participate or not, all the services you receive at this clinic will continue and nothing will change. If you choose not to participate in this research project, you will be offered the treatment that is routinely offered in this hospital. If you agree to participate, you may change your mind *at any time* and stop participating even if you agreed earlier.

Study Procedures and Data Collection

You were referred to me because your CD4 cell count has been done in the HIV clinic and found to be less than 350. If you agree to participate in the study, you will be requested to provide a blood sample and a urine sample. These samples will be sent to the main laboratory for further testing. The first step is to carry out a second CD4 test using a different machine called a FACS Calibur. If your CD4 count on this second test

is found to be less than or equal to 200, then you will be eligible for secondary enrolment in the study and a test will be conducted on your blood and urine called Cryptococcal Antigen Lateral Flow Assay (CrAg LFA). If your CD4 count is found to be more than 200, then your blood and urine samples will not be tested.

The results of the CrAg LFA test will determine the next steps for you, as follows:

1. If your CrAg LFA tests are negative, then you will not be required to return to the hospital for further tests related to the study but will continue with your routine HIV care. If you have not been contacted within 3 days then you can assume that your tests were negative.
2. If your CrAg LFA results are positive, you will be called within 3 days and will be asked to return as soon as possible for a follow-up appointment with the doctor and further tests. If you are unavailable or do not answer the call, your next of kin will be contacted and requested to inform you to visit the clinic to collect your results. If you do not have a telephone, or do not arrive at the clinic, then a home visit will be made within 7 days of clinic receiving results from the laboratory to encourage you to collect your results and receive care from the clinic.

When you return to the hospital, you will be first be seen by a doctor for clinical examination, and then you will be requested to undergo a procedure called lumbar puncture to help the doctors decide if the disease is affecting your brain. A lumbar puncture (LP) is a medical procedure where a needle is inserted into the lower part of the spine, in order to look for evidence of conditions affecting the brain, spinal cord or other parts of the nervous system. **Refer to the attached illustration of the Lumbar Puncture procedure.** A fluid called CSF will be collected from the spine and the same Cryptococcal Antigen Lateral Flow Assay (CrAg LFA) test will be carried out on this CSF fluid in the laboratory.

If these results show that you *do have* disease of the brain, then you will be given treatment as recommended by the Ministry of Health. If the results show that you *do not have* the disease of the brain, you will be given medication to prevent the development of disease.

Confidentiality

The information that you have provided will be kept confidential. Only people involved directly in your care and those conducting the study will have access to your information. Your name will not be used and you will be identified using a unique number. Information collected electronically will be stored on password protected computers.

Risks

The study does not require any additional risk than that which is usually associated with standard patient care. On some occasions, participants may experience discomfort when undergoing phlebotomy and in rare instances, swelling or infection at the puncture site. The lumbar puncture procedure may result in lower back pain or a headache or in rare instances, swelling or infection at the site of the LP.

Benefits

The study will provide an easy to use and quick test to determine if you have been infected by the fungus that causes infection to the brain. If you are identified to have infection, further tests will be done to determine if you have developed the disease of the brain and you will be provided with prompt treatment. If you have been infected but have not yet developed disease, treatment will be provided to prevent disease development.

Findings from this study will also provide evidence for improving treatment and care for PLHIV in Swaziland.

Reimbursements

You will not be provided any payment or incentive to take part in the study.

Dissemination of the Results

The study results and findings will be shared with relevant stakeholders including, but not limited to, clinical service providers. Your name will not be used when the results are shared but rather the findings from the study will be described. Findings will be published and will contribute to knowledge globally.

Right to Refuse or Withdraw

You do not have to take part in this study if you do not wish to do so, and choosing to participate will not affect your treatment now or in the future. You may stop participating in the study at any time that you wish without any negative impact to you.

Who to Contact

The procedures for conducting the study have been reviewed and approved by committees that are responsible for ensuring that participants (like you) are protected. The committees overseeing the study include the Swaziland Scientific and Ethics Committee (SEC), the University Research Institutional Review Board (URC IRB) and the Center for Disease Control and Prevention Institutional Review Board (CDC IRB). If you want to know more about the Swaziland Scientific and Ethics Committee (SEC) contact Sisi Lukhele or Babazile Shongwe at 24047712. If you have questions or want to know more about the study you can call the study investigators Dr Samson Haumba at 24047154 or Ms. Gugu Maphalala (SHLS) 24042190.

Like any of the information you have provided, questions and other information you have told us will be kept confidential. You are free to ask questions even when you are not here.

If you wish, you can ask me any other questions about the study. Do you have any questions?

