Phased implementation of a public health programme: cryptococcal antigen screening and treatment in South Africa

Acronym: CAST-NET

(Cryptococcal Antigen Screening and Treatment National Evaluation Team)

Funding: NIH R01AI118511

Principal Investigator: Nelesh Govender

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4 January 2017

STATEMENT OF COMPLIANCE

The study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46; 21 CFR Part 50, 21 CFR Part 56, and 21 CFR Part 312)
- ICH E6; 62 Federal Register 25691 (1997)
- NIH Clinical Terms of Award

All key personnel (all individuals responsible for the design and conduct of this study) have completed Human Subjects Protection Training.

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LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
CRF	Case Report Form
DHHS	Department of Health and Human Services
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Form
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
ICF	Informed Consent Form
IND	Investigational New Drug Application
IRB	Institutional Review Board
MIC	Minimum Inhibitory Concentration
MOP	Manual of Procedures
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
PHI	Protected Health Information
PI	Principal Investigator
PK	Pharmacokinetics
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event/Serious Adverse Experience
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
US	United States
WHO	World Health Organization

PROTOCOL SUMMARY

Title:	Phased implementation of a public health programme: Cryptococcal antigen screening and treatment in South Africa (Short title: CAST-NET)
Population:	Persons with cryptococcal antigenemia in South Africa
Rationale:	Persons with cryptococcal antigenemia have high mortality and are at high risk for cryptococcal meningitis. A national reflex laboratory CRAG screening and treatment programme has been rolled out in South Africa (from 1 November 2016). We propose to implement enhanced surveillance to determine the outcomes of this programme.
Study Duration:	5 years
Objectives:	**Primary objective (Years 1 and 2):** To implement enhanced surveillance at selected sites to determine the outcomes (overall and for pre-specified subgroups) of a national reflex laboratory CRAG screening and pre-emptive treatment programme in South Africa. **Primary objective (Years 2-5):** To implement and test programmatic interventions to improve 6-month cryptococcal meningitis-free survival. Examples of programmatic interventions may include: a) Enhanced diagnostics, e.g. quantification of CRAG titre, to customize or differentiate patient care b) Healthcare provider training c) Enhanced laboratory services: Determine benefit of enhanced methods to deliver CD4 and CRAG results to requestors **Secondary Objectives:** 2. Evaluate cost-effectiveness of a real-world CRAG screening and pre-emptive treatment programme on the ground in South Africa.

	3. Evaluate risk factors for treatment failure. **Exploratory Objectives:** 4. Measure the impact of same-day HIV testing and treatment on timing of ART initiation among CRAG+ and CRAG-negative individuals. 5. Describe the prevalence of opportunistic infections in CRAG+ and CRAG-negative individuals
Primary endpoint:	Among those with a CRAG+ result, to estimate the 6-month cryptococcal meningitis free-survival (overall and among pre-specified subgroups)
Secondary endpoints:	1. 6-month retention in care 2. Operational challenges of implementation including: a. Time from CD4 testing, CrAg testing, antifungal therapy, and ART initiation among those with a CRAG+ result b. Proportion of CD4/ CRAG results that are received, acknowledged and acted upon by a health practitioner c. Proportion of patients with advanced disease receiving TB screening d. Proportion of CRAG+ persons who develop symptomatic cryptococcal meningitis e. Completion of lumbar puncture when clinically indicated f. Availability of lumbar puncture and appropriate antifungal therapy in routine clinical practice g. Loss to follow-up after CRAG screening and/or during antifungal therapy h. Proportion of clinicians and staff trained on the CRAG screening algorithm and CRAG lateral flow assay (LFA) (the latter only for facilities that are distant from a laboratory) i. Adherence to CRAG screening and pre-emptive treatment guidelines by healthcare practitioners 3. Costs of CRAG+ treatment programs, Incremental cost-effectiveness ratio (ICER) of CRAG screening

Exploratory endpoints:	1. Time to ART initiation in HIV test and treat programs amongst HIV-infected persons with advanced disease. 2. Prevalence of opportunistic infections in HIV-infected persons with advanced disease
Description of Study Design:	This is a monitoring and evaluation programme of the existing national reflex laboratory CRAG screening and pre-emptive treatment programme in South Africa. In years 1 and 2, we will perform enhanced surveillance at selected sites to determine 6-month outcomes of those identified as CRAG positive. We estimate performing enhanced surveillance at approximately 400 clinics out of 4500 clinics nationally. A list of surveillance facilities will be agreed upon in discussion with provincial departments of health. Data will be collected by two methods: A) primary (retrospective) data collection by chart/ record review at facility level by partners B) secondary analysis and triangulation of existing laboratory and surveillance datasets. In years 2 through 5 we will implement and test programmatic interventions to improve 6-month outcomes.
Estimated Time to Complete Enrollment:	5 years (2017-2021)

Key Roles

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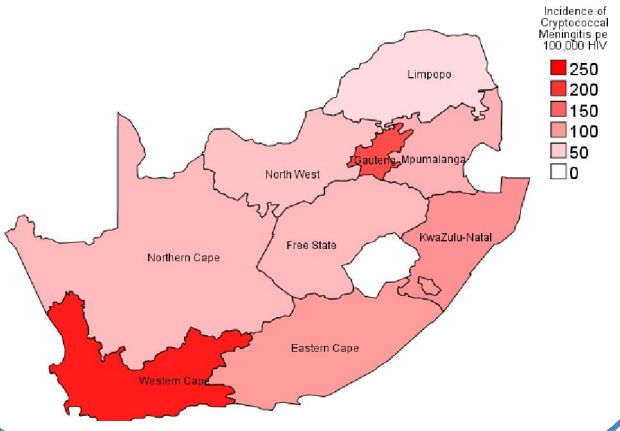
1 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

1.1 Background Information

Cryptococcus neoformans is a major opportunistic pathogen and a leading cause of death among AIDS patients in Sub-Saharan Africa.(1-5) *C. neoformans* is the most common cause of meningitis among adults in Africa, more common than bacterial meningitis.(6) Across the developed world, introduction of antiretroviral therapy (ART) has been associated with a decline in the incidence of cryptococcal meningitis.(7) However, despite major efforts to expand ART access over recent years,(8) in many resource-limited settings CD4+ T-cell (CD4) counts of persons entering HIV care remain low,(9, 10) and there is a high risk of new AIDS events and early mortality. In South Africa, the National Institute for Communicable Diseases (NICD) has reported a median CD4 count of approximately 320 among those entering HIV care. Between 8%-26% of patients die during the first year of ART, with most deaths occurring during the first few months of ART.(9, 11) Cryptococcal disease remains a leading contributor to this early ART mortality throughout Sub-Saharan Africa,(11-15) and thus prevention efforts should be a priority. **Cryptococcosis accounted for 13- 18% of all deaths in 4 cohorts of HIV-infected persons from Uganda** (2, 3, 11, 16) and **44% of all deaths in a South African cohort**.(4) While the incidence of TB is higher, the mortality burden due to cryptococcosis may approach that of tuberculosis.(1, 4, 9, 17)

Despite the roll out of ART in South Africa with now >80% ART access for persons with CD4 count <350 cells/µL,(8) **the incidence of cryptococcal disease has only minimally decreased** based on national surveillance by the NICD . From 2011 to 2013, the incidence of *reported* laboratory-confirmed cryptococcal disease decreased from 117 to 108 per 100,000 HIV-infected persons.(18, 19) In Gauteng, KwaZulu-Natal, and Western Cape provinces, the incidence of cryptococcal meningitis actually increased from 2011 to 2013 **(Figure 1)**. (18, 19) Thus, cryptococcal disease is still a public health problem in South Africa.

Figure 1. Cryptococcal Meningitis Incidence per 100,000 HIV-infected persons, 2013



Cryptococcal Disease Prevention

One strategy to prevent the early mortality in ART programs is to screen asymptomatic persons for subclinical cryptococcosis using CRAG testing in the blood, and then preemptively treat those with subclinical disease with fluconazole.

