Vietnam Cryptococcal Retention in Care Study (CRICS)

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Table of contents

Acronyms 4
Protocol Summary 6
Investigators and collaborating institutions 9
1. Introduction 10
1.1 Background on cryptococcal infection 10
1.2 HIV and cryptococcal infection in Vietnam 11
1.3 Diagnosis of cryptococcal infection in Vietnam 12
1.4 Rationale for study 12
2. Study Objectives and Outcome Measures 14
3. Study Design and use LFA in study and non-study sites 17
4. Study population 19
4.1. Inclusion criteria: 19
4.2. Exclusion criteria: 19
5. Study sites 20
6. Sample size calculation: 23
7. Study procedures 24
8. Study Implementation 40
8.1. CrAg screening program launch meeting 40
8.2 Pre-study training 40
8.3. Additional training in Phase 2 41
8.4 Monitoring visits 42
8.5 Management Meetings 43
9. Specimen collection and processing 43
9.1. Sample collection 43
9.2. CD4 and CrAg Lateral Flow Assay (LFA) testing 44
9.3. LFA in CSF during evaluation of CM 46
9.4. Quality Assurance for LFA and CD4 count 47
9.5. Measurement of cryptococcal antigen titres 48
10. Data Collection 48
10.1Patient variables 50
10.2. Cost and cost-effectiveness analysis data 52
10.3. Talaromyces Manaffei Antigenemia in Stored Samples (Sub-study) 57

10.4.Datastorage 58
10.5. Data transfer 59
10.6 Data ownership, governance, sharing, dissemination 60
10.7 Data quality assurance 60
11. Data Analysis 60
12. Expanding implementation of CrAgscreening: Feasibility 63
13. Ethical considerations 63
13.1 Ethical review and informed consent 63
13.2. Waiver of consent (specific items) 65
13.3Confidentiality 65
13.4Benefits/Risks 66
14. Safety monitoring and adverse event reporting 67
15. Sponsor Monitoring 67
16. Budget 70
17. Implementation schedule 71
References 72
List of appendices 74

Acronyms

AE	Adverse event
AIDS	Acquired Immune Deficiency Syndrome
ALT or SGPT	Alanine transaminase or serum glutamic-pyruvic transaminase
ART	Antiretroviral therapy
ARV	Antiretroviral
AST or SGOT	Aspartate transaminase or serum glutamic oxaloacetic transaminase
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
CM	Cryptococcal meningitis
CNS	Central nervous system
CRA	Clinical research associate
CrAg	Cryptococcal antigen
CRF	Case reporting form
CSF	Cerebrospinal fluid
DEFF	Design effect
DOB	Date of birth
EFV	Efavirenz
ELISA	Enzyme-linked immunosorbent assay
HIV	Human Immunodeficiency Virus
HRQOL	Health-related quality of life
ICC	Intraclass correlation coefficient
ICER	Incremental cost-effectiveness ratio
ID	Identification
IRB	Institutional Review Board
IRIS	Immune reconstitution inflammatory syndrome
LFA	Lateral flow assay
LNMP	Last Normal Menstrual Period
LP	Lumbar puncture
MOH	Ministry of Health
NHTD	National Hospital for Tropical Diseases
NVP	Nevirapine
OI	Opportunistic infection

OPC	Outpatient clinic
OUCRU	Oxford University Clinical Research Unit in Vietnam
PC	Personal computer
PEPFAR	President's Emergency Plan for AIDS Relief
PI	Principal Investigator
PLHIV	People Living with HIV
POC	Point of contact
QALY	Quality-adjusted life-year
SAE	Serious adverse event
SOC	Standard of care
SOP	Standard Operating Procedure
TB	Tuberculosis
TDH	Tropical Diseases Hospital
TmAg	*Talaromyces marneffei*
USD	United States Dollar
VAAC	Viet Nam Administration of HIV/AIDS Control
WHO	World Health Organization

Protocol Summary

Title: **Vietnam Cryptococcal Retention in Care Study (CRICS).**

Purpose: This is a multicenter prospective cohort evaluation of the implementation of a cryptococcal antigen (CrAg) screening program at selected outpatient HIV clinics (OPCs) and network laboratories in Vietnam. The project will be implemented in 2 phases;

Phase 1: From August 2015 to March 2017 [projected], HIV-infected patients who present for HIV care and undergo CD4 testing will be reviewed to determine the proportion of newly presenting patients with advanced disease (CD4 ≤100 cells/μL). Reflex CrAg screening will be performed using Lateral Flow Assay (LFA) for those with CD4≤100 cells/μL, per Vietnam national guidelines.

Patients with CD4≤100 cells/μL who present for ART at a study OPCs—CRICS Sites— will be recruited into the longitudinal study and followed up with assessments and the collection of routine and supplemental data for 12 months or through September 2017 (whichever comes sooner).Those who are CrAg-positive, but have no features of central nervous system (CNS) disease, will be treated with high-dose fluconazole. Those with symptoms of CNS disease will be treated according to national guidelines. Survival, retention in care, and other clinical outcomes will be documented for patients who test CrAg-positive and are treated with fluconazole and those who test CrAg-negative. Data from those tested at participating labs but not eligible for enrollment in the longitudinal study will contribute to estimation of the prevalence of CrAg.

Phase 2: From April 2017[projected] to September 2017, we will perform a cost and cost-effectiveness analysis of CrAg screening, and continue screening as a routine service at existing sites and expand to additional sites (preferentially to hospitals affiliated with Phase 1 OPCs and to other OPCs whose CD4 testing is conducted at laboratories already conducting CrAg screening as part of Phase 1). CrAg tests will also be made available to screen all patients with CD4≤100 cells/μL including those who are treatment-experienced. The test will also be made available for use among symptomatic patients for diagnostic purposes, including CSF and bloodtesting. We will monitor prevalence at each testing site, but screened patients will not be enrolled in longitudinal follow-up. Phase 2 will last for at least 6 months based on availability of funding and fluconazole for those who screen CrAg positive and the availability/stability of CD4 testing. [Note that follow up of patients enrolled in Phase 1 will continue during this time period, but is considered to be part of Phase 1 rather than Phase 2. Also, sites included in Phase 2 may

change over time as a results of the instability of CD4 testing (e.g., if participating laboratories stop conducting CD4 testing, those sites might no longer be included; if participating laboratories begin CD4 testing for other sites, those sites might be included).]

Objectives:

Primary objectives:

- To estimate the proportion of people living with HIV (PLHIV) who have advanced disease (CD4 ≤ 100 cells/μL) at presentation to HIV care;
- To determine the prevalence of CrAg-positivity among HIV infected patients with CD4 ≤ 100 cells/μL newly enrolled in care in Vietnam;
- To determine clinical outcomes including common causes of mortality for PLHIV with CD4 ≤ 100 cells/μL who are enrolled in a programmatic rollout of screening for CrAg;
- To estimate the 12-month mortality among two groups of HIV-infected patients with CD4 ≤ 100 cells/μL who are newly enrolled in care and treatment:
    - Those who are Crag-positive and are treated with high-dose fluconazole
    - Those who are CrAg-negative

Secondary objectives:

- To estimate the 12-month retention in care among two groups of HIV-infected patients with CD4 ≤ 100 cells/μL who are newly enrolled in care and treatment:
    - Those who are CrAg-positive and are treated with high-dose fluconazole
    - Those who are CrAg-negative
- To identify challenges associated with implementation of routine plasma CrAg screening in clinics providing HIV care.
- To disseminate lessons learned with participating sites to estimate the costs of implementing CrAg screening based on data collected at 22 OPCs participating in Phase 1and provider costs associated with cryptococcal meningitis (CM) treatment.
- To conduct an incremental cost-effectiveness analysis of CrAg screening compared with a standard of care (SOC)(no CrAg screening, and treatment for symptomatic CM only).
- To project potential cost savings from implementing CrAg screening and financial resources required to implement CrAg screening under different scale-up scenarios and for national rollout. To determine the prevalence *Talaromyces* (formerly *Penicillium*) *marneffei* antigenemia in stored sera and assess the impact of Talaromyces antigen (TmAg) positivity on mortality[1]

[1] This objective is part of a sub-study and study procedures related to these objectives have a separate funding source.

Study participants and sites:

HIV-positive ART naïve and experienced adults (>18 years of age) with a CD4 count ≤100 cells/μL or WHO stage 3 or 5 enrolling in HIV care at 22 or more outpatient clinics (see Table 2) in the North, Middle, and South of Vietnam (Phase 1), laboratories serving patients from non-CRICS sites; HIV positive ART-naïve and experienced hospitalized patients who have CD4 ≤100 cells/μL or WHO stage 3 or 4 disease or those with have symptoms of CNS infection (Phase 2).

Investigators and collaborating institutions

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CDC Project Officer

Aaron Zee, MPH. Aaron is the project officer for this study and will provide technical support during implementation of the project, review of reports generated, participate in abstract and manuscript writing and monitor compliance with USG guidelines

1. Introduction

1.1 Background on cryptococcal infection

CM is a fungal opportunistic infection (OI) that is one of the most common causes of death among human immunodeficiency virus (HIV)-infected persons [1]. In Southeast Asia, the prevalence of CM in HIV-infected patients presenting to care might be as high as 10-20%; an estimated 120,000 cases of CM and 66,000 deaths attributable to CM occur in SE Asia each year; this exceeds mortality from HIV-associated tuberculosis [1].CM occurs not only before a patient is on antiretroviral therapy (ART), but also afterwards, as an immune

reconstitution inflammatory syndrome (IRIS) [2, 3]. The incidence of CM has declined in some settings for patients who have access to ART [4]. Benefits of ART in patients with HIV will remain suboptimal as long as significant co-morbidities exist. CM is among the significant causes of poor patient outcomes and discontinuation of care. Linkage to care and retention in an ART program in South Africa following an episode of CM was poor, in part because of high rates of mortality[5].

Increasing access to ART, which has resulted in significant reduction in incidence of cryptococcal disease in some settings, has not uniformly reduced overall morbidity and mortality from CM in part due to late HIV testing and treatment and inability to diagnose the disease early. An estimated 17-30% of patients who develop CM do so after the initiation of ART[2, 3, 6], in the context of IRIS.