Part II: Certificate of Consent

I have been asked to participate in a study on testing for Cryptococcus on my blood and urine.

I have read the information, or it has been read to me. I have understood and have had the opportunity to ask questions about it and all questions I have asked have been answered to my satisfaction.

Print Name & Surname of Participant _____

Signature of Participant _____

Date (DD/MM/YY) _____

If the research participant is illiterate or unable to sign a document, thumb prints are an acceptable alternative.

Print Name & Surname of Participant _____

Thumb print of participant



A witness is required to observe the consent process and sign below only if written informed consent is not possible and the patient has provided verbal consent. The signature of the witness below means that another person has observed the consenting of the participant. The witness must be impartial and not part of the evaluation staff.

Print Name & Surname of Witness _____

Witness Signature: _____

Date (DD/MM/YY): _____

Appendix B: FGD and Interview Topic Guides

VCT clinic staff

1. What do you understand about the CrAg screening programme?
2. Do you think CrAg screening is worthwhile? (probe why; understanding of importance etc)
3. How do you think the study went overall? (Probe successes and failures)
4. In your experience, how do patients respond to screening and treatment if applicable? (probe reasons for refusal, if applicable; feedback from patients generally and tolerance/adherence to drugs; discussion around IEC material- does it help?)
5. Do you have any concerns about the feasibility of roll-out of CrAg screening at this facility or recommendations if this were to be done ? (probe: problems obtaining samples/fluconazole, resources, patient compliance, staff compliance, training, communication with lab....?)
6. How do you think the screening would fit into existing workload? (Fitting into adherence counselling appointments schedule etc).
7. Do you have any concerns about the feasibility of roll-out of CrAg screening at a national level? (probe challenges at different staff cadres ie management; doctors; nurses and different levels ie clinic, health centre, hospital).
8. What do you think could make routine screening possible and make it more likely to be successful? (probe discussions around preferred model: lab driven vs provider initiated; additional resource requirements; awareness raising)

Laboratory Staff

1. How do you understand CrAg screening using Lateral Flow Assay?
2. Do you think CrAg screening is worthwhile?
Probe why; understanding of importance etc
3. How did you feel the study went overall?
Probe: did you receive adequate training/materials on cryptococcal disease and screening when this program started?
4. What was your experience in performing the test?
Probe:
 - Any difficulties/challenges?
 - Adequate training
 - Orientation
 - Technical expertise
 - Quality Control
5. If CrAg screening were introduced as a routine test, what would you say would be required? (eg trainings, materials, personnel, refresher trainings, etc)
6. How would you envision screening fitting into existing work load?
Probe:
 - Provider initiated vs lab driven; What about point of care (using whole blood)
 - Which department/whose responsibility? Decentralisation
 - Sample transport issues
 - Turnaround time
7. Do you have any concerns about the feasibility of roll-out of CrAg screening at this facility or recommendations if this were to be done?
Probe: problems obtaining samples; reagents; training needs; HR; communication with VCT

Pharmacy

1. Have you heard of screening for Crypto Antigen? Or What is your understanding of screening for CrAg?
2. How does Swaziland currently obtain fluconazole supply for government health facilities?
3. Can you describe the Diflucan partnership in Swaziland?
4. Is the generic currently available and in use in the government facilities?
5. How would you rate consistency in availability of fluconazole? Frequency of stock-outs etc?
 - probe here for stories/anecdotal accounts of stock outs and the ramifications that follow
6. Can you explain the drug forecasting system and cycle?
 - What about fluconazole? Is it different? If so, how?
7. If we were to roll out CrAg screening nationally, how could a provision be added to:
 - procure additional fluconazole supplies for preventative therapy (not just treatment)?
 - procure additional medicines eg Flucytosine? (through government system)
8. Can you explain the essential medicines list; what it includes and what determines included items
9. How is your relationship with SNAP?

Appendix C: FGD Informed Consent Form

Informed Consent for Focus Group Participation: A Pilot Cryptococcal Antigenemia (CrAg) Screening Program among HIV-Infected Patients Attending Mbabane Government Hospital: Prevalence of Cryptococcal Antigenemia, Clinical Utility, Feasibility and Implications for National Roll Out of a CrAg Screening Program

Investigators: Samson Haumba, Gugu Maphalala, Charles Azih, Peter Ehrenkranz, Tom Chiller, Rachel Smith, Benjamin Park, Charmaine Khudzie Mlambo.