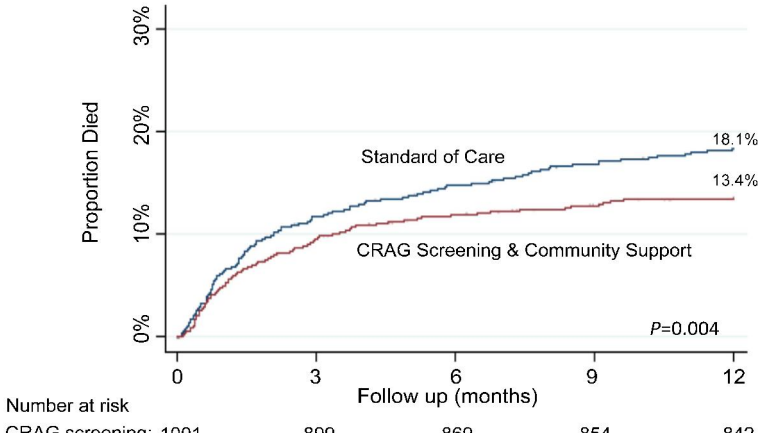
In 2011, the WHO guidelines recommended CRAG screening in those with a CD4 <100 cells/μL.(20) followed by preemptive therapy for those who were asymptomatic and CRAG+.

> **WHO recommended pre-emptive therapy:** 800 mg fluconazole daily for 2 weeks, followed by 400 mg daily for 8 weeks and then 200 mg daily until immune reconstitution. This was adopted by the Southern African HIV Clinicians' Society and in 2014, by SA's National Department of Health.

The WHO acknowledged in the guidelines that the optimal management of CrAg+ persons was not known.

This CRAG screen-and-treat strategy has proven to be efficacious in one published randomized clinical trial and two cohorts in Tanzania and Zambia.(21-23) In the trial, CRAG screening and 4 weeks of ART adherence support resulted in a 28% relative reduction in mortality among persons with a CD4 count <200 cells/μL **(Figure 2)**.

Figure 2. CRAG screening improves survival



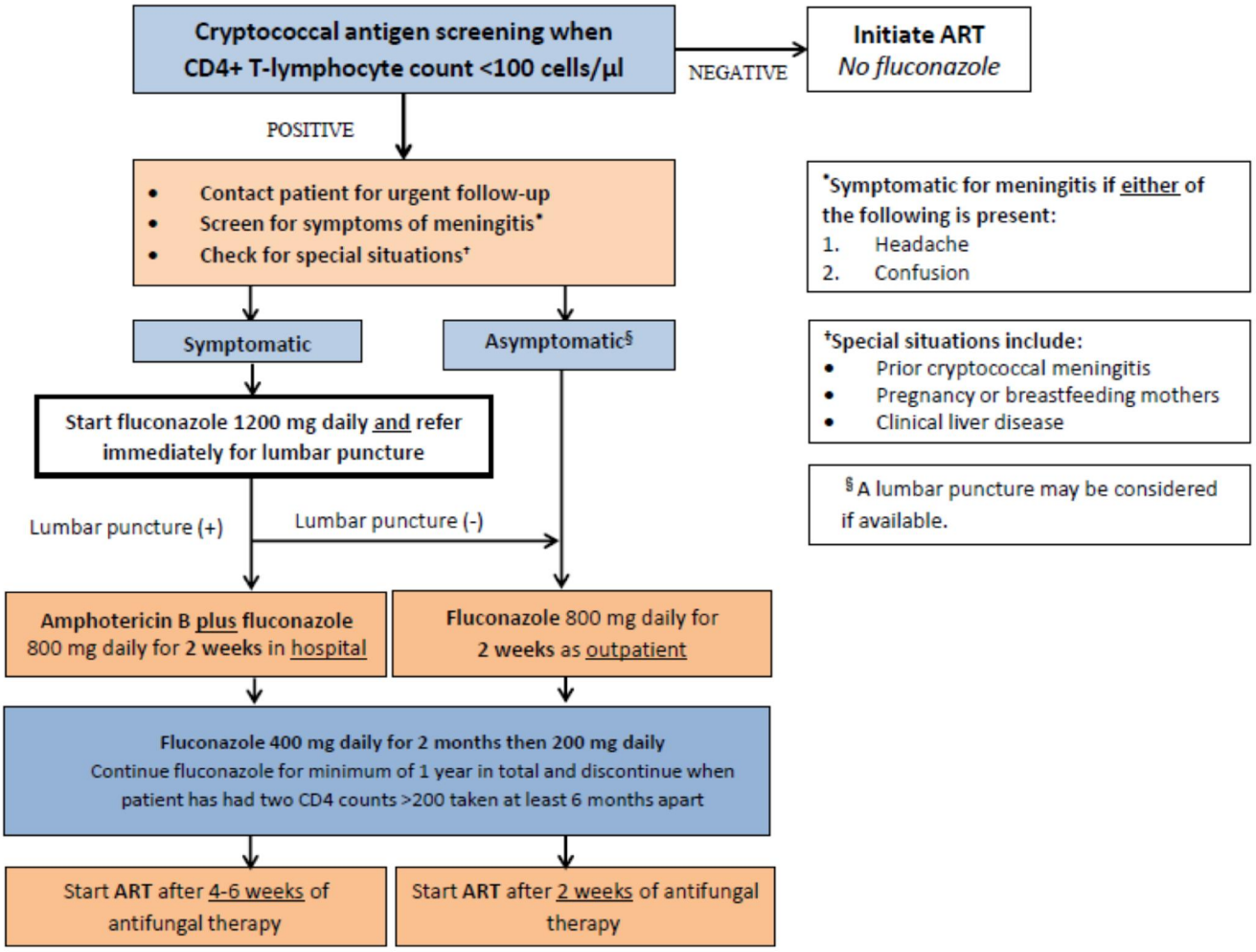
Based on South African outcome and cost data, Jarvis *et al* estimated that CRAG screening saves $156 (95%CI: $119 to $197) for every person with a CD4 count <100/μL,(24) and this results in 40%-80% better 5-year survival.(22, 25) In Cape Town, 73% of ART-naïve patients

presenting with cryptococcal meningitis had already been diagnosed with HIV, a median of 4 months earlier.(26) CRAG screening could have identified these patients, allowing for pre-emptive antifungal therapy and fast-tracking for ART initiation – an issue of particular priority, given the exceptionally high mortality of South African patients in this pre-ART initiation period.(26-28) If all patients who had previously tested HIV-positive (both those on ART and the 73% who were known to be HIV-infected but not on ART) had been CRAG screened and subsequently preemptively treated if CRAG+, up to 78% of cases of cryptococcal meningitis could have been averted, with significant numbers of lives saved and hospitalization costs averted.

Due to the overwhelming cost-benefit of this relatively simple intervention, South Africa has included CRAG screening in 2014 national HIV guidelines.

The current recommended algorithm of CRAG screening and care in South Africa is provided in **Figure 3**.

Figure 3.



We have demonstrated that in South Africa, reflex CrAg screening in the laboratory (on remaining CD4 specimen sample) is a preferred approach to provider-initiated screening (29). **As of 1 November 2016, reflex screening was implemented at all South African public-sector CD4 laboratories.** An estimated 250,000 patients with advanced HIV disease (CD4 count <100 cells/µL) will be screened through this laboratory screening programme per annum. However, the logistical challenges of widespread implementation into health systems are not known.