Screening HIV-infected patients for early cryptococcal disease by detecting circulating cryptococcal antigen (CrAg) and subsequently treating them with fluconazole shows promise as a public health strategy to reduce death and disseminated disease [7], and potentially improve patient retention in care. Studies have demonstrated that CrAg is present in peripheral blood weeks to months before the development of meningitis[8]. Treatment at this stage may be accomplished using oral fluconazole; treatment for meningitis, however, requires a toxic medication (intravenous amphotericin B) with substantial resource utilization (toxicity monitoring, frequent lumbar punctures (LP), and hospitalization). Screening strategies that detect CrAg in blood before clinical meningitis develops have been shown to be highly cost-effective in Cambodia, Uganda, and Vietnam[7, 9, 10]. In 2011, the World Health Organization (WHO) recommended that screening for cryptococcal antigenemia be considered in areas with a high prevalence of cryptococcal disease, among high-risk patients (adults with CD4 counts $\leq$100cells/μL)[11]. As a result, several countries are now investigating the feasibility of implementing CrAg screening as part of HIV care.

1.2 HIV and cryptococcal infection in Vietnam

The HIV epidemic in Vietnam remains concentrated primarily among three key populations: people who inject drugs, men who have sex with men, and female sex workers. As of 30 November 2012, HIV cases had been reported in all 63 provinces of Vietnam, 98% of districts and 79.1% of communes with an estimated 208,866 people living with HIV (PLHIV), 59,839 acquired immunodeficiency syndrome (AIDS) cases, and 62,184 AIDS-related deaths[12].

Data on the overall burden of OIs including CM and mortality in Vietnam is limited. Cryptococcus was reported as the most common OI among HIV-infected persons in a study of patients presenting to a single hospital in Ho Chi Minh City [13].In that same study, *Cryptococcus neoformans* was the leading cause of fungal meningitis and fungemia and was associated with the highest mortality rate (16.8%) among OIs in HIV patients [13].In a study in a northeastern province of Vietnam during 2007—2010, two of 49 AIDS-related deaths were attributed to CM[14]. However, the data on prevalence of CM has not been reported.

Only one retrospective study has evaluated CrAg prevalence in Vietnam. There appears to be some geographical variation across different regions; the estimated CrAg-positivitywas6% in the South and 2% in North Vietnam[10]. The prevalence in the middle of Vietnam has not been determined. The reasons for differences in prevalence of cryptococcal disease between regions in Vietnam are not known but could be related to environmental or host factors.

Vietnam is currently undergoing substantial changes in financing for and delivery of HIV services as funding is transitioned from international donors to national government sources. Additionally, as viral load monitoring is being rolled out, CD4 testing is being scaled down. Some laboratories now require patients to pay for CD4 testing, some have reported stock out of CD4 reagents or technical failures. Some laboratories have transitioned CD4 testing to other laboratories.

1.3 Diagnosis of cryptococcal infection in Vietnam

In Vietnam, the diagnosis of CM relies on clinical criteria, a positive cerebrospinal fluid (CSF) India ink stain or a positive CSF culture[15].However, clinics within Vietnam have variable abilities to conduct LP and CSF analysis. Historically, CrAg tests have not been utilized in countries such as Vietnam, due to cost and need for laboratory infrastructure and training.

In July 2015, the Vietnam MOH released new guidance on HIV/AIDS management which currently recommends cryptococcal antigen screening among ART naïve patients with CD4≤ 100 cells/μL and pre-emptive therapy with fluconazole for those who are CrAg (+) [16].

1.4 Rationale for study

In Vietnam almost 52.7% of PLHIV who started ART in 2010 had a CD4 count of less than 100 cells/μL[17], and were therefore at risk of development of severe OIs including CM.

Knowledge of OIs and mortality among patients presenting with late-stage HIV will be useful for clinical care and service delivery. In particular, implementing new prevention strategies for CM are critical to improve the success of HIV treatment programs.

The evaluation of this cryptococcal screening program will provide an assessment of the, survival, other clinical outcomes, and retention in care in of patients with CD4 count ≤100 cells/μL who test positive and negative for plasma CrAg; an estimate of the prevalence of cryptococcal antigenemia in Vietnam; and also an understanding of clinical outcomes in HIV patients with CD4 count ≤100 cells/μL.

We hypothesize that implementing plasma CrAg screening in clinics providing routine HIV care will enable identification of Vietnamese adult patients with advanced HIV (CD4 ≤100cells/μL) who have early cryptococcal disease, enable prompt preemptive treatment with high-dose fluconazole, and improve survival. The program will demonstrate the feasibility. The program will provide important programmatic information and economic data for the scale-up of CrAg screening and treatment in the rest of Vietnam and provide additional information on the burden of OIs and causes of mortality among patients presented with late-stage HIV.

2. Study Objectives and Outcome Measures

Table 1. Study objectives and outcome measures

Primary objectives	**Outcome measures**
1. To estimate the proportion of people living with HIV (PLHIV) who have advanced disease (CD4 ≤ 100 cells/μL) at presentation to HIV care	• Proportion of HIV-infected adults who have CD4 count ≤ 100 cells/μL
2. To determine the prevalence of CrAg-positivity among HIV-infected patients with CD4 ≤100 cells/μL	• Proportion of patients who are CrAg-positive among all HIV-infected adults who have CD4 count ≤ 100 cells/μL
3. To determine clinical outcomes including common causes of mortality for PLHIV with CD4 ≤ 100 cells/μL who are enrolled in a programmatic rollout of screening for CrAg	• Reported causes of death • Proportion of patients with HIV-related hospitalizations at 6 and 12 months • Proportion of patients with new AIDS-defining OIs/conditions at 6 and 12 months
4. To estimate the 12-month mortality among two groups of HIV-infected patients with CD4 ≤ 100 cells/μL who are enrolled in care and treatment: • Those who are CrAg-positive and are treated with high-dose fluconazole; • Those who are CrAg-negative.	• Twelve (12) month all-causes and CM-related mortality among patients who screen CrAg-positive and CrAg-negative

Secondary objectives	Outcome measures
5. To estimate the 12-month retention in care among two groups of HIV-infected patients with CD4 ≤ 100 cells/μL who are newly enrolled in care and treatment: • Those who are CrAg-positive and are treated with high-dose fluconazole • Those who are CrAg-negative	• Twelve (12) month retention among patients who screen CrAg-positive and CrAg-negative
6. Identify challenges associated with implementation of routine plasma CrAg screening in clinics providing HIV care	• Percentage of patients with CD4≤ 100 cells/μL who are lost to follow-up or have incomplete documentation • % of patients with no documented clinic visit 30, 60, and 90 days after date of the scheduled clinic appointment
7. To disseminate lessons learned with participating sites	• Reflection and transition workshop
8. To estimate the costs of implementing CrAg screening based on data to be collected at 22 participating OPCs participating in Phase 1 and provider costs associated with CM treatment.	• Total costs and unit cost per person screened, per CrAg+ treated by site, lab facility type, and cost component
9. To conduct an incremental cost-effectiveness analysis of CrAg screening compared with a standard of care (no CrAg screening, and treatment for symptomatic CM only).	• Incremental cost-effectiveness ratio (cost per CM death averted and cost per quality adjusted life year (QALY))

10. To project potential cost savings from implementing CrAg screening and financial resources required to implement CrAg screening under different scale-up scenarios and for national rollout.	• Total cost savings and amount of financial resources required to implement CrAg screening
11. To determine the prevalence of *Talaromyces marneffei* antigenemia (TmAg) in stored CRICS samples using the Mannose phosphate isomerase 1 (MP1) enzyme-linked immunosorbent assay (ELISA)	• Proportion of stored samples that test positive for TmAg
12. To assess the impact of TmAg positivity on mortality	• Six (6) and twelve (12) month all-causes and TM-related mortality among patients who screen TmAg-positive and TmAg-negative

3. Study design and use LFA in study and non-study sites

This is multicenter, prospective, cohort evaluation of a CrAg screening program among HIV-infected patients with CD4 ≤100 cells/μL who are newly enrolled in care at selected HIV OPCs in Vietnam, conducted in collaboration with the Vietnam Administration for HIV/AIDS Control (VAAC). The evaluation will be implemented in 2 phases.

Phase 1: Enrollment in the longitudinal study will take place from August 2015-March 2017 [projected approval of the protocol amendment for Phase 2]. In Phase 1, patients with CD4≤100 cells/μL who are newly presenting for HIV care at 22 outpatient sites in the North, Middle, and South of Vietnam have been screened for cryptococcal disease by testing their plasma for CrAg using LFA and enrolled into the study for 12 months' follow-up.

Phase 2 will start in April 2017 or when the protocol amendment is approved and will continue for up to 3-6 months. After 3-6 months, transition of the program will be discussed with VAAC for continued programmatic rollout. . During this phase we will perform a cost and cost-effectiveness analysis of CrAg screening and continue CrAg screening as a routine service without enrollment in prospective follow up. CrAg tests will be made available to screen patients with CD4≤100 cells/μL including those who are treatment experienced in selected existing and additional sites, and to screen hospitalized patients with CD4 ≤100 cells/μL and/or suspected CNS infections in hospitals affiliated with the CRICS OPCs. CrAg testing will also be used for diagnostic purposes among patients with symptoms or signs of meningitis. We will monitor the prevalence during Phase 2 but screened patients will not be followed up prospectively in the study. As CrAg screening is a WHO and Vietnam MOH national recommendation, it is considered SOC for persons with advanced HIV (CD4≤100 cells/μL), and reflexive testing does not require informed consent. Testing will be expanded during Phase 2 to screen other patients at selected Phase 1 and additional sites without enrolling them into follow up.

Use of LFA at study and non-study facilities and laboratories in Phase 2

CrAg screening will continue to be performed upon the completion of Phase 1. However, screened patients will not be followed prospectively.

To facilitate the implementation of CrAg screening nationally, laboratory staff and clinical providers will be made aware of the availability of LFA for screening and diagnosis. CRICS laboratories will use the LFA provided by the study to perform reflexive CrAg

screening with study LFA on specimens from patients with CD4≤100 cells/μL from OPCs included in Phase 1 and from other OPCs that are in their network, i.e., that routinely transfer specimens to these labs for CD4 count testing. LFA kits will also be used as needed for outpatient and inpatient medical wards affiliated with the participating laboratories for screening HIV patients with CD4≤100 cells/μL or with WHO stage 3 or 4 disease and for diagnostic purposes among patients with suspected meningitis.

During Phase 2, for patients who test CrAg positive, participating laboratories will inform the corresponding OPCs or inpatients wards of the results as soon as possible. The patient's primary providers will be responsible for discussing results with the patients and for prescription of fluconazole and follow up according to national guidelines. If the patient's OPC or hospital does not have fluconazole available (because of the current uncertainty of the supply of medications for opportunistic infections during the transition of financing from international donors to Vietnamese sources), fluconazole for identified CrAg positive patients will be provided by the study.

Numbers of tests and results will be documented through participating CRICS labs and tallied and will be used to in estimating the CrAg prevalence.