Purpose of the Project: The MoH is conducting a study on ways to improve detection of a disease called Cryptococcal infection in people living with HIV (PLHIV) using a new easy to use test which has been recommended by the World Health Organization (WHO). Cryptococcus is a fungus which, when it infects the body, can cause disease in the brain, lungs, skin, and bones, especially in immune-compromised people, such as HIV/AIDS patients. Meningitis (which is an inflammation of the tissue surrounding the brain) is the most common form of cryptococcal disease in HIV/AIDS patients. The test used in the study can identify Cryptococcus infection *before* it causes disease and therefore enables doctors to provide you with medicine to *prevent* the infection from causing disease. We have collected data on the prevalence of Cryptococcus infection among patients at the Mbabane Government Hospital (MGH) VCT, and now would like to understand how best a screening programme can be rolled out at the MGH VCT facility and nationally by discussing with the healthcare workers from the VCT, the laboratory and the pharmacy.

Explanation of Procedures

If you decide to participate in this study, you will take part in a focus group discussion with 5-7 other participants, which will be led by a focus group facilitator. A focus group assistant/observer will also be present during the focus group session. We will audio-tape the session and make a written copy for later analysis. The questions that the focus group facilitator will ask will address your opinions about how best a screening programme can be rolled out. You also will complete a brief survey that will request information about your age, occupation and educational background. The focus group session will last approximately 1- 1 ½.

Confidentiality

The information collected in this study will remain confidential. This means that your identity as a participant will not be revealed to people other than the investigators listed above. Any references to information that would reveal your identity will be removed or disguised prior to the preparation of the research reports and publications. All research materials will be kept in a locked office at URC office. All audio recordings will be erased at the completion of the study.

Risks and Discomforts

We do not anticipate that participation in this study will pose physical or psychological risks beyond what you encounter in everyday life. However, if you are uncomfortable answering a particular question, you are free to refuse to answer the question, and you are free to quit the study at any time.

Benefits

You will not receive any benefits from participating in this research. However, the final results of the study will be shared with key national HIV stakeholders and may help to improve provision of Cryptococcal Meningitis screening. We will also provide the facility with a copy of the study findings.

Freedom to Withdraw Participation

Participation in this study is voluntary; you will not be penalized if you decide not to participate. You are free to withdraw consent and end your participation in this project at any time.

Contact Information

If you have concerns about this study please contact 2404 7156.

Your signature below shows that you understand the above and agree to participate in this focus group discussion.

Please print your name _____ Witness signature

Please sign your name _____ Date _____

Appendix D: Interview Informed Consent Form

Informed Consent for Interview Participation: A Pilot Cryptococcal Antigenemia (CrAg) Screening Program among HIV-Infected Patients Attending Mbabane Government Hospital: Prevalence of Cryptococcal Antigenemia, Clinical Utility, Feasibility and Implications for National Roll Out of a CrAg Screening Program

Investigators: Samson Haumba, Gugu Maphalala, Charles Azih, Peter Ehrenkranz, Tom Chiller, Rachel Smith, Benjamin Park, Charmaine Khudzie Mlambo.

Purpose of the Project: The MoH is conducting a study on ways to improve detection of a disease called Cryptococcal infection in people living with HIV (PLHIV) using a new easy to use test which has been recommended by the World Health Organization (WHO). Cryptococcus is a fungus which, when it infects the body, can cause disease in the brain, lungs, skin, and bones, especially in immune-compromised people, such as HIV/AIDS patients. Meningitis (which is an inflammation of the tissue surrounding the brain) is the most common form of cryptococcal disease in HIV/AIDS patients. The test used in the study can identify Cryptococcus infection *before* it causes disease and therefore enables doctors to provide you with medicine to *prevent* the infection from causing disease. We have collected data on the prevalence of Cryptococcus infection among patients at the Mbabane Government Hospital (MGH) VCT, and now would like to understand how best a screening programme can be rolled out at the MGH VCT facility and nationally by discussing with the healthcare workers from the VCT, the laboratory and the pharmacy.

Explanation of Procedures

If you decide to participate in this study, you will take part in an interview, which will be led by a facilitator (Rosanna Jeffries/Kelly Clarke). An assistant/observer (Rosie Jeffries) will also be present during the interview. We will audio-tape the session and make a written copy for later analysis. The questions that the facilitator will ask will address your opinions about how best a screening programme can be rolled out. You also will complete a brief survey that will request information about your age, occupation and educational background. The interview will last approximately 1 hour.

Confidentiality

The information collected in this study will remain confidential, however as we are interviewing only one pharmacist in the study, it may be possible to infer the source of information. We will therefore send you the draft research report prior to dissemination to check that you are happy with how information from the interview has been represented. All research materials will be kept in a locked office at URC office. All audio recordings will be erased at the completion of the study.