The Optimal Cascade of Care

For cryptococcal antigen screening and treatment programmes to be successful, the following steps need to be optimized:

1) Maximize the number of those with a CD4<100 cells/µL who are CRAG screened

- Requires reflexive testing in the lab, repeated lab training
- Requires feedback from the laboratory to the health care worker if the specimen for CD4/ CRAG testing is rejected so that a second specimen can be submitted

2) Ensure that all CRAG results return to patients' medical charts
   - A CRAG+ result should be considered a critical value
   - Health care workers should be notified of the result by the laboratory
   - Facility data capturers should capture the CRAG result on tier.net
   - Patients should be contacted to return to clinic urgently
3) Ensure that healthcare providers act on the CRAG result
   - Patient needs to be assessed for symptoms of meningitis
   - If symptomatic, consider lumbar puncture to evaluate for fulminant meningitis
4) If asymptomatic, ensure that fluconazole is prescribed
   - Ensure no contraindications to fluconazole (i.e. pregnancy)
5) Ensure patient receipt and completion of fluconazole
   - Ensure adequate supply of fluconazole in pharmacy
   - Manage side effects (e.g. nausea, vomiting)
6) Ensure that ART is initiated
   - Return to clinic for ART, and cotrimoxazole
   - Ensure adequate supply of ART

Figure 4. Cascade of CRAG+ Preemptive Therapy

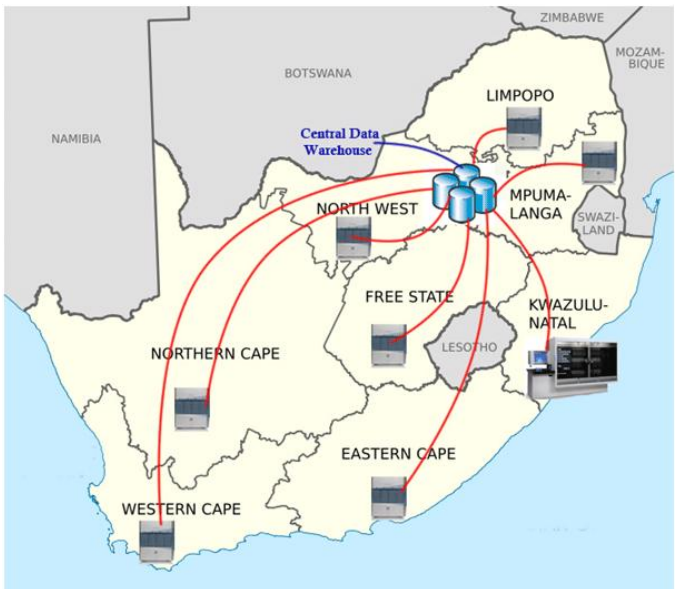
Systems currently in place to monitor cryptococcal disease in South Africa:

1. NHLS CDW
2. NICD-GERMS
3. DHIS
4. Tier.net
5. Statistics SA death register
6. National CrAg laboratory dashboard developed by NICD/ NHLS

National Health Laboratory Service Corporate Data Warehouse

The National Health Laboratory Service (NHLS) is a single parastatal entity comprising of a large integrated national network of diagnostic pathology laboratories that provide clinical services to government hospitals and clinics. This captures >90% of HIV care delivery in South Africa. Lab tests, including CD4 and CRAG, are archived in the NHLS Corporate Data Warehouse (CDW). Relevant identifying information (contained on the original specimen request form) accompany these records, e.g. medical record (hospital/ facility) number, age, sex, NHLS lab testing site, and clinic site. The CDW servers are located at the NHLS in Sandringham, Johannesburg, where the NICD is co-located on the NHLS campus (**Figure**). This system has been used for monitoring HIV viral load testing, CD4 count testing, GeneXpert MTB testing and for the prevention of mother-to-child transmission (PMTCT) therapy to generate aggregate and granular performance data, down to the individual site level. In 2013, 362,000 CD4 counts <100 cells/μL were resulted in the NHLS CDW of which approximately 80% came from unique individuals. The proportion of CD4<100 cells/ μL in 2013 was ~10% of 3.8 million CD4 counts run by the NHLS in South Africa, representing a sizeable population. Other routine laboratory results are also available through the CDW, which can potentially be used as a proxy for retention-in-care.

Figure 5. NHLS Corporate Data Warehouse (CDW) Structure



With national implementation of CRAG screening, data from the NHLS CDW will be used to: 1) determine CRAG+ prevalence by NHLS CD4 lab, province, district, sub-district and clinic level; 2) determine the number eligible for CRAG screening and the number who are CRAG screened (i.e. screening coverage). Within Gauteng and Free State provinces between September 2012 and September

2015 (Phase 1) through this NHLS CDW system, we know that approx. 50,000 persons with a CD4<100 were CRAG tested, and 3% (n=1271) have been identified as new CRAG-positive. In Phase 1, approximately 840 persons were CRAG screened per month, and 30 to 35 were found to be CRAG+. Unfortunately, since September 2012, only 63% of CRAG+ persons have been treated with fluconazole, reflecting further training is necessary. In 2016, a live CRAG dashboard was developed by NICD to monitor the national CRAG programme by reporting the 2 above-mentioned indicators (CRAG prevalence and screening coverage). This dashboard presents data mined directly from the CDW and is integrated in the "all ages" NHLS CD4/ VL dashboard.

NICD-GERMS laboratory-based surveillance for cryptococcal meningitis

Currently nested within the NHLS, the National Institute for Communicable Diseases (NICD) is South Africa's national public health government organization for communicable diseases. NICD runs the GERMS-SA surveillance programme. This comprises of a nationwide network of approx. 200 clinical microbiology laboratories (in the public and private sector) which report cases through the NICD/GERMS-SA active lab-based surveillance programme for a number of diseases of public health importance, including cryptococcal meningitis since 2005. The denominator for this surveillance is assumed to be the entire population of SA (approximately 53 million people). In recent years, NICD has obtained surveillance data for the public sector from NHLS CDW. Additionally, at 25 hospitals in all 9 provinces, NICD has employed nurses to collect additional clinical and epidemiological information on lab-confirmed cases of cryptococcosis (from 2014 onwards, for only 3 months a year).

In addition, there are several other surveillance systems that could be used to monitor the cascade of care among those screened for CRAG with advanced HIV disease

District Health Information System (national)
Sampling will occur within clinics to capture longitudinal follow up of CRAG+ persons using the District Health Information System (DHIS).(30) This is the public health data system used in South Africa (and 40 other countries). The DHIS system is used for many health metrics, for example, this system is what is used to track completion of TB therapy. Similarly, we will use DHIS to assess whether persons are retained in care. Between the NHLS CDW and DHIS systems, these will provide a large volume of a limited dataset. The NHLS CD4/ VL dashboard currently pulls in data from DHIS.

TIER.net in selected provinces

The TIER.net system was originally developed by the University of Cape Town to integrate three paper-based data collection tools into one electronic recording system. It is now used more widely across SA to track patients through the cascade of care and monitor those patients who are lost to follow up. Facility-based data capturers enter patient data from facility records into TIER.net. The system is then able to generate monthly/quarterly reports for facility

operational managers to track and monitor progress of patients through the system. Data entry follows the pathway as indicated below **(Figure 6)**. TIER.net uses a standardised data exchange template that is used to transfer data into other software programmes.