Enrollment in the follow-up study

In Phase 1, enrollment in the longitudinal cohort will be offered to eligible patients who have CD4≤100 cells/μL enrolled at and plan to receive ongoing outpatient care at one of the selected study OPCs (Table 2). Those who test plasma CrAg-positive and have no symptoms of meningitis will be treated with high-dose fluconazole. Study participants will be followed from the time of plasma CrAg screening for a minimum of 6 months and up to12 months depending upon when they are recruited with respect to the study expected duration. Routine clinical data will be abstracted from medical records and supplemental data will be collected to determine treatment outcomes and related factors. Fluconazole will be provided by the study for patients enrolled in the follow-up program during Phase 1 for the duration of the study. For those who tested CrAg-positive and are still receiving maintenance fluconazole at the end of their longitudinal follow up, fluconazole will be provided through routine sources.

In Phase 2,screened patients will not be followed up prospectively. However, fluconazole supply for identified CrAg-positive patients will be provided by the project if patients do not otherwise have access through their OPCs.

Definition of end points

Retention in care: Patient known to be alive and have visited the clinic in the prior 90 days.

Dead: Patient confirmed or reported to have died.

Lost to follow-up: Follow up will be described in two ways. From the time of enrollment in the study and from ART initiation (routinely collected through retention national indicators). Patients have not returned to the clinics > 90 days from the last clinical appointment for those who initiated ART and > 6 months for those who have not initiated ART without any follow up contacts with their OPCs, have not been reported or confirmed dead, or known to have transferred to other clinics.

4. Study population

Eligibility criteria for Phase 1.

4.1. Inclusion criteria:

- Aged ≥ 18 years (having passed 18th birthday using Western calendar)
- Confirmed HIV infection using National Testing Algorithm
- CD4 ≤100 cells/μL
- Able to provide written informed consent
- Enrolled at and plan to receive ongoing outpatient care at one of the selected study OPCs

4.2. Exclusion criteria:

- History of prior CM
- Receipt of systemic antifungal medication for more than 4 consecutive weeks within the past 6 months
- Receipt of ART for more than 4 consecutive weeks within the past year
- For CrAg-positive patients only: Known to be currently pregnant or planning to become pregnant during the study period

Eligibility criteria for phase 2

In Phase 1, patients were prescreened for eligibility ***to enroll in the longitudinal study***, and CrAg testing was only performed on patients who met study eligibility criteria (as above) and for whom the clinician filled out a CrAg requisition form as part of the study.

Phase 2, has no longitudinal component, and CrAg screening will be implemented in a programmatic manner. The national guidelines recommend "screening for serum *Cryptococcus neoformans* antigen (CrAg) in adults who have not received ART with CD4 count below 100 cells/μL." Thus, in phase 2, as per national guidelines, CrAg screening will be performed reflexively on all HIV positive ART Naïve and experienced adults with CD4<100 cells/μL or with WHO stage 3 or 4 who are enrolled for care at the CRICS OPCs or are receiving inpatient or outpatient care at a facility whose CD4 testing is conducted by one of the CRICS labs. CrAg screening will also be done if requested by providers based on CD4 or clinical criteria.

5. Study sites

Phase 1

Eligible patients will be enrolled consecutively from 22 participating OPCs. See sites by region and number of patients starting ART in 2013, an indication of the volume of patients at each site. Program data are not disaggregated by CD4 ≤ or >100cells/μL; approximately 1/3 to ½ are expected to have CD4 ≤100 cells/μL [16]. The sites have been purposefully selected to represent: (1) district, provincial or national level; (2) facilities able to provide primary or tertiary care; (3) rural, peri-urban, and urban location; (4) facilities with a sufficient number of patients; and (5) different capacity for diagnostic investigations. For logistical reasons, only two sites will be recruited from the middle of Vietnam, a reflection of the relatively lower prevalence of HIV and lower number of PLHIV and treatment facilities in this region. The prevalence of cryptococcal disease in this region has not been previously assessed and findings from these sites will help to determine if another prevalence study will be required.

Table 2.List of Phase 1 study sites by region and number of patients starting ART in 2013

	Name of OPC	Name of province	Region	Facility level of care	Funder	Estimated # of ART initiators in 2013
1	Tropical Diseases Hospital	Ho Chi Minh City	South	Provincial	PEPFAR	446
2	National Hospital for Tropical Diseases	Hanoi	North	National	PEPFAR	366
3	Binh Thanh District Outpatient Clinic	Ho Chi Minh City	South	District	PEPFAR	236
4	Health Center of Hoc Mon District	Ho Chi Minh City	South	District	PEPFAR	231
5	An Giang Provincial Hospital	An Giang	South	Provincial	PEPFAR	214
6	A Hospital	Thái Nguyên	North	Provincial	Global Fund	212
7	Bach Mai Hospital	Hanoi	North	National	PEPFAR	208
8	District 8 Hospital	Ho Chi Minh City	South	District	PEPFAR	183
9	Binh Chanh District Hospital	Ho Chi Minh City	South	District	PEPFAR	183
10	Thu Duc District Outpatient Clinic	Ho Chi Minh City	South	District	PEPFAR	171
11	Binh Duong Provincial Hospital	Bình Dương	South	Provincial	PEPFAR	164
12	Tan Binh Preventive Medicine Center	Ho Chi Minh City	South	District	PEPFAR	162
13	Vinh Phuc Provincial AIDS Center	Vĩnh Phúc	North	Provincial	Global Fund	162
14	Quang Ninh Provincial Hospital	Quảng Ninh	North	Provincial	PEPFAR	159
15	Tan Chau District Hospital	An Giang	South	District	PEPFAR	142
16	Thanh Hoa Provincial AIDS Center	Thanh Hóa	Mid	Provincial	Global Fund	140
17	Mai Son Hospital	Sơn La	North	District	PEPFAR	132
1	District 4 Outpatient Clinic	Ho Chi Minh City	South	District	PEPFAR	130
19	Nghe An Provincial Hospital	Nghệ An	Mid	Provincial	PEPFAR	128
20	Binh Tan Preventive Medicine Center	Ho Chi Minh City	South	District	Global Fund	110
21	Ha Dong Hospital	Hanoi	North	District	PEPFAR	97
22	09 Hospital	Hanoi	North	Provincial	National targeted program	66
	Total					4042

Phase 2: Types of study sites

For purposes of this study and as described above we have characterized three potential types of sites:

(i) **CRICS sites**- This includes the 22 OPCs participating in Phase 1. Based on number of CrAg-positive patients identified at given OPCs, resources, and logistics, CrAg screening may not continue at all 22 Phase 1 sites during Phase 2.

(ii) **Inpatient wards affiliated with the 22 OPCs**- These wards are often co-located with the OPCs. Patients with CD4≤100 cells/μL or with WHO stage 3 or 4 will be screened for CrAg at the discretion of their providers. Patients with symptoms of meningitis will be tested for diagnostic purposes (blood and/or CSF) regardless of CD4 count.

(iii) **Expanded OPCs**- Reflex CrAg screening will be expanded to additional OPCs (preferentially to OPCs whose CD4 testing is conducted at laboratories already conducting CrAg screening as part of Phase 1).

Participation of sites and patients might not be consistent during Phase 2:

- Because of transition of finances from international donors to Vietnamese sources and from CD4 to viral load monitoring, CD4 testing may be unstable:
    - Participating laboratories might temporarily run out of reagents
    - If CD4 machines break down, resources might or might not be available to repair them
    - Some laboratories might cease CD4 testing altogether

  When this happens, if a different laboratory assumes CD4 testing for a given OPC, CrAg screening at those OPCs might not continue if that laboratory has not been conducting CrAg screening. In these cases, CrAg screening will be performed when requested by the primary provider for those with WHO stage 3 or 4 or those with overt symptoms of meningitis.
- If a given laboratory assumes CD4 testing for multiple other laboratories, the burden of CD4 testing might preclude CrAg testing.
- Some laboratories might charge patients for CD4 testing. If the patient does not have health insurance or ability to pay for CD4 testing, individual patients might not receive CD4 testing (and then would not receive CrAg testing).

Such sources of instability will be monitored as factors related to feasibility of CrAg screening.

6. Sample size calculation:

The two primary outcomes of the study are 1) the estimate of the percentage of prescreened patients who have CD4≤100 cells/μL and 2) Of those with CD4≤100 cells/μL, the estimate of the percentage who are CrAg+. For these primary outcomes, data will come from both Phase 1 and Phase 2. From study initiation to October 31 2016 (approximately 13 months), we prescreened 2,612participants, and 1068 of these had a CD4 ≤100 cells/μL. Of those, 957 enrolled, and 25(2.6%) were CrAg+. Assuming a similar enrollment rate (74 per month), upon the completion of Phase 1 (March 2017), we will have prescreened 3,014 participants and would expect 1,232to have CD4≤100 cells/μL and 1,104 to be enrolled in the follow up study. Of these, we would expect approximately 29to be CrAg+.

For Phase 2 (presumably from February 2017 to September 2017), plan to prescreen an additional 1,808 patients. Taking both data from Phase 1 and Phase 2 into account, we would expect to prescreen 4,822 patients and to document 1,972 patients with CD4≤100 cells/μL. In total, we would expect approximately 46 patients to be CrAg+.

Table 3presents confidence limits for the two primary outcomes based on these assumptions and incorporating a design effect (DEFF) to account for the within-facility clustering of observations. The design effect is computed as [1+(m-1) ICC] where m is the average number of patients per facility and ICC is the intraclass correlation coefficient. There is no local data on which to base assumptions about the magnitude of the within-clinic correlation in Vietnam, but a recent publication reports intraclass correlation coefficients (ICCs) of about .02 or smaller for health-related variables in an HIV-positive clinic population in sub-Saharan Africa [4]. Taking data from Phase 1 into account only, for the first primary outcome, m is 137 (3014/22) and for the second primary outcome, m is 56 (1,232/22). Taking data from both Phase 1 and Phase 2 into account, m is 219 (4822/22) and is 90 (1972/22) for the first and the second primary outcomes respectively.

Table 3. Precision for primary study outcomes based on projected enrollment in Phase 1 (through March2017) and Phase 1 + Phase 2 (projected through September 2017)

Pre-screened	% with CD4≤100	Design Effect	95% confidence limits	Number tested for CrAg	% CrAg-positive	Number CrAg+	Design Effect	95% confidence limits
Phase 1 (projected through Dec 31, 2016)								
3,014	40.9	3.72	37.5, 44.3	1,232	2.6	29	2.10	1.30, 3.90
Phase 1 + Phase 2 (projected through September 2017 among 22 study OPCs only)								
4,822	40.9	5.36	37.7, 44.1	1,972	2.6	46	2.78	1.40, 3.80

Note: Confidence limits are inflated by a design effect of [1+(m-1) ρ] where m is the average number of patients per facility andρ is the intraclass correlation coefficient.