Risks and Discomforts

We do not anticipate that participation in this study will pose physical or psychological risks beyond what you encounter in everyday life. However, if you are uncomfortable answering a particular question, you are free to refuse to answer the question, and you are free to quit the study at any time.

Benefits

You will not receive any benefits from participating in this research. However, the final results of the study will be shared with key national HIV stakeholders and may help to improve provision of Cryptococcal Meningitis screening. We will also provide the facility with a copy of the study findings.

Freedom to Withdraw Participation

Participation in this study is voluntary; you will not be penalized if you decide not to participate. You are free to withdraw consent and end your participation in this project at any time.

Contact Information

If you have concerns about this study please contact 2404 7156.

Your signature below shows that you understand the above and agree to participate in this interview.

Please print your name _____ Witness signature

Please sign your name _____ Date _____

Appendix E: Cause of death reports

Report on participant WRD 010: On admission, the patient was admitted in very weak state, confused with reduced level of consciousness. The patient's retroviral disease status (RVD) status was unknown and the diagnosis was query Psychosis and pulmonary TB. In the hospital there is the HTC department that comes to do HTC to clients with an unknown RVD status but the patient did not have HTC the whole first week of her admission. On Sunday the 31/08/14 an LP was done because the patient was not responding well to treatment and was still confused. The results returned on 04/09/14 were suggestive of viral infection on CSF, with no indication of fungal infection. The patient was then done HTC and tested positive on the 05/09/14 and was enrolled in the study. The results returned plasma CrAg positive on the 05/09/14 after 3:30pm but when the nurse research assistant reached the wards at 5:00 pm the doctor said they had done LP on the patient some days before and based on the patient's condition, wasn't sure if it should be done again. After some deliberation on whether the LP should be done, the patient demised Tuesday 09/09/14 morning at 3:15am before any fluconazole treatment was initiated. The cause of death is stated in the file to be aspiration pneumonia and contributing circumstances were RVD and bacterial meningitis.

Report on participant WRD 011: The patient was enrolled on 05/09/2014. Results showed the patient to be plasma CrAg positive but, after lumbar puncture, the CSF CrAg results was negative. Upon disclosure of results and next steps, the patient expressed a desire to go home and was not interested in pursuing treatment of any kind (fluconazole or otherwise). The patient signed their refusal of all hospital treatment and went home without starting any fluconazole. The next of kin's phone was out of reach for a long time and the patient was considered lost to follow-up until the research assistant called the next of kin on 27/1/15 and were informed that the patient had died in November 2014.

Report on participant WRD 041: The patient was admitted on the 24/11/14. The patient was diagnosed with RVD and severe anemia and was enrolled on the 25/11/14 at 15.30 pm and study samples (blood and urine) were collected on the 26/11/14. Results were received on 27/11/14 and was found to be plasma CrAg positive. The patient consented to LP and samples were collected on 28/11/14, and results were received on the same day at 1630hrs and all doctors had left. The results were CrAg negative on CSF. Other investigations conducted were TB and malaria which were both negative. Liver function tests: GGT – 28, ALT – 21, AST – 63 (high) Albumin – 20, and proteins – 52. The patient was started on treatment 01/12/14 (Fluconazole 400mg twice daily = 800mg/day).

The patient demised on the 02/12/14 at 1:30am. The cause of death was severe anemia, generalized infection (lymphadenopathy) and a low CD4 count of – 86c/μl (6%).

Report on participant WRD 044: The patient was admitted on the 24/11/14. The patient was diagnosed with RVD and acute diarrhea. The patient was enrolled on the 27/11/14 and samples were collected on the same day. Results were received on 28/11/14 and was found

to be plasma CrAg positive. The patient consented to LP and samples were collected on 01/12/14, and results were received on the same day at 1600hrs and all doctors had left. The patient was CrAg negative on CSF. Treatment was started on the 03/12/14 (Fluconazole 400mg bd). Other medications used were metronidazole 400mg bd, amoxyl 500mg bd, diclofenic acid 75mg bd, loperamide 4mg stat, MVT and folifer. The patient demised on the 09/12/14, 6th day of treatment. The cause of death was acute diarrhea. Exacerbating factors were OPC and RVD (CD4 – 7c/μl (1%).