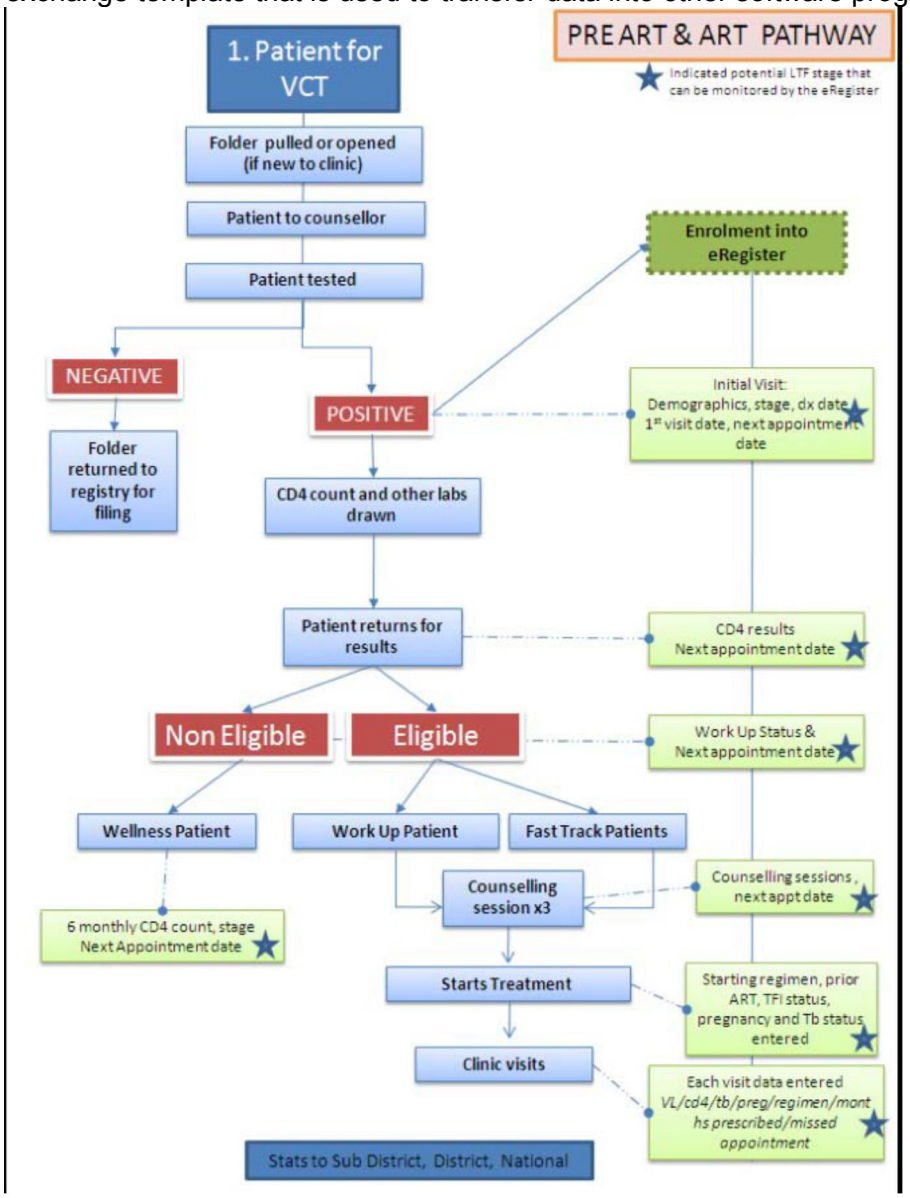


Figure 6

Integrated ART/ TB/ NHLS database in the Western Cape

The Western Cape's electronic eKapa TB (ETR.net) and ART database integrates TB and ART patient information and then links these to NHLS CDW data using probabilistic matching. A study conducted by Vallabhaneni et al using data from WC provincial HIV program revealed that between September 2012

and August 2013, a total of 4395 patients were eligible for CRAG screening; out of those, 1170 (26.6%) were screened. The overall CRAG prevalence was 2.1 % from the total screened.

Statistics SA death register

Stats-SA houses the national data statistics warehouse, where completed death notification forms from the Department of Home Affairs (DHA) are collected. Data verification shows that about 94% of these data are complete. All deaths are compiled in accordance with WHO regulations and follow the ICD-10 coding system. Patient national identification numbers are captured through this system. It is possible to obtain death status for a person if the person's SA ID number is known.

The table below shows death distribution by causes:

Table: Number and percentage distribution of deaths by main groups of causes of death, 2014*		
No. Main groups of underlying causes of death (based on ICD-10)	Number	Percentage
1 Certain infectious and parasitic diseases (A00-B99)*	98 817	21,8
2 Neoplasms (C00-D48)	39 143	8,6
3 Diseases of the blood and immune mechanism (D50-D89)	9 594	2,1
4 Endocrine, nutritional and metabolic diseases (E00-E90)	29 642	6,5
5 Mental and behavioural disorders (F00-F99)	1 996	0,4
6 Diseases of the nervous system (G00-G99)	10 274	2,3
7 Diseases of the eye and adnexa (H00-H59)	16	0,0
8 Diseases of the ear and mastoid process (H60-H95)	54	0,0
9 Diseases of the circulatory system (I00-I99)	78 258	17,3
10 Diseases of the respiratory system (J00-J99)	45 381	10,0
11 Diseases of the digestive system (K00-K93)	11 928	2,6
12 Diseases of the skin and subcutaneous tissue (L00-L99)	793	0,2
13 Diseases of the musculoskeletal system etc. (M00-M99)	1 619	0,4
14 Diseases of the genitourinary system (N00-N99)	8 772	1,9
15 Pregnancy, childbirth and puerperium (O00-O99)	1 027	0,2
16 Certain conditions originating in the perinatal period (P00-P96)	9 363	2,1
17 Congenital malformations (Q00-Q99)	2 138	0,5
18 Symptoms and signs not elsewhere classified (R00-R99)	56 784	12,5
19 External causes of morbidity and mortality (V01-Y98)	47 761	10,5
Total 453 360 100		
* Including deaths due to MDR-TB and XDR-TB.		

Knowledge gaps in the implementation of CRAG screening and treatment interventions

1) Retention-in care: In a pilot study in Soweto, important challenges to real-world implementation were identified, including high rates (48%) of loss-to-follow-up among CRAG+ patients (31). Here, linkage and retention-in-care was essential to the success of the CRAG screening program.

2) Linkage to laboratory results: Poor linkage to laboratory (CD4 and CRAG testing) results was identified as a major operational barrier during an evaluation of the first phase of reflex CRAG screening at 200 health facilities in 4 South African health districts (approximately 20%).

3) Integration with WHO universal HIV test-and-treat guidelines: South Africa adopted the WHO universal HIV test-and-treat guidelines in September 2016. While the National Department of Health has indicated that a baseline CD4 count should be ordered for all patients starting ART, in practice many patients are initiated on ART on the same day as HIV diagnosis, well before the baseline CD4 and reflex CrAg results are available. In KwaZulu-Natal, the provincial department has recently recommended routine same-day ART initiation at all its facilities.

   It is unknown if same-day ART initiation among those with advanced HIV would have an adverse impact on patient outcomes at a programmatic level (as a result of unmasking IRIS). Conversely, even reflex CRAG screening may potentially delay ART initiation for those who ultimately screen CRAG negative but should be fast tracked onto ART to reduce risk of HIV progression and other OIs. We have previously described shorter median times to ART initiation amongst those CRAG-screened compared to those not screened (32).

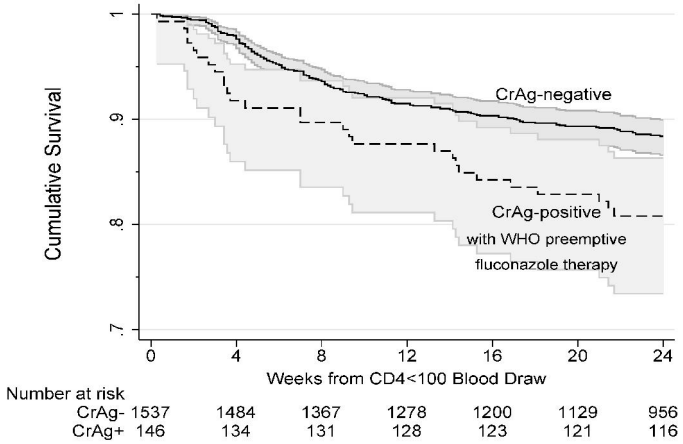
4) Impact of training: We have previously implemented training workshops for health care providers (doctors, nurses, pharmacists, counsellors) with the goal of improving clinical management of cryptococcal disease. However the impact of these workshops on patient outcomes has not been measured.