7. Study procedures

7.1. CrAg Screening Implementation (Reflex testing, return of results, communicating results and enrolment into the prospective cohort)

7.1.1. CrAg screening in study OPCs in Phase 1

As CrAg screening is a WHO and Vietnam MOH national recommendation, it is considered SOC for persons with advanced HIV (CD4≤100 cells/μL), and reflexive testing does not require informed consent. All adult patients presenting for ART evaluation and who provide a sample for CD4 testing will be educated about the risks of OIs such as CM and tested for cryptococcal antigen in accordance with national guidance. They will be specifically informed about CrAg screening prior to their blood draw using a short script (See Appendix 1: Screening , Appendix 2A: Script informing patients of CrAg screening (English)and Appendix).

Patients will be told that those with low CD4 whose CrAg results are positive will be contacted by the clinic to invite them back for an earlier appointment. Results of the CD4 and CrAg tests will be sent to the respective clinics within 24 hours of the tests, and made available to physicians (Appendix 15: CrAg Testing Request Form). Results of the CD4 and CrAg tests will be recorded daily into the evaluation logs at the participating laboratories and respective clinics (see Appendix and Appendix 16: CrAg testing log for the lab).

Once the clinic receives the test results, OPC staff will be responsible for contacting the patients who are CrAg-positive by telephone with the results within 24 hour sand request

them to return to the clinic for an earlier appointment. Three attempts will be made to reach the patient by phone or Short Message Service (SMS) and if not reachable, the patient will be seen at his routine scheduled appointment which is normally within two weeks of the date of CD4 testing. When patients with CD4 ≤100 cells/μL return to the clinic, they will be informed of the results of both the CD4 and plasma CrAg testing. Procedures for the prospective cohort evaluation and data collection will be described. If the patient agrees to participate, s/he will sign the consent form for enrollment (see Appendix 3A: Inform Consent Form (English)) and will be assigned a study identification (ID) number. This will be considered a study visit since in routine practice, patients only return for their CD4 at the next scheduled visit, usually 1-2 weeks after that date of sample collection.

7.1.2. CrAg screening in Phase 2

In Phase 2, CrAg screening will be performed as a routine service, and physicians will not prescreen patients according to study eligibility criteria. Rather, all routine laboratory requisition forms from the participants sites will be pre-marked with a note to the laboratory to perform reflex CrAg screening on any specimen with a CD4 ≤100 cells/μL. Providers will have a checkbox to mark the indication for the CD4 test on the requisition form, i.e., whether the CD4 test is being ordered for (1) new enrollment; (2) routine monitoring; or (3) presumed treatment failure. Laboratories will use the LFA provided by the study to perform reflexive CrAg screening with study LFA on specimens from patients with CD4≤100 cells/μL or signs/symptoms of meningitis or among outpatient or hospitalized patients with WHO stage 3 or 4 diseases at the discretion of the provider.

Results of the CD4 and CrAg tests will be recorded daily by the participating laboratories, and results sent to the requesting OPC or hospital along with the CD4 count. All laboratories participating in Phase 2 will record the CD4 count, CrAg result, and indication for CrAg test along with the code for the requesting site and other data that are routinely included on the requisition form (typically age and birth year). Because Phase 2 is being implemented programmatically, laboratories will have the choice whether to incorporate the CRICS data (e.g., CrAg result and indication for CD4) in their routine laboratory logs or to use logs provided by CRICS. The data will be collected by the project team on a regular basis.

Under Phase 2, participating CRICS laboratories are expected to inform the corresponding OPCs or inpatients wards, as well as the project team, of any CrAg-positive results as soon as possible. The patient's primary providers are responsible for prescription of fluconazole and follow up according to their standard care procedures and national

guidelines. Fluconazole supply for identified CrAg-positive patients will be provided by the study if not readily available for the patient at the participating sites. We will monitor the prevalence of CrAg positivity through labs without enrolling patients into the study.

7.2. Subject ID assignment (Phase 1)

The assignment of participant ID will be only applied in the study sites. Each patient enrolled in the evaluation will be given a subject ID. This subject ID will be independent of the patient's OPC ID, which is assigned to each individual when they are registered at the OPC. The subject ID will take the following format :<XX>-<YYY>

- XX: the letter code(s) assigned to each participating site using alphabetical letters.
- YYY: the number which is assigned to an individual patient. This part has three characters from 001 to 999.

Sequentially numbered subject IDs for each site will be printed and, after consent, assigned to each enrolled participant. A sticker with a pre-printed subject ID will be placed at the top of the first page of all case report forms (CRFs). Accurate records of assigned numbers will be kept and updated into Enrollment log at OPCs (see Appendix 14B: Enrollment Log for OPC) to ensure that no two patients are assigned the same subject ID. The study staff will be responsible for securing the patient's information and minimize the risk of re-identification of the subject.

In Phase 2, screened patients will be given a unique ID which is made of patient's name in abbreviation, age, and OPC code. This would allow the study team to identify patients with duplication of CrAg results and track patients with positive CrAg results for their uptake of fluconazole.

7.3. Management of a negative CrAg result

a. Phase 1: Patients CD4 ≤100 cells/μL who have a negative plasma CrAg result will be managed in accordance with national guidelines which include the assessment for other OIs, adherence preparation, and starting ART. They will be followed up for a minimum of 6 months and up to 12 months depending on the duration of the study period. Baseline data and follow-up data (2-.6-, and 12- month follow ups) will be collected on the enrollment and follow-up forms (see Appendix 4: Enrollment and Appendix 6: Follow-up).

b. Phase 2: Patients are not enrolled for follow-up. No clinical data will be collected apart from CD4 count, indication for CD4 test, and CrAg result. In addition, we will document number tested, number who are CrAg positive, and the respective CD4 counts.

7.4. Management of a positive CrAg result

a. Management of a CrAg-positive result - Phase 1

Asymptomatic patients

Patients with a positive CrAg test, and who consent to study participation will undergo routine examination at the OPC including assessment for presence of symptoms of meningitis (see Appendix). Patients who are plasma CrAg-positive but do not have symptoms of meningitis will be started on high-dose fluconazole as treatment for early cryptococcal infection.

The optimal antifungal regimen in the population with isolated plasma CrAg remains to be determined.[11] The WHO Rapid Advice recommends that patients with isolated plasma CrAg-positivity should be treated with fluconazole 800 mg/day (or 12 mg/kg/day up to 800 mg/day if aged below 19 years) for two weeks, then 400 mg/day (or 6 mg/kg/day up to 400-800 mg/day if aged below 19 years) for eight weeks, and continued maintenance with fluconazole 200 mg/day is recommended [11]. In this program, maintenance fluconazole will be continued until CD4>200 cells/μL for at least 6 months.

In Vietnam, the 150 mg tablet of fluconazole is the standard formulation and widely available for use in the national HIV treatment and care program for the treatment of CM. For this programmatic roll out, fluconazole dosage differs slightly from that recommended by the WHO recommendations but is in line with readily available formulations (150 mg tablets) in Vietnam, and with the updated national guidance [16]. The initial dosage of fluconazole in CrAg-positive patients therefore will be 900 mg taken each day for 2 weeks (WHO recommends 800mg). This will be followed by fluconazole 450 mg orally each day for 8 weeks. Finally, maintenance treatment with fluconazole 200mg orally each day will continue until CD4 >200 cells/μL for at least 6 months. In accordance with the 2016 WHO guidelines, if HIV viral load monitoring is not available, fluconazole treatment will be continued until patient is stable and adherent to ART and antifungal maintenance therapy for at least one year. If HIV viral load monitoring is available, fluconazole treatment will be continued until patient is stable and adherent to ART and antifungal maintenance treatment for at least one year and has a suppressed viral load. Maintenance treatment after 6 months will be supported by VAAC with dosage of 150 mg per day as same dose as recommended by national

guideline for treatment of cryptococcal meningitis during maintenance therapy. VAAC is responsible for coordinating ART and OIs drug throughout the country, and they commit to support a full supply of fluconazole for patients enrolled in the screening program. Study findings will inform supply chain and other fluconazole logistical issues. During treatment, development of adverse events (AEs) or side effects related to the use of this drug will be assessed during routine visits to each OPC and managed according to national protocols.

LPs in asymptomatic patients

In most study OPCs, asymptomatic, CrAg-positive patients will not be offered or referred for LP and CSF analysis since Vietnam National HIV Guidelines do not support the routine use of this invasive procedure in persons who do not have clear clinical features of a CNS infection [15]. Additionally, after discussions with multiple stakeholders at hospitals, conducting LPs on all asymptomatic CrAg-positive patients was felt to be unfeasible due to the limited availability of skilled clinicians and supplies for the procedure. However, patients who are CrAg-positive will be informed by the provider of the possibility of subclinical meningeal disease which can only be diagnosed by LP. If the treating doctors suggest LP to any patients who wish to pursue an LP outside of the program, they will be referred to an appropriate hospital. Study LFA may be used on serum or CSF as part of work up for patients with suspected meningitis.

Patients who refuse fluconazole

Patients who meet the indications for preemptive treatment with fluconazole but decline the treatment will have a one-on-one meeting with a designated clinic staff to ensure they understand the benefits of the antifungal therapy as well as the risks of not taking recommended treatment. If, after this meeting, patients still decline treatment with fluconazole, they will be strongly counseled about the need to return to the clinic for evaluation for symptoms consistent with cryptococcal disease. Patients will continue to be enrolled in the study and the collection of outcomes will continue.

Symptomatic patients

Plasma CrAg-positivity in a person with symptoms consistent with meningitis increases the suspicion for CM. If symptoms of meningitis (e.g., fever, headache, stiff neck) are determined by the treating physician to be present in a CrAg-positive patient, the patient will be referred to the nearest hospital capable of performing an LP and CSF analysis according to national protocols. Data from the hospitalization will be collected for analysis (Appendix 7:

Hospitalization); however, evaluation and treatment of study participants with symptoms of meningitis will be determined by the treating physician in accordance with national guidelines. Patients with laboratory-confirmed CM will receive induction, consolidation, and maintenance phase therapy as per the Vietnam National HIV Guidelines. These might include intravenous amphotericin B 0.7-1mg/kg/day or high-dose fluconazole for two weeks (induction phase), followed by fluconazole 900 mg/day for 8 weeks (consolidation phase). At the end of the consolidation phase, fluconazole 200mg/day will be prescribed as secondary prophylaxis until CD4 >200 cells/µl for at least 6 months or after viral suppression. This is according to the national protocol and is not considered part of this evaluation [15, 16].