Report on participant VCT 158: The patient came to VCT on 22/12/14. CD4 count was done and was enrolled. Patient disappeared with urine specimen bottle and did not come for second class counselling for ART initiation. On the 23rd patient was called following serum CrAg positive results. The patient promised to come the following day but did not. The patient was called again on the 23rd but did not pick up the phone both with the next of kin. On the 29th till the 1st of January it was the same story (phone not picked up). On the Friday the 2nd of January the patient picked up and promised to come on Monday (7/01/15). On the 7th the LP was done and blood for LFT, FBC and KFT were collected and was sent for a chest X-ray. When all results came later in the day, they showed huge elevation of all values. The chest X-ray showed chest infection, and the patient had productive coughs with chest pains, shortness of breath, night sweat and severe weight loss. The CSF was CrAg negative, no AFBs seen and a negative Indian ink. The patient also complained of diarrhoea.

The patient was then diagnosed with acute renal failure, acute liver failure, acute pulmonary infection (query TB), RVD and acute diarrhoea. The patient was admitted in ward 18 with rocephin, flagyl, brufen, iron and folic acid supplements, and multivitamins with an IV fluid bolus with furosemide 40mg. The patient was also given sputum specimen bottles for Gene Xpert. The doctor advised against high dose fluconazole given both kidney and liver failure and the fact that the patient was being investigated for TB, which in any case required treatment which cannot be combined with fluconazole high dose. The nurse research assistant continued making follow-up visits in the ward but the patient was deteriorating fast.

The patient demised on the 09/01/15, in the early morning hours (1:15 am). The cause of death was acute diarrhea, acute liver failure, and pulmonary infection. Exacerbating factors were acute renal failure and RVD.

Appendix F: Symptoms of Patients with Positive Plasma CrAg Results

Participant Number	Did patient report any of the following symptoms:										Did patient present with any of the following signs:	
	Fever	Headache	Neck stiffness	Confusion	Photophobia	Nausea	Nightsweat	Cough	Vomiting	Shortness of Breath	Skin Papules	Kernings
WRD10	Yes	Yes	No	Yes	No	No	Yes	No	No	Yes	No	No
WRD11	Yes	Yes	No	No	No	No	Yes	Yes	No	Yes	No	No
WRD41	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	Yes	No	No
WRD44	Yes	No	No	No	No	Yes	Yes	No	Yes	No	No	No
VCT57	Yes	Yes	Yes	Yes	No	No	Yes	No	No	No	No	No
VCT100	No	No	No	No	No	No	No	Yes	No	No	Yes	No
VCT101	Yes	No	No	No	No	No	No	Yes	No	Yes	No	No
VCT158	Yes	Yes	No	No	No	No	Yes	Yes	No	Yes	No	No

Appendix G: Qualitative analysis thematic framework outline

Theme	Codes	Components of code	Participants' potential solution
1. Screening is valued	Evidence of value	Desire to extend to all patient groups equally	-
		Other labs demanding CrAg screening	-
	Value defined by health resource input versus patient benefit	Diagnostic challenges (avoiding missing a CM diagnosis)	-
		Minimising invasive procedures	-
		Prevalence as a measure of value	-
		Simplicity and low resource requirements of CrAg LFA	-
	Mortality/morbidity costs	-	
Lack of consensus on the purpose of screening	-	HCW sensitisation/training	
2. Patient acceptability is multi-faceted	Acceptability dependent on time efficiency	-	Need to integrate into routine pre-ART screening
	Acceptability improved with patient sensitisation	Terminology; IEC materials; Impact on relatives and friends	Morning health talks as an existing forum for sensitisation
	Uptake of screening influenced by symptom burden	-	-
	Fear of Lumbar puncture	-	-
3. Provider-driven model is preferable	Patient interaction motivates use of results	Do not trust the lab to manage	-
		Do not trust lab to bring back in time	-
	Concerns about quality and work burden associated with POC model	-	-
4. Roll out of the screening programme may burden existing resources	Sample influx as a burden on lab HR	-	Perform a needs assessment and fill the gaps/Phased roll out to avoid overwhelming lab resources
	Loss of a focal person	Loss of study-provided facility and lab assistants	Serology suggested as focal department
	Anticipating reagent stock outs	-	Needs assessment, timely procurement in lab tender
	Concerns about fluconazole procurement	-	CMS solutions including forecasting, procurement of generic and SNAP leadership
	Lack of skills at clinic level for decentralisation of screening	-	Task-shifting and establish referral pathway
	Additional burden on NSTS	-	-
5. Lab-facility communication barriers compromise	Poor stock out communication compromises HCW-patient relationship	-	Forum for shared development of roll out plan
	Lack of feedback on use of results	-	Data management training and monitoring by SNAP