5) Optimal treatment regimen for asymptomatic CRAG+ persons. Although CRAG+ persons receiving fluconazole generally do better than if not treated at all, 25%-30% go on to develop meningitis or die despite the WHO-recommended antifungal therapy and ART. A 2.9-fold worse survival was observed in asymptomatic CRAG+ vs. CRAG- persons with CD4 count <200 cells/μL in Tanzania and Zambia (33) **(Figure 7)**.

   The current WHO regimen for asymptomatic CRAG+ patients was based on expert opinion and extrapolated from management of symptomatic cryptococcal meningitis.

This regimen likely requires further refinement. As an example, preliminary results from the ORCAS trial in Uganda suggest that persons with CRAG titres ≥160 and CD4 count <50 cells/μL are more likely to develop subsequent cryptococcal meningitis compared with those with a lower CRAG titre/ higher CD4 count when receiving current WHO-recommended antifungal therapy. Customized or more intensive therapy for those with high titres may be a useful strategy to reduce CRAG+ mortality **(Figure 8)**.

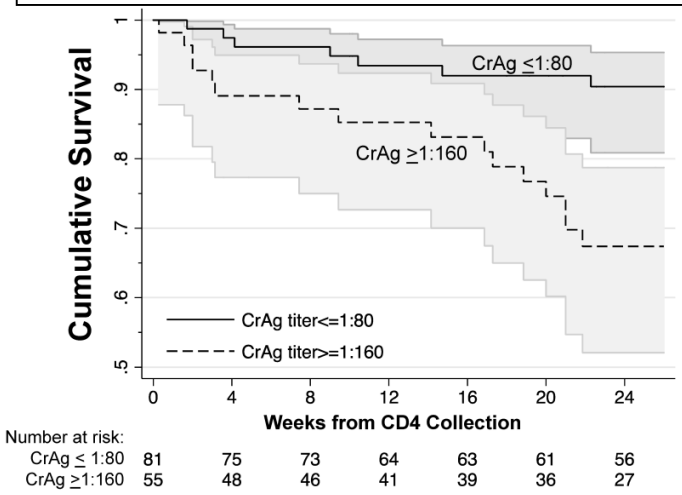
Figure 7. Survival by CRAG status in Uganda & REMSTART trial in Tanzania & Zambia



6) Risk factors for progression to cryptococcal meningitis:

Asymptomatic CRAG+ patients are at increased risk of meningitis and death. But who progresses to meningitis and the expected time frame of this progression has not previously been studied. Lumbar punctures have been proposed as a valuable tool to investigate potential CNS involvement. However, lumbar punctures are generally very poorly accepted in African populations, and the majority of clinics do not have the capacity or healthcare worker resources to perform lumbar punctures on a number of asymptomatic outpatients. One group in Tanzania performed lumbar punctures in 31/32 CRAG+ persons, and identified 2 out of 12 patients without any neurologic symptoms who had meningitis in CSF. Thus, symptoms of meningitis are not necessarily predictive alone. CRAG titres have been found to predict risk of meningitis and death. Other risk factors have not yet been evaluated.

Figure 8. Survival stratified by CRAG titre in Ugandan ORCAS trial



1.2 Rationale

Despite the commendable effort of rolling out national reflex CRAG screening in laboratories, there will likely be considerable **gaps in the HIV cascade of care** that will **hinder the potential survival benefit** from CRAG screening and treatment. We seek to evaluate how best to **optimize implementation** of reflex laboratory CrAg screening in real-world facilities across South Africa in order to **improve the HIV care continuum** and thus **maximize the survival** of persons living with AIDS.

1.3 Potential Risks and Benefits

1.3.1 Potential Risks

This study involves little/no risk. We are monitoring 6-month outcomes of an existing national programme. Cryptococcal meningitis is a life-threatening condition, and CRAG screening and pre-emptive treatment has been implemented by the national government and NHLS to prevent this. We seek to first evaluate the success and challenges of that programme and then to test programmatic interventions to optimize the impact of this national programme.

Data will be collected retrospectively, then analyzed in a de-identified manner, and aggregated prior to presentation. Individual identifying information will not be available to CDC investigators.

1.3.2 Known Potential Benefits

Benefits of this research are collection and presentation of detailed 6-month outcomes on an already implemented CRAG screen-and-treat programme across South Africa. This will highlight gaps in the care cascade, and areas for improvement of the programme in the future.

2 OBJECTIVES

2.1 Study Objectives

We seek to evaluate how best to optimize implementation of reflex laboratory CrAg screening in real-world facilities across South Africa in order to improve the HIV care continuum and thus maximize the survival of persons living with AIDS.

Primary objective (Years 1 and 2): To implement enhanced surveillance at selected sites to determine the outcomes (overall and for pre-specified subgroups) of a national reflex laboratory CRAG screening and pre-emptive treatment programme in South Africa.

Primary objective (Years 2-5): To implement and test programmatic interventions to improve 6-month cryptococcal-free survival.
Examples may include:

a) Enhanced diagnostics to customize or differentiate patient care
    a. Using CRAG titre results (e.g. ≥160 titre) to guide when to recommend to perform lumbar puncture (LPs) to detect subclinical meningitis
    b. Using CRAG titre results to guide recommendations for enhanced antifungal therapy
    c. Validate CRAG diagnostics
b) Healthcare provider training
    a. Training in an online/digital creative format
    b. Refresher training: Determine frequency of refresher training associated with adherence to national protocols and patient outcome
    c. Patient education
c) Enhanced laboratory services: Determine benefit of enhanced methods to deliver CD4 and CRAG results to requestors
    a. Using SMS to healthcare providers (or SMS alerts to patients to return to the facility for urgent results)
    b. Contacting patients directly with CRAG results with provision of simple instructions for required next steps
    c. Confidential “results for action reports” to district and facility managers (i.e. line lists of CRAG+ patients and patients with a CD4<100 but no CRAG test result)

Secondary Objectives:

2. Evaluate cost-effectiveness of a real-world CRAG screening and pre-emptive treatment programme on the ground in South Africa.

3. Evaluate risk factors for treatment failure.

Exploratory Objectives:

4. Measure the impact of same day test and treatment on timing of ART initiation among CRAG+ and CRAG negative individuals.

5. Describe the prevalence of opportunistic infections in CRAG+ and CRAG negative individuals

2.2 Study Outcome Measures (Overall and For Pre-Specified Subgroups)

2.2.1 Primary Outcome Measures (years 1-5)

1. 6-month cryptococcal meningitis free-survival

2.2.2 Secondary Outcome Measures

1. 6-month retention in care

2. Operational challenges of implementation including:
    a. Time from CD4 testing, CRAG testing, antifungal therapy, and ART initiation among those with a CRAG+ result
    b. Proportion of CD4/ CRAG results that are received, acknowledged and acted upon by a health practitioner
    c. Proportion of patients with advanced disease receiving TB screening
    d. Proportion of CRAG+ persons who develop symptomatic cryptococcal meningitis
    e. Completion of lumbar puncture when clinically indicated
    f. Availability of lumbar puncture and appropriate antifungal therapy in routine clinical practice
    g. Loss to follow-up after CRAG screening and/or during antifungal therapy
    h. Proportion of clinicians and staff trained on the CRAG screening algorithm and CRAG lateral flow assay (LFA) (the latter only for facilities that are distant from a laboratory)
    i. Adherence to CRAG screening and pre-emptive treatment guidelines by healthcare practitioners

3. Costs of CRAG+ treatment programs, Incremental cost-effectiveness ratio (ICER)

2.2.3 Exploratory outcome measures:

1. Time to ART initiation in test and treat programs amongst HIV-infected persons with advanced disease.

2. Prevalence of opportunistic infections in HIV-infected persons with advanced disease

3 STUDY DESIGN

This is an evaluation of an ongoing national screening programme. In years 1 and 2, we will implement an enhanced metrics and evaluation project at selected sites in all provinces across South Africa to measure 6-month outcomes of this programme, along with operational challenges. In years 2 to 5 we will implement programmatic strategies to maximize 6-month cryptococcal meningitis-free survival.