Symptomatic CrAg-positive patients who refuse to have an LP or in whom an LP cannot be done will be offered the same treatment as laboratory-confirmed cases of CM, as outlined in the National Guidelines. If the patient refuses treatment for CM, they will be given oral fluconazole at the same dosage/length as asymptomatic CrAg-positive patients as a final option.

Patients admitted for treatment of CM will remain in the study, and the study poin to contact (POC) at the OPC will be responsible for keeping in contact with the hospital to monitor the progress and document the patient's outcome. The OPC staff will abstract information from the inpatient medical record and fill in the study Hospitalization Form (see Appendix)

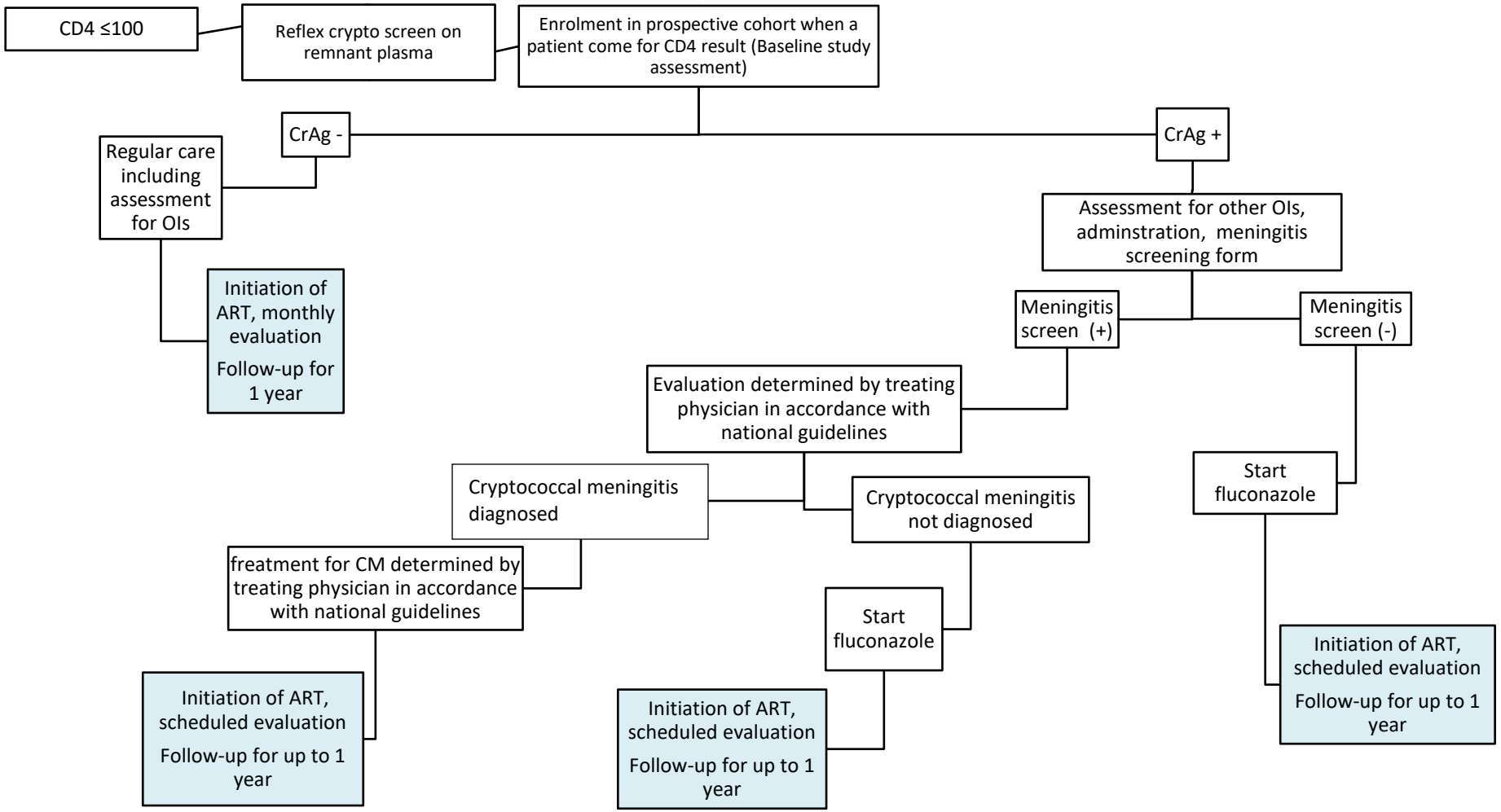
If, based on laboratory testing, the diagnosis of CM is excluded in patients suspected to have had meningitis (based on presence of fever, headache, or stiff neck) on initial evaluation, they will be treated by using the same protocol as asymptomatic CrAg-positive patients, i.e., started on fluconazole treatment for early cryptococcal disease (900 mg daily for 2 weeks) immediately after CM has been ruled out. This may be started during hospitalization (if patients remain hospitalized for other indications) or upon discharge from the hospital (if no other indication for hospitalization exists). All patients in whom CM is excluded should be provided with fluconazole upon discharge from the hospital so that treatment is not interrupted between hospital discharge and the next OPC appointment. After discharge, patients shall return to the OPC within two weeks to continue pre-emptive treatment as shown in Figure 1 below;

b. Management of a CrAg-positive result- Phase 2

Under Phase 2, upon the receipt of CrAg positive test results from CRICS labs, the patient's primary providers will be responsible for prescription of fluconazole and follow up according to their standard care procedures and national guidelines. Fluconazole will be donated to selected facilities for CrAg-positive patients if not otherwise readily available free of charge at the participating site.

Patients will not be enrolled in longitudinal follow up. No clinical data will be collected apart from CD4 count, indication for CD4 test, and CrAg result, and in CrAg-positive patients, whether fluconazole treatment was initiated. In addition, we will document number tested, number who are CrAg positive, and the respective CD4 counts.

Figure 1: Management of Patients Enrolled in the CrAg Screening Program in Phase 1



7.5. Fluconazole therapy

Toxicity

Fluconazole is a safe, widely used antifungal medication. The main side effects are nausea, headache, skin rash, abdominal pain, vomiting and diarrhea[18].In a study of high-dose fluconazole for HIV-associated CM in Uganda, fluconazole at both dosages of 800 mg/day and 1200 mg/day appeared to be well tolerated. No patients discontinued fluconazole therapy because of suspected AEs before completing 14 days of treatment. Although fluconazole can cause liver dysfunction, in this study there was no statistically significant difference between the 2 dosage groups in the percentage change in alanine aminotransferase (ALT) and serum glutamic pyruvic transaminase (SGPT) level [19].

Special populations

Pregnancy: Fluconazole, a US Federal Drug Administration Category D medication, has been shown to be teratogenic in the first trimester of pregnancy. For this reason, it is contraindicated during the first trimester. Further, the effects of prolonged high-dose fluconazole later in pregnancy have not been confirmed.

According to the Vietnam Guidelines for Diagnosis and Treatment [15]all ART-eligible female patients should have an initial assessment with obstetric, gynecological history and use of contraceptive methods documented. A urine pregnancy test is done based on need. For this study, a urine pregnancy test will be performed for all CrAg+ women of reproductive age (18–49 years old) at the enrollment visit and during follow up in those where pregnancy is suspected (last normal menstrual period (LNMP) is > 5 weeks). Pregnancy is an exclusion criterion for study enrollment of CrAg+ women.

Women who enroll in the study (Phase 1) will be given informed consent regarding the effects of fluconazole in pregnancy and its contraindication in pregnancy. During the consent process, they will be told that pregnancy testing will be required at enrollment and at any follow-up visits if they have missed a period. They will be strongly encouraged to use a modern contraceptive method during the course of the study, and will be told to stop fluconazole immediately and to come to the clinic if they suspect pregnancy.

At each study visit, for CrAg+ women of reproductive age, study staff will highlight the importance of not getting pregnant, the effect of fluconazole for pregnancy, and discuss contraceptive methods. Each of these women will be asked about her LNMP and her

contraceptive use. These data will be noted in the study records. If a female participant's LNMP is > 5 weeks before the current visit, a urine pregnancy test will be performed to rule out pregnancy.

Fluconazole will be discontinued immediately for women who become pregnant during the study period because of the drug's teratogenicity during the first trimester. Pregnancy in these study participants will be considered a serious adverse event (SAE), reportable to the institutional review boards (IRBs) and funder.

Other relevant contraindications: Patients with other relative contraindications will be advised accordingly. Patients with known liver disease, patients starting ART on anevirapine (NVP)-containing regimen, and patients on certain anti-tuberculosis (TB) medications will be given the following information and advised accordingly.

- **Patients with a history of liver disease**: Patients with cirrhosis, hepatitis, jaundice, or abnormal liver enzyme tests (>2x upper limit of normal) will receive careful monitoring for signs of liver damage after beginning fluconazole treatment. These signs include right upper quadrant pain, nausea/vomiting, and jaundice. If signs of liver damage are present, liver function tests will be ordered and evaluated.
- **Patients starting ART**: Patients on fluconazole starting ART will not be started on an NVP-containing regimen to avoid the synergistic hepatotoxicity of NVP and fluconazole treatment. For these patients, efavirenz (EFV) will be considered if there are no contraindications.
- **Patients with renal failure:** Fluconazole is primarily renally excreted, thus dosing in patients with impaired kidney function needs to be adjusted based on the patient's creatinine clearance. Discussion with a senior physician is recommended for patients who have impaired creatinine clearance to appropriately dose their fluconazole.
- **Patients with TB/HIV Co-infection**: Because both fluconazole and TB medications can cause hepatotoxicity, patients co-infected with TB will be started on an EFV-based rather than an NVP-based ART regimen. Patients will be monitored closely for signs and symptoms of hepatotoxicity such as right upper quadrant abdominal pain, nausea/vomiting, or jaundice. If there are signs of toxicity, then liver function tests will be ordered and evaluated and managed as outlined in the sections below.