Per South African standard of care, those with a CD4<100 cells/μL will reflexively get a CRAG test performed on remaining blood specimen. We expect the majority of these persons to be asymptomatic outpatients presenting for routine HIV care, though a small proportion will be persons who present to care because they feel unwell. All participants will receive standard of care treatment for asymptomatic cryptococcal antigenaemia, per South African HIV guidelines.

Primary Objective (years 1 and 2): To implement enhanced surveillance at selected sites to determine the outcomes (overall and for pre-specified subgroups) of a national reflex laboratory CRAG screening and pre-emptive treatment programme in South Africa. We estimate performing enhanced surveillance at **approximately 400 clinics out of 4500 clinics nationally**. A list of surveillance facilities will be agreed upon in discussion with provincial departments of health.

We will assess the cascade of care from CD4 testing to CRAG testing, to receipt of CRAG results by healthcare provider, assessment for symptoms, lumbar puncture if indicated, pre-emptive treatment for those asymptomatic, and 6-month outcomes.

The initial surveillance performed in Objective 1 will establish **baseline 6-month CRAG+ outcomes** (Year 1 and 2) from which subsequent programmatic interventions will seek to improve.

Subgroup Analyses

A number of subgroup analyses will be performed to understand optimal CRAG screening and treatment programmes. For example, we will compare outcomes amongst CRAG+ persons who:

a) initiated on ART before CD4/ CRAG results are known (same-day ART initiation in the context of universal HIV test-and-treat) to those who have ART delayed until a minimum of 2 weeks of antifungal treatment completion
b) rural clinics vs. urban centres
c) high titres (≥160) vs. low titres (<160)

d) clinics that routinely perform lumbar punctures to those that are unable to perform lumbar punctures (stratified by titre)
e) development of culture-positive cryptococcal meningitis. These lab results will be captured by the NHLS database, and additional queries by NICD personnel of medical records of CSF CRAG+ persons will be performed for secondary data review and quality assurance on the rate of capture of meningitis episodes. The GERMS surveillance system run by NICD will continue to capture cryptococcal meningitis among those lost to follow up or who transfer care.
f) Cryptococcal meningitis-free survival of those who receive secondary prophylaxis vs. none
g) Special populations: pregnant women, those with liver disease.

Such information will be particularly nuanced to the South African CRAG screening and treatment programme, but will also be informative to other sites in sub Saharan Africa that are initiating similar national programmes.

Active enhanced surveillance

Data will be collected by two methods:
A) primary (retrospective) data collection by chart/ record review at facility level by partners and
B) secondary analysis and triangulation of existing laboratory and surveillance datasets.

Primary data collection by partners
We will collaborate with partners in South Africa to conduct active surveillance of all CRAG+ persons at selected facilities using a standardized data collection tool (attached – version 1.0, 4 January 2017). Please see appendix for list of partners, and potential roles. Partners may assist with monitoring and evaluation, capturing health information, contact tracing of those who are lost-to-follow up, and/or collecting operational information.

Partners will review patient demographics and outcomes of those who are CRAG+ by retrospective chart/ record review. This will be performed approximately 3 times over a 6-month period, to obtain information about CRAG result, CD4 result, presence of CNS symptoms, receipt of fluconazole if appropriate, and triage to lumbar puncture if symptomatic, along with timing of ART initiation, 6-month survival, retention-in-care, and incidence of cryptococcal meningitis.

Surveillance will not occur at all facilities across South Africa, but at selected facilities within each South African province. A list of surveillance facilities will be agreed upon in discussion with provincial departments of health.

Secondary analysis and triangulation of existing datasets noted on pages 13 and 14.

- Cohort of those with advanced HIV (CD4 <100), incl. CRAG results
    - Subset of those with a positive CRAG result
    - Subset of those with cryptococcal meningitis

Primary objective (Years 2-5): To implement and test programmatic interventions to improve 6-month cryptococcal-free survival.

Starting in Year 2, we will use the data generated from the preceding years regarding the barriers in the cascade-of-care to identify areas which may require programmatic interventions to improve 6-month CRAG+ outcomes. Such interventions will be implemented at selected enhanced surveillance facilities to determine their effectiveness (pre/post intervention) as well as compared with facilities without the intervention. The exact intervention(s) to be implemented will be dependent on the challenges observed during the initial surveillance. Examples may include:

Intervention 1: Stratification by CRAG titre
For a subset of CRAG+ persons in the enhanced surveillance cohort, we will perform CRAG titres. We anticipate performing these retrospectively (batched) initially, and then for a short time prospectively to evaluate whether this is feasible in the lab. CRAG titres will not be reported to treating clinicians initially. Once data have been collected in years 1 and 2 regarding outcomes of those with high CRAG titres vs. low CRAG titres, we may then routinely report CRAG titre results with CD4 results. An information sheet may be attached to the lab results educating healthcare workers of the CRAG+ result and suggestions for management. Alternatively, this

information will be included in routine healthcare worker training materials. We may recommend that those with a CRAG titre ≥160 have a LP performed to evaluate for meningitis, given that these persons are at high risk for meningitis and death. The outcome will be 6-month survival.

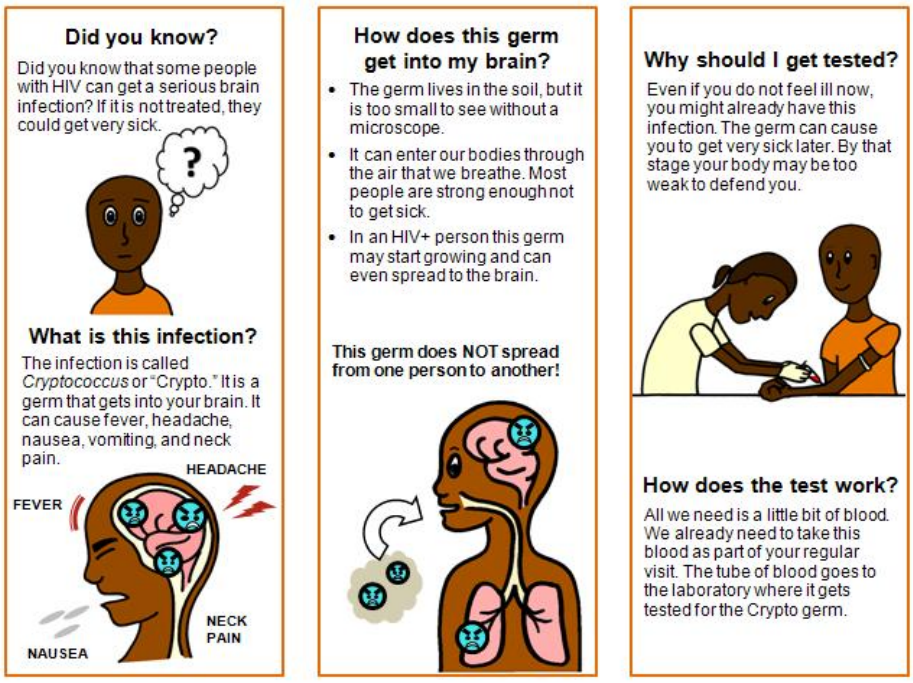
We hypothesize those with CRAG titres ≥160 will benefit from recommended lumbar punctures to detect occult meningitis (defined as CSF CRAG+ and/or culture-positive) in comparison with those who decline LPs. Conversely, we hypothesize those with low CRAG titres <160, do not have a survival benefit when receiving LPs (i.e. due to low prevalence of asymptomatic occult meningitis).

Intervention 2: Healthcare provider training

We will create online/digital training programs for laboratory workers, health care providers, and clinic staff regarding CRAG screening and pre-emptive treatment. Alternatively, we will partner with existing initiatives such as Project ECHO (continuing professional education and clinical mentoring via zoom video conferencing). These educational programs will be implemented at clinics to evaluate whether gaps in the cascade of care can be minimized.

We will create refresher training for lab workers, health care providers, and clinic staff regarding CRAG screening and pre-emptive treatment, and measure if more frequent training sessions improve adherence to national protocols and patient outcome.

Finally we will create patient education materials in the form of posters that can be hung in clinic waiting rooms, short presentations that can be performed by community healthcare workers in clinic waiting rooms/ as part of focus groups, and digital training programs for patients. We will measure overall clinic adherence to national protocols and patient outcomes. Sample educational materials below.



Intervention 3: Determine benefit of enhanced methods to deliver CD4 and CRAG results

a) SMS may be used for clinic appointment reminders, medication adherence reminders, and general information, as a potential method to enhance retention in care of CRAG+ persons. SMS may be used by clinics to enhance the cascade of care, for example, to report anticipated fluconazole stockouts, or to remind providers to perform TB screening for those with advanced HIV disease.

b) Reports for each district and facility managers with line list of CRAG+ patients, and those with a CD4<100 without CRAG testing (compare outcomes in districts/ facilities with Result for Action reports vs. no Result for Action reports)

Intervention 4: Enhanced antifungal therapy based on CRAG titre. After years 1 and 2, outcomes of those with high/low CRAG titres will have been summarized. We hypothesise that those with high CRAG titres would have higher mortality and would be at higher risk for breakthrough meningitis, as seen in multiple other studies.

If this is the case, we may then select a small number of high volume sites to perform CRAG titres in real time, and recommend enhanced antifungal therapy for those with high CRAG titres. Please note: This will be a **separate ethics committee submission and will include informed consent from participants**.

Secondary Objectives:

1. Evaluate cost-effectiveness of real-world CRAG screening and pre-emptive treatment programmes in South Africa.

Cost-effectiveness of CRAG screening and pre-emptive treatment has been evaluated in South Africa using mathematical modelling. However, real-world costs and implementation have not been accounted for. For example, this prior model did not account for the repeated training sessions required for healthcare workers. Theoretical models typically don't account for challenges that are seen on the ground, such as fluconazole stock outs, point-of-care CD4 testing, and variable performance and acceptance of lumbar punctures. Effectiveness will be obtained from Objective 1. Analysis will be from the perspective of the health care payer. We will map out the flow of CRAG screen and treat through the HIV cascade of care.

Costs will be collected on health care workers' salaries and percent effort dedicated to CRAG screening, training, lab testing, transport of lab results, fluconazole, and further evaluation of symptomatic patients such as lumbar puncture will be incorporated. Cost of hospitalization and treatment of cryptococcal meningitis will be an important consideration, and will likely vary depending on hospital site. Cost-outcome measures will include annual cost per patient, cost per death prevented, cost of meningitis-free survival, and cost per quality-adjusted life year gained. Cost effectiveness ratios will be stratified by CRAG titre threshold. Incremental cost-effectiveness ratio (ICER) with interventions from Aim 2 will be compared to Aim 1.

Costs and effectiveness will be stratified by subgroups. For example, we expect variable cost and efficacy amongst:

a) Rural vs. urban clinics
b) Clinics where LPs are feasible vs. those that are not
c) Patients that receive secondary prophylaxis vs. those who stop fluconazole without prophylaxis
d) High CRAG titre vs. low CRAG titre
e) Education and Retention-in-care: Efforts for education of patients and healthcare workers, and efforts to improve retention in care such as using SMS will likely be more costly at the clinic level. However, if proven to be efficacious (compared to without these interventions) may be cost-effective interventions.

Probabilistic sensitivity analysis will be performed thereafter to account for uncertainty in parameter such as loss to follow up, or performance of lumbar punctures –which will likely vary by site. This will yield more nuanced data regarding the efficiency of CRAG screening programs, and important barriers to overcome for comprehensive, cost-effective care.

2. Evaluate risk factors for treatment failure.

The purpose of this objective is to define high-risk populations that may require customized, intensive care in the future. We hypothesize that high initial CRAG titres in blood (≥160), is a risk factors for meningitis and death. Preliminary data from the original ORCAS study in Uganda from 2012 to 2014 suggests that CRAG titre is a risk factor for death.

Other risk factors may include rapid or same-day ART initiation, delay in ART initiation >2 weeks from initial CD4 blood draw, delays in initiation of fluconazole beyond 2 weeks from initial CD4 blood draw, stock outs of fluconazole, etc.

3.1 Exploratory studies

We will evaluate the impact of CRAG screening programs on the timing of ART initiation, particularly in the context of a same-day HIV test-and-treat strategy. We plan to perform a case-control study, of 2 CRAG negative persons for each CRAG positive person, matched by clinic, CD4 count, and CD4 date, and will evaluate time to ART initiation from CD4 testing. This case-control study will be conducted at a small subset of facilities in each province. Data for the case-control study will be collected retrospectively, as specified on page 29.

Additionally we will describe the prevalence of opportunistic infections amongst CRAG+ and CRAG negative persons. Opportunistic infections of particular interest include tuberculosis, toxoplasmosis, other fungal infections. These data will be obtained from laboratory records: toxoplasmosis serology, MTB/Rif GeneXpert results, beta d glucan results, urine LAM, etc.

4 STUDY ENROLLMENT AND WITHDRAWAL

Study population will predominantly be from outpatient clinics. Those identified as CRAG+ by lab test will typically be HIV-infected outpatients presenting for routine care. However, there will be some who present with complaints of feeling unwell.

4.1 Subject Inclusion Criteria

- Blood CRAG test positive

4.2 Subject Exclusion Criteria

- Age <18 years

Rationale for Criteria:

This project will generate an epidemiological dataset and will then explore the primary endpoint for pre-specified subgroups, along with operational challenges amongst specific subgroups.

5 STUDY EVALUATIONS

We will collect 3 types of data: patient-level, clinic-level, and laboratory-level.

Patient-level data

Chart review will occur approximately 3 times over a 6 month period, with the goal of capturing the patient-level information on CRAG+ persons. Timing of these chart reviews will be variable.

The chart abstractor will ensure that the subject has a positive CRAG result. If so, additional clinical information will be extracted from the chart (see below).

Informed consent will not be obtained for this study, as we are retrospectively collecting demographics and outcomes for CRAG+ and CRAG- patients in routine care. Please see Informed Consent section below.

Baseline demographic information will be obtained, including age, sex, date of birth, location, distance from clinic. Thereafter information about CRAG screening will be obtained. Namely, whether CRAG results were returned to the medical chart and how the healthcare provider acted on these results. If symptomatic, were they offered an LP? If asymptomatic were they prescribed fluconazole? What were the doses and dates of fluconazole therapy, and were there any side effects. Timing of initiation of ART and active treatment for TB will be obtained from the chart. Survival status, along with the date that the patient was last known to be alive will also be collected.