Management of hepatotoxicity

Patients with hepatotoxicity will be managed according to the national guidelines**[15].**

- Management of hepatotoxicity when patients are on ART:
  - If ALT increases to grade 3 and the clinical status of the patients is stable, ART will be continued with less hepatotoxic drugs, such as EFV; with monitoring of ALT levels closely (every 2 weeks) and of the clinical symptoms;
  - HIV/HBV co-infected patients on 3TC, TDF will be monitored closely if they have to stop antiretroviral (ARV) drugs, for any reason.
- Management of NVP Hepatotoxicity:

Grade	ALT	Management
Grade 1(Mild)	1.25 - 2.50 times upper normal limit	Continue with NVP. Monitor ALT closely in every 2 weeks
Grade 2 (Moderate)	2.60 – 5 times upper normal limit	
Grade 3 (Severe)	5 - 10 times upper normal limit	**STOP NVP immediately**. Continue with other 2 drugs for 7 days, then replace NVP by EFV if ALT improves or If ALT is not improved, stop other 2 drugs. Restart ART only when ALT has improved; replace NVP with EFV-based regimen.
Grade 4 (Severe life-threatening)	> 10 times upper normal limit	Take into account both ALT and clinical signs and symptoms for appropriate management. The ARV regimen can be stopped, patient hospitalized, or referred to higher level. Restart ART with NVP substituted with EFV or TDF or LPV/r on case-by-case basis

7.6. ART

Since 2011, the recommended first-line prioritized ART regimen in Vietnam includes tenofovir, lamivudine, andoneno-nucleoside reverse transcriptase inhibitor (NNRTI), either EFV

or NVP. This regimen is used for all ART initiators by national guideline unless there are contraindications to any of the drugs[20].

The optimal timing of ART initiation among patients with isolated CrAg-positivity (no evidence of CM) has not yet been established. In this program, initiation of ART will be delayed by 2 weeks in those who are Crag-positive to enable completion of 2 weeks of pre-emptive therapy with fluconazole. This delay is intended to prevent development of IRIS. The ART regimen selected will follow national guideline with a preference for EFV over NVP. CrAg-negative patients will be evaluated at those clinics according to routine protocols and evaluated and initiated on ART, as appropriate.

For patients with CM, the optimal timing of ART initiation has also not been established. However, the Cryptococcal Optimal ART Timing Study (ClinicalTrials.gov identifierNCT01075152), conducted in Uganda, aimed to evaluate early ART initiation after CM diagnoses (7-11 days after receipt of amphotericin B) versus late ART initiation (5 weeks after receipt of amphotericin B). This trial was stopped early after interim results from the study's Data Safety Monitoring Board review showed increased mortality among patients who were in the early ART initiation group[21]. Thus, in this program ART initiation will be deferred for at least 2-5 weeks after the start of antifungal treatment in order to comply with the best evidence to-date and national guidance [16].

7.7. Schedule of visits – Phase 1

Under Phase 1, all enrolled participants will attend routine scheduled visits at the OPCs. Evaluation procedures at each CRICS's visit are indicated in the table and described in details in the sections below. There will be regular contacts by calls by OPC staff if a patient misses scheduled visits as a routine practice.

(Note that under Phase 2, patients screened for CrAg are followed according to the routine OPC procedures, and no additional follow-up visits are conducted or data collected for CRICS apart from whether CrAg-positive patients receive fluconazole.)

Visit 0 (V0) – Patients presenting for enrollment in HIV care during implementation period

Patient presenting for enrollment in HIV care at a participating clinic will be briefly informed about cryptococcal diseaseand CrAg screening on the remnant plasma from their CD4 count sample. (See **7.1. CrAg Screening Implementation (Reflex testing, return of results, communicating results and enrolment into the prospective cohort)**. For patients who have already had a CD4 test done in the2 weeks before the enrolment date, the remnant plasma from other routine tests ordered on the day of enrollment will be used for CrAg screening. If no other tests are requested on the day of enrollment, the patient will be asked to provide a fresh sample of blood for CrAg screening.

Baseline Study Enrollment Visit– All Patients with CD4 ≤ 100 cells/µL

On this visit, the patient will receive their CD4 count and plasma CrAg results. After having the study described to them and the opportunity to ask questions, patients will be asked to sign written, informed consent forms for participation in the study. A urine pregnancy test will be performed for women of childbearing age who are found to be CrAg-positive as described under special populations. A study ID will be assigned to those who have CD4 count ≤100cells/µL. A full physical examination will be performed and the Study Enrollment Form will be filled out (Appendix 4: Enrollment). Patients who are CrAg-positive and have no evidence of meningitis will be prescribed and provided with fluconazole treatment for early cryptococcal disease. This will consist of 900mg per day for two weeks. Patients will be supplied with enough fluconazole to last until their next clinical visit (2 weeks). Patients who are CrAg-negative will follow standard clinic care.

Patients who have symptoms suggestive of meningitis or other OI that requires hospitalization will be referred to hospital for evaluation as is routine practice in Vietnam. These patients will remain in the study and hospitalization data will be collected (Appendix 7: Hospitalization)

Follow-up Visits

- CrAg-positive patients without symptoms of cryptococcal disease will be seen back at clinic **after two weeks** for evaluation and changing of their fluconazole dose to 450 mg per day (to be taken for 8 weeks) and initiation on ART as per the evaluation guidelines (see Appendix 5: Starting ART Form). They will subsequently return to clinic for an

evaluation **eight weeks later,** during which time a dose reduction in their fluconazole to 200 mg per day maintenance dose will be completed.

- Follow-up visits for fluconazole dose changes should occur 2 weeks after fluconazole initiation and then 8 weeks after. If these do not coincide with routine OPC appointment, then non-routine appointments should be made. Patients should be told the importance of coming back to the OPC at those designated times for fluconazole dose changes. CRICS evaluation will be conducted and follow-up data will be collected at these 2 types of follow-up visits.
- CRICS evaluation and follow-up data collection should also be collected at routinely scheduled OPC visits that occur at ART initiation (if not at one of the above follow up visits), 2-, 6- and 12-months after ART initiation. Therefore, all enrolled CrAg-positive patients will be re-evaluated at 2-week and 10-weekafter fluconazole initiation and 2-, 6-, and 12-month follow-up after ART initiation (referred to as CRICS's visit). The specific date of these CRICS's visits might be decided in consideration of the patients' regularly scheduled clinic visit. In these visits, a short follow-up form (see Appendix 6: Follow-up) which incorporates information on current symptoms, side effects of fluconazole (if they are on fluconazole) and other clinical outcomes should be filled out.
- CrAg-negative patients will be re-evaluated at routinely scheduled visits for ART initiation and 2-. 6-, and 12-month follow-up visits. The follow-up data collection form should be filled for each of these visits.
- All visits will be documented using Appendix 18: Patient's visit logbook

Table 4. Schedule of select procedures and tests

Procedure	**Pre-Study**	**V0**	**Baseline visit (Study enrollment)**	**Follow-up visits**
HIV testing	X			
CD4 testing*		X		X (6, 12 months)
Viral load testing*				After 6 and 12 months on ART (If available)
Patient informed about availability of Crag screening		X[a]		
Patient provided with CrAg test result			X[a]	
Written consent			X[a]	
A urine pregnancy test			X[a](for CrAg+ women)	X[a] (for CrAg+ women whose LNMP is more than 5 weeks prior)
Enrollment Form			X[a]	
ART initiation form				At the time of ART initiation as recommended by national guideline
Health-related quality of life Form			X[a]	At baseline, 6- and 12- month follow-ups [a]
Follow-up Form				For CrAg-positive patients: At 2-week[b], 10 week[b], 2-[a], 6-[a] and 12 month [a] follow-ups For CrAg-negative patients: At 2-[a], 6-[a] and 12 month [a] follow-ups
Starting preemptive therapy of fluconazole-patient with CrAg (+)			X[b]	

Reducing dose of fluconazole				X[a] (At the first day of 9th week)
Off-study Form				X[b] (12 months after ART initiation)

[a]Study-related procedures, but conducted on patients' routine visits for HIV care.

[b]Study-related procedures for CrAg-positive patients that may occur at a time separate from a routine OPC visit.

*-MOH is in the process of implementing routine viral load monitoring. Results from CD4 and viral load test that are conducted routinely will be collected for CRICS.

Minimizing loss to follow-up

For patients who cannot be reached for a follow-up appointment or miss their follow-up appointment, a study-related red-coloured sticker will be placed on medical records to remind study staff at OPC of scheduled routine clinic visit or study visit. The note will be addressed to the nurse or physician and will be placed in the front of the file reminding them to inform the social worker or other assigned staff who routinely contact patients who miss appointments. We will collect information about routine attempts to contact the patient and immediate outcomes of each attempt will be documented on a study form (see Appendix 8: Late Attendance, Missed Appointment).

Health-related quality of life (HRQOL)

Since assessment of clinical outcomes (OIs, immune status CD4, survival rate) might not be reflect non-medical aspects of PLHIV, such as interpersonal, mental health and social functions, health-related quality of life will be used to measure the change in theses dimensions among participants. In this study, we employ EQ5D-5L (The EuroQoL-5 Dimensions-5 Levels*)* for measuring health-related quality of life of patients. This measurement provides a health profile and a single index value for health status, which includes five dimensions: Mobility, Self-care, Usual activities, Pain/Discomfort and Anxiety/Depression. EQ5D has been used among PLHIV in Vietnam, and shown to be valid for measuring HRQOL for PLHIV[22]. Moreover, EQ5D also provides a health utility score that is very useful for evaluating cost-effectiveness of the intervention. EQ5D will be assessed at the time of enrollment and every 6months (at 6- and 12-month follow-ups) upon patient's choice. The HRQOL Form will be filled after follow-up form

(see Appendix). For patients who enrolled before HRQOL questionnaire was used, the EQ5D will be assessed only at 6- and 12-month follow-ups.

8. Study Implementation

8.1. Crag screening program launch meeting

A meeting will be held to launch the screening project and to discuss implementation. The meeting will be attended by investigators, representatives from all sites, representatives from Centers for Disease Control and Prevention (CDC), other United States government agencies, other donors and implementing partners and will be chaired by VAAC.

8.2 Pre-study training

A training course is mandatory for the study implementation. It will be conducted before study implementation begins and after the launch meeting has been done. During implementation of the study, refresher training will be conducted in order to maintain competencies in the study protocol and address gaps in care and difficulties that arise in implementation.