Patient-level data to be collected

Evaluations	Monitoring Visit
CRAG+, titre	X
CD4 count	X
Baseline demographic data	X
If symptomatic, receipt of LP	X
If asymptomatic, receipt of fluconazole	X

Evaluations	Monitoring Visit
Completion of fluconazole	X
Initiation of ART (timing)	X
Concurrently on TB treatment	X
Incidence of cryptococcal Meningitis	X
6-month retention in care	X
6-month vital status	X

5.1 Clinic Evaluations

Clinic-level factors will be collected to further understand challenges to CRAG screening. This information will be extracted from existing data systems, and from primary data collection. For example, we will collect

- Distance from clinic to nearest NHLS CD4 lab – already mapped out by NHLS
- Number eligible for CRAG screening at clinic site
- Number CRAG screened, by clinic site
- Number who received TB screening
- Timing from CD4 testing to receipt of CRAG result
- Proportion who required LP, and had LP performed
- Acceptability of LP by patients
- Resources for LP performance
- Healthcare worker training (nurses, doctors, counselors, pharmacists) by clinic site

- Clinic standard of care for initiation of ART (test and treat vs. wait for CD4 results)
- Frequency of fluconazole stock outs

5.2 Laboratory Evaluations

Information from each NHLS CD4 laboratory will be obtained to further understand barriers to reflex CRAG screening. Such information may include:

- Number of CD4<100 cells/uL per week/month
- How CRAG screening performed (batched, performed daily)
- Ease of performing CRAG titres in real-time (prospectively)
- Procedures after CRAG+ result identified

6 ASSESSMENT OF SAFETY

As we are retrospectively monitoring outcomes in routine care, there will be no assessment of safety. CRAG screening and preemptive treatment have previously been studied to be safe and efficacious. This is now standard of care in South Africa; no additional safety monitoring will be pursued.

7 CLINICAL MONITORING

N/A

7.1 Site Monitoring Plan

N/A

8 STATISTICAL CONSIDERATIONS

Primary Endpoint

1) 6-month cryptococcal meningitis free survival
    - Those who die of any cause are failures
    - Those developing symptomatic meningitis are failures

8.1 Statistical Analysis Plan

The rate (with 95% confidence interval) for the primary and secondary events will be estimated overall and for pre-specified subgroups during the first 2 years of the study. Those rates will subsequently be compared with the rates after programmatic changes have been made (during years 2-5) to increase the effectiveness of the national screening program.

For the subgroups we will also use time-to-event methods to compare the primary and selected secondary endpoints. The table below reports detectable hazard ratios for the primary event with 80% and 90% power, assuming a two-sided alpha=0.05, 70% CM-free survival in the control group, and the stated number of subgroup participants. For example, if CSF CRAG titre data are available for 1200 participants (with 300 having a titre ≥ 160), then we can detect a hazard ratio as small as 1.37, for an almost 10% difference in event-free survival.

Detectable Hazard Ratios for Subgroups (Assuming two-sided α=0.05 and 70% event-free survival in the control group)					
		80% Power		90% Power	
Subgroup	N per group	HR	Proportion Surviving	HR	Proportion Surviving
High CSF CRAG titre (≥1:160)	300	1.37	0.61	1.44	0.60
Control (CSF CRAG titre < 1:160)	900				

The a priori subgroups of interest are:

- High CRAG titres (≥1:160) vs. low titres (<1:160)
- Male vs. Female
- Pregnant vs. Non-pregnant
- Liver Disease (ALT>3x Upper Limit of Normal) vs. No liver disease

- Geographic Location (urban vs. rural)
- Timing of ART Initiation
    - On pre-existing ART
    - Initiated on ART before CD4/ CRAG results are known (same-day ART initiation in the context of universal HIV test-and-treat)
    - ART initiated at 2 weeks
    - ART initiated at >=4 weeks
- Clinic-level outcomes (i.e. cluster) based on proportion of CRAG+ persons receiving Lumbar Punctures.
- Duration of fluconazole use
- Fluconazole secondary prophylaxis vs. none

8.2 Sample Size Considerations

Sample size will depend on number of persons CRAG screened under the national CRAG screening program. Within 4 districts in Gauteng and Free State provinces between September 2012 and September 2015 through this NHLS CDW system, we know that 50,000 persons with a CD4<100 have been CrAg tested, and 3% have been identified as CRAG-positive. With national CRAG screening, we expect 6000 to 8000 asymptomatic CRAG+ cases to be detected per year and 15000 to 17000 symptomatic CRAG+ or cryptococcal meningitis cases per year.

Quality Control and Quality Assurance
Data verification will occur in the first 2 years of the study, where we will evaluate a sample of clinic and laboratory records in detail, and describe the baseline rate of error that can be expected from our data.

Ongoing data quality control (QC) and quality assurance (QA) activities will occur via data management system. **Clinical Quality Management Plan** (CQMP) reports will include:

- Summary of Data Completeness
- Case Report Form (CRF) Delinquency Report
- Delinquent CRFs by Participant ID

9 ETHICS/PROTECTION OF HUMAN SUBJECTS

9.1 Ethical Standard

The investigators will ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and/or the ICH E6; 62 Federal Regulations 25691 (1997). This study will be in compliance with the Declaration of Helsinki, CIOMS, International Ethical Guidelines for Biomedical Research Involving Human Subjects (2002).

9.2 Ethics Committee Review

We are retrospectively monitoring an ongoing national screening programme that is considered standard of care in South Africa. Data collection will only be performed retrospectively, thus informed consent will not be obtained for this study.

If further interventions that are beyond the standard of care are pursued, an amendment will be made to this protocol, and further review will be pursued with the local ethics committees.

9.3 Subject Confidentiality

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover clinical information relating to participating subjects.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party.

9.4 Future Use of Stored Specimens

Specimens will not be stored beyond the length of this study. Specimens may be tested and batched for testing of: CRAG titre, CRP, and other laboratory parameters that may be risk factors for treatment failure.

10 DATA HANDLING AND RECORD KEEPING

This study will use REDCap system for multisite data collection. REDCap is a secure web application for managing online databases. While REDCap can be used to collect data (including 21 CFR Part 11, FISMA, and HIPAA-compliant environments), it is specifically geared to support online or offline data capture for research studies and operations. Data will be entered directly from patient files into the REDCap database.

The partners affiliated with this study (see Appendix) may use their own secure data management systems.

The investigators are responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL.

Copies of the electronic CRF (eCRF) will be provided for use as source documents and maintained for recording data for each subject enrolled in the study. Data reported in the eCRF derived from source documents should be consistent with the source documents or the discrepancies should be explained

10.1 Data Management Responsibilities

A data manager at the NICD/ University of Minnesota will work with the study statistician to conduct Quality Assurance activities and prepare reports.

All source documents will be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete.

10.2 Data Capture Methods

Clinical data and clinical laboratory data will be entered into a RedCap database. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

10.3 Essential/Source Documents

The site will maintain appropriate medical and research records for this study, in compliance with ICH E6, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects.

Source data are all information, original records of clinical findings, observations, or other activities in a study necessary for the reconstruction and evaluation of the study. Examples of these original documents and data records include, but are not limited to, hospital records, clinical and office charts, laboratory notes, pharmacy dispensing records, recorded data from automated instruments, digital media, radiographs, and subject files and records kept at the pharmacy, at the laboratories.

For this study, the source documents will be the REDCap CRFs completed by monitoring staff. It is acceptable to use CRFs as source documents, when the study personnel are making their original observations and recording on CRFs. In the event of transcription from another data source, e.g. laboratory report or hospitalization records, these records are the source document. A copy of any external record should be obtained and stored in the study chart.

Routine clinic records completed by non-study staff are **not** source documents, unless they are being used to transcribe data.

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APPENDIX

LIST OF PARTNERS (INCLUDE BUT ARE NOT LIMITED TO):

1. Foundation for Professional Development (FPD):
    a. Provides facility- and district-level support in 3 high-burden HIV SA health districts
    b. Employs M&E mentors who supervise the activities of facility-level data clerks who capture health information via the DHIS
    c. In addition, contact tracing of those lost to follow up may be performed in order to supplement the NHLS CDW and DHIS data
    d. Operational information for CrAg+ persons will be collected, as above. Enhanced surveillance data will be collected on case report forms (CRFs) via RedCap web-based data management system. CRFs will be completed by personnel employed by FPD.
2. SA Medical Research Council/ Epicentre:
    a. Epicentre is subcontracted to collect data for MRC studies
    b. Employ data collectors who are trained to do field work.
    c. Enhanced surveillance data will be collected on case report forms (CRFs) via Mobenzi Researcher.
    d. QC officers will collect 10% of data from source again to re-check data.
    e. Data will be cleaned by data management team and will then be shared with the study team.