Clinician and laboratory staff training

1. Before training, each participating OPC, laboratory, and hospital will be asked to select a POC person who will be in charge of coordinating the cryptococcal screening program at their site. Although others at each site may additionally be involved in training, the POC will be responsible for communication between their site and the other sites (OPC, lab, and hospital), and ensuring that data are collected appropriately and in a timely fashion. Additionally, a back-up POC will be identified at each site, in the event that the POC is away, leaves the facility, or cannot fulfill their duties.
2. Training for both clinicians and laboratory staff will be carried out in parallel in Hanoi and Ho Chi Minh City by the investigators and subject matter experts from CDC-Vietnam, CDC-Atlanta, and NHTD. Initial trainings will take place prior to the implementation of the cryptococcal screening program with planned refresher trainings as needed to account for personnel turnover and quality assurance.
3. Clinician training will include outpatient-site training at the participating OPCs, and

inpatient-site training among referral hospital. Training will be conducted for each OPC and any relevant hospital staff. The following topics will be covered:

a. Basic information about cryptococcal disease, including early and disseminated infection
b. Explanation of the rationale behind the implementation of this screening program
c. Review of the algorithm for implementation
d. Role-playing using different clinical scenarios in order to ensure proper adherence to the algorithm and flow of patients.
e. Good clinical practice

4. Laboratory training will be inclusive of both outpatient participating laboratory staff serving the OPCs participating in the implementation of this screening program, and in-hospital laboratory staff who will be performing CrAg LFA testing on CSF specimens at Tropical Diseases Hospital and National Hospital for Tropical Diseases. Training for laboratory staff will include:
    a. Instructions and practice in performing and interpreting the LFA CrAg test
    b. Review of the protocol for communicating CSF CrAg results to OPCs
    c. Reviewing the standard operating procedures (SOP) portions relevant to the laboratory staff.

Training of all laboratory and clinical staff at all OPCs and laboratories will occur in a two-week period preceding the program roll-out; in-country staff from NHTD, TDH, and CDC will return to the node laboratories, OPCs, and hospitals to provide refresher training as needed. The refresher training and training for new study staff will be performed on site as needed.

8.3. Additional training in Phase 2

Training for relevant staff from network laboratories, non-CRICS sites and affiliated inpatient wards will be conducted by the investigators and experts from CDC-Vietnam, CDC-Atlanta, NHTD and TDH, and other local experts as relevant. The training contents will cover:

- Screening procedures, roles and responsibility of each party in Phase 2
- How to communicate CrAg results with OPCs and handle CrAg-positive results
- Data collection

- Reporting requirements regarding the number of screened patients and CrAg+, as well as the any factors that affects the stability of CD4 testing (e.g. the availability of reagents, the functioning of CD4 count machines and any changes to the list of sites that the lab is conducting CD4 testing for) on a regular basis.

8.4 Monitoring visits

a. Phase 1

Every 1-2months, the study coordinators/research associates/research assistant/data manager or other study staff will visit each of all study sites to supervise the study and collect data. Every six months, the regular visit will also includePrincipal Investigators or lead-coordinators and refresher training will be included as needed. The monitoring tasks include:

- Checking that patients with CD4≤100 cells/μL have all been screened for CrAg
- Checking the number of newly-enrolled patients
- Checking if all patients enrolled since the last clinical research associate (CRA) visit have signed the informed consent forms.
- Checking that fluconazole dosage of CrAg-positive patients is being changed according to protocol (section 7.4)
- Checking that all CRFs are properly filled.
- Checking the number of newly enrolled patients.
- Recording all emerging issues during implementation, including ascertainment of changes to routine CD4 or viral load testing due to financing or other reasons.

b. Phase 2 labs will be the focal point of contact in Phase 2 as all study activity related to CrAg screening and data collection will take place at labs. Thus, lab monitoring visits will be conducted on a monthly basis. The purpose of such monitoring visits is to:

- The number of CD4 tests performed and number of tests having CD4 count ≤100 cells/μL
- The number of patients tested for CrAg and those with positive CrAg results
- Obtain an updated lists of OPCs and sites that are served by the lab
- The availability of reagents, the functioning of CD4 testing machines, and any factors that might influence the lab's ability to perform CD4 testing

- Working closely with lab staff to ensure regular updating the expansion progress and timely data collection.

8.5Management Meetings

A meeting of the study management team will be held annually after implementation of the program. The meeting will involve the PIs and site investigators. Other meetings will include:

- A quarterly teleconference with all investigators
- A monthly meeting with the study investigators in Vietnam

The topics will include:

- Reporting the progress of implementation.
- Reporting emerging issues during implementation.
- Proposing solutions and recommendations for the remaining period.

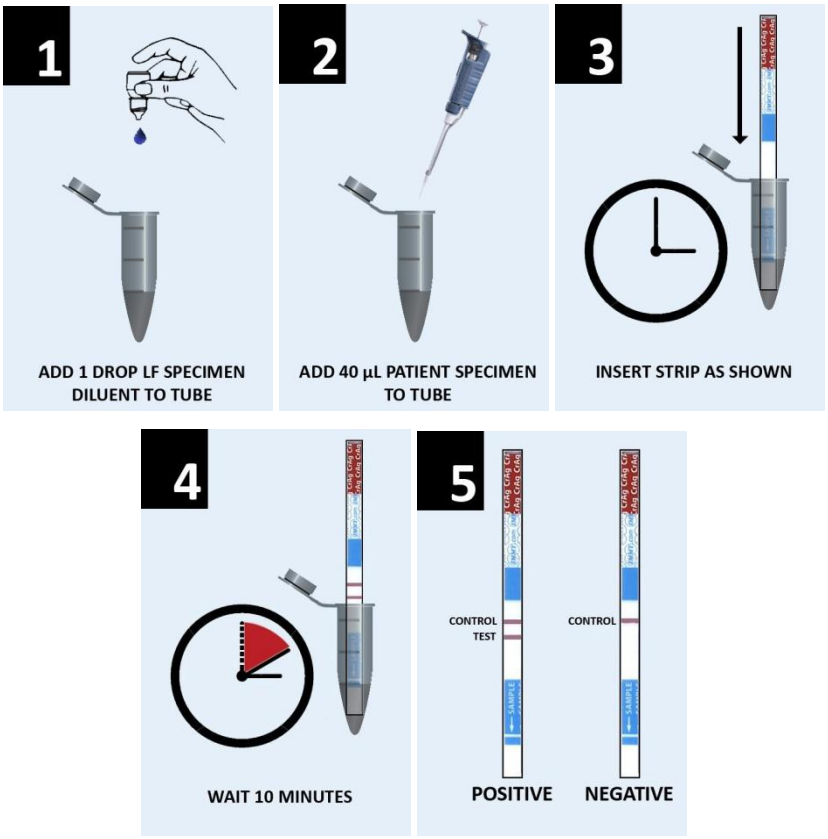
9. Specimen collection and processing

9.1. Sample collection

- Blood specimens will be collected from all patients at the time that routine CD4 count testing is being done. As mentioned above under visit 0, patients who have already had CD4 tests done in the2 weeks before the enrolment date, the remnant plasma from other routine tests ordered on the day of enrollment will be used for CrAg screening. If no other tests requested on the day of enrollment patients will be asked to provide a fresh sample of blood for CrAg screening.
- CSF specimens will only be collected in patients with suspected meningitis on the basis of symptoms and who consent to LP.
- Urine samples for pregnancy tests will be taken from CrAg+ women who are within the reproductive age group (under 55 years) at the enrollment visit and at follow-up visits for those whose last normal menstrual period began > 5 weeks prior.
- Collection, transportation and storage of specimens will follow national CD4 testing guideline.

9.2. CD4 and CrAg LFA testing

The CrAg LFA is a dipstick sandwich immunochromatographic assay. Specimens and specimen diluent are added into an appropriate reservoir, such as a test tube, and the lateral flow device is placed into the reservoir. The test uses specimen wicking to capture gold-conjugated, anti-CrAg monoclonal antibodies and gold-conjugated control antibodies deposited on the test membrane. If CrAg is present in the specimen, then it binds to the gold-conjugated, anti-CrAg antibodies. The gold-labeled antibody-antigen complex continues to wick up the membrane where it will interact with the test line, which has immobilized anti-CrAg monoclonal antibodies. The gold-labeled antibody-antigen complex forms a sandwich at the test line causing a visible line to form. Positive test results create two lines (test and control). Negative test results form only one line (control). If a control line fails to develop then the test is not valid.



- Blood specimens for CD4 counts will be sent to the participating laboratory performing the CD4 count, with a request to test for cryptococcal antigen in those samples with CD4 ≤100 cells/µL. Remnant plasma from all specimens with CD4 ≤100 cells/µL will be

tested for CrAg. If the patients already had CD4 ≤100 cells/μL within the last 2 weeks, specimens of the remnant plasma from other routine tests or additional blood draws will be used.

- In Vietnam, blood specimens for CD4 counts are kept in storage for quality control purposes as routine practice before being destroyed. For all patients who will undergo CrAg screening (those with CD4 ≤100 cells/μL), remnant plasma after of specimens with will be stored at -20°C until CD4 count result is returned to patients. The samples are only identified by a "serial laboratory number" and not names. We are referring to this as "***temporary routine storage.*** Standard logbook of CD4 testing will be kept at the laboratory and individual results will be returned to the OPC as MO mandates.
- When patients return to the clinic—usually within 2-3 weeks— to receive their CD4 results, those with CD4 ≤100 cells/μL will be consented for "enrollment into the study". Consent will also be sought for "storage of remnant plasma".
    a. Patients who consent for study enrollment and for sample storage: Out Patient Clinic (OPC) Staff at participating sites will inform the lab about sample storage for patients who have consented. Their samples will be retrieved from ***"temporary routine storage"***; a Study ID will be affixed and the laboratory number erased permanently. All samples will now be stored as "Study Samples in storage". No other identifying information will be recorded and kept at the laboratory.
    b. The OPC will inform the lab periodically if patients do not come within the scheduled time usually 2-3 weeks. Their samples will subsequently be destroyed by the lab following the laboratory protocols for disposal of biological specimens. These samples will not bear a study ID.
- The laboratory POC will contact the POC at the OPC by telephone each day with a list of patients and the results of CrAg testing. In addition, the laboratory will return the CrAg and CD4 test results as per their normal CD4 result notification process (e.g., paper laboratory reports delivered weekly).
- Each laboratory will keep a facility list of all patients that were screened for plasma Crag from each participating OPC and the results of the CrAg test. A copy of this list will be retrieved by the OPC during the regular collection of CD4 results.

- Any unused plasma specimen for CD4 counts and leftover CSF will be stored frozen upon patients' consent. All specimens will be securely stored at the NHTD for future testing for 10 years and will be destroyed by incineration after that. The specimens of participants who do not consent to long-term storage and additional testing will be discarded as using standard protocols. Approval for further testing will be sought prior to further testing from the relevant IRB.
- The leftover specimen from non-enrolling patients will be destroyed after CrAg testing without storage.
- **Quality control**: Participating laboratories will conduct internal quality control testing for the LFA reagents each day when samples are run or a new packet of kits is opened. CrAg-positive and negative control samples included in the kit will be tested to ensure the quality of the reagents and good testing practices. If the positive or negative controls do not yield the expected results, a new packet of LFA tests will be opened for use and the old packet discarded. If this does not result in appropriate positive and negative control results the matter will be reported to the study team for necessary corrective measures prior to further patient testing for CrAg.

  For CD4 count: Participating laboratories have participated in EQA program and performed IQC everyday of testing per national testing guideline to ensure quality of testing.

9.3. LFA in CSF during evaluation of CM

Clinical and laboratory training on the use and interpretation of the LFA in CSF will be conducted at participating hospital sites. For study participants who are referred to hospital for evaluation of meningitis, if an LP is done, the study will provide LFA for use in CSF in order to make a diagnosis of CM in study participants. Additional testing should be done per the hospital's routine evaluation for CM. As LFA CrAg testing is not available routinely for the diagnosis of CM in Vietnam, guidance on interpreting the LFA in the context of other CM diagnostic test (e.g., India ink and culture) will be included in clinical and laboratory training (**Table 5**).

Table 5: Guide to interpretation of CSF laboratory test results for the diagnosis of CM

LFA	India Ink	Culture	Interpretation
+	-/+	-/+	Positive for CM
-	+	-/+	Positive for CM
-	-/+	+	Positive for CM
-	-	-	Negative for CM

9.4. Quality Assurance for LFA and CD4 count

Training on quality assurance will be included into pre-study implementation training. Quality assurance site visits to the laboratory sites will be conducted by laboratory coordinators within one month after implementation starts, at least after one month, and then at least every three months to evaluate clinical and laboratory adherence to the SOP and laboratory technique. Laboratory quality assurance will be accomplished through:

1. Direct onsite monitoring of assay performance by observing procedural accuracy, workflow, and appropriate biosafety practices and through employment of personal protective equipment and universal precautions during specimen handling.
2. Proficiency testing (PT) via retesting of remnant specimens from consenting patients. Two remnant plasma or CSF specimens will be selected at each site, coded and retested using LFA and the results compared to initial test results as a measure of operator proficiency. If PT testing fails, refresher training will be provided.
3. External quality assurance (EQA) tools, comprised of dehydrated CrAg antigen tubes (positive, low positive, and blank) will be prepared for all participating laboratories. During assurance visits, EQA tubes will be coded and rehydrated in physiological buffer, then tested with the CrAg LFA to ensure test kit quality, operator proficiency, and inter-laboratory reproducibility.
4. EQA program for CD4 testing will be performed routinely following national CD4 testing guideline.

Other measures of quality control that will be monitored and assessed during these visits include effective clinician-laboratory communication and potential protocol drifts. Corrective actions and follow-up will be implemented as needed to assure quality patient service. CDC-Atlanta and CDC-Vietnam will assist with this basic program evaluation and provide input into

adjustments that will help improve process and flow of the CrAg LFA testing program implementation. A brief survey will be conducted among testers and personnel involved in the Quality Assurance program to evaluate.

9.5. Measurement of CrAg titres

The serial monitoring of CrAg may play a limited role in the management of HIV patients with CM[23]. In this study we will not be doing routine LPs on those who are Crag positive and serum CrAg titers will not be measured in real time but may be assessed on stored samples to evaluate correlation between the titers and clinical outcomes.

10. Data Collection

Phase 1

Overview

Demographic, clinical, treatment, and follow-up data will be obtained through chart abstraction into standardized CRFs. Data from CRFs will then be entered into Epi-Info (CDC, Atlanta).

- At enrollment, eligible patients will have an 'Enrollment Form' (see Appendix 4: Enrollment **Error! Reference source not found.**) filled out by OPC clinic staff. Baseline patient variables included in this form are listed in the section **"**10.1. Patient variables**."** Both CrAg-positive and –negative patients are also evaluated for quality of life (see Appendix 17: Health-related Quality of Life Form) on the day of their enrollment.
- ART initiation form (Appendix 5: Starting ART Form):
- A 'Follow-up Form' (Appendix 6: Follow-up **Error! Reference source not found.**) will be filled out for both CrAg-positive and –negative patients. All patients will be followed from enrollment for a minimum of 6 months and up to 12 months after ART initiation. The frequency of follow-up will be similar for both CrAg-positive and negative patients except for an additional visits to (i) reduce the dose of fluconazole to 450 mg and begin ART initiation 2 weeks after the baseline visit and (ii) for reduction of fluconazole to 200 mg 8 weeks after the start of that dose (Refer to 7.4. Management of a positive CrAg

result**).** Specifically, for CrAg-positive patients, follow-up forms should be filled out at 2-week after fluconazole, 10-week after fluconazole, 2-, 6-, and 12- month follow-ups after ART initiation. Whereas, for CrAg-negative patients, follow-up forms should only be filled out at 2-6-, and 12- month follow-ups after ART initiation. Follow-up patient variables are listed in the section "up. The history of appointment in all patients and history of fluconazole pre-emptive treatment among CrAg (+) patient will be recorded using AppendixandAppendix 19: Fluconazole preemptive treatment log respectively.

- Patients' quality of life will be re-evaluated at 6- and 12-month follow-ups after ART initiation.
- If a patient requires hospitalization at any point during their 12-month follow-up period, data about hospitalization will be collected in a 'Hospitalization Form' (Appendix 7: Hospitalization **Error! Reference source not found.**). The clinical staff at the study site from which the patient is transferred will take charge of collecting these data after the patient has been discharged or died in the hospital (Appendix 11: Off **Error! Reference source not found.**).
- An 'Off-Study Form' (Appendix 11: Off **Error! Reference source not found.**) will be used to collect data related to patient's status, reason for study termination and to assess endpoints.
- All follow-ups will be performed actively, to minimize patient loss to follow-up. Patients will receive a phone call one day before each scheduled visit to remind them of the appointment. These procedures are intended to minimize loss to follow-up and to ascertain patient status in those with a missed study visit at following enrolment into the screening program.

In this amendment, a number of additional items are added to Enrolment Form, Follow-up Form and Off-study Form (health insurance, date of first registration with the OPCs, number of hospitalizations, and source of causes of death report). Such information will be collected prospectively on those who are enrolled after the approved amendment only.

Phase 2

For Phase 2: patients screened for CrAg will be managed by their providers according to the routine OPC procedures, and no additional study related follow-up visits will be conducted. No

additional data will be collected for CRICS, but the following data will be abstracted from the laboratory form or other log; CD4 count, indication for CD4 test (baseline or follow up), CrAg result, and for those who test CrAg-positive, whether fluconazole treatment was initiated)

10.1. Patient variables

a. Phase 1

10.1.1. Individual variables – Baseline

Sociodemographics

- Gender
- Date of birth (DOB)
- Age (calculated field, if DOB is entered)
- Marital status
- Education level
- Occupation
- Monthly net income from all sources
- Distance from home to clinic
- Health insurance status

Medical and past history

- HIV-related (Date HIV testing/diagnosis, CD4 count, HIV Staging, OIs)
- Past history (diabetes, hypertension, kidney failure, heart failure, stroke, chronic obstructive pulmonary disease

Current symptoms:

- Headache, seizures, night sweat, vomiting, nausea, abdominal pain, constipation, diarrhea, jaundice, cough, dyspnea, blurred vision, skin rash, fever, photophobia

Performance status, Physical examination

- Vital signs: Glasgow coma score, pulse rate, blood pressure, temperature, respiratory, weight, height
- Chest examination, abdominal examination, neurological examination (neck stiffness, Kernig'ssign, cranial nerve palsies), visual examination, and fundoscopy

Laboratory tests

- Hemoglobin
- WBC, neutrophil %, lymphocyte %
- Platelet count
- Serum creatinine and serum urea
- Serum aspartate transaminase (AST/SGOT) and alanine transaminase (ALT/SGPT)
- Serum total bilirubin
- Hepatitis B surface antigen (HBsAg), anti- hepatitis C virus (HCV)
- Plasma CrAg test result
- Pregnancy test result

Initial management plan:

Referral to hospital for LP

- Initiation of fluconazole pre-emptive therapy
- Dose of fluconazole, formulation of fluconazole

10.1.2 Individual variables – Follow-up

Missed visits: Number of visits patients are supposed to attend and the actual number of visits they made

HIV Staging and OIs

Current symptoms: headache, seizures, vomiting, constipation, skin rash, fever, photophobia

Physical examination: Glasgow coma score, temperature, weight, neurologic examination, and skin exam, performance status

Laboratory tests

1. Hemoglobin
2. WBC, neutrophil %, lymphocyte %
3. Platelet
4. Serum creatinine and serum urea
5. AST/SGOT and ALT/SGPT

ART

1. Date of ART initiation

2. ARV drugs and dosage
3. ART adherence: using self-report questions and visual analog scale

Management plan:

1. Referral to hospital for LP?
2. Referral hospital

Fluconazole pre-emptive therapy

4. Current fluconazole dose
5. Timing current symptoms to fluconazole AEs
6. Fluconazole adherence: using self-report questions and visual analog scale

c. Phase 2

The following data will be abstracted from the laboratory form or patient record: CD4 test, CD4 indication, CrAg result, and whether fluconazole treatment was initiated for patients who test CrAg-positive. Sex and birth year/age will be collected when available in the routine laboratory data (e.g., from lab requisition forms).

10.2. Cost and cost-effectiveness analysis data

Retrospective program cost data associated with CrAg screening, pre-emptive fluconazole treatment and CM treatment will be collected using standardized input type categories. A cost assessment will be done to assist policy makers to understand the costs per patient screened, cost per CrAg+ treated and cost per person treated for CM. Cost drivers in CrAg screening and CrAg+ treatment will also assessed across 22 OPCs, CRICS laboratories, and inpatient wards at CRICS-affiliated hospitals. The findings will assist policy makers in both understanding the benefits and resources necessary to implement CrAg screening and provide CrAg+ treatment. These data will be useful for advocating with the Government of Vietnam for inclusion of CrAg screening as part of a package for patients with advanced HIV in the list of services for HIV patients that are reimbursed by social health insurance.

Data to be collected at all 22 OPCs, CRICS laboratories and inpatients wards at CRICS-affiliated hospitals include: cost information, beneficiary volume, and site characteristics. We will collect data on costs from existing data systems or interviews with facility staff. We will use

a standard method ingredient-based cost analysis to derive the total cost and unit cost. We will retrospectively collect at a minimum 12-months of data from each OPC, CRICS labs and inpatient wards at CRICS affiliated hospitals. The timeframe may have to expand to capture costs associated with CrAg screening and CrAg+ treatment, but will be within the study enrollment period at each OPC. If patients were referred to other facilities for CM treatment, data collection for treatment costs will also be conducted at those facilities. Given the small number of CrAg positives who developed CM within the project so far, in order to achieve a better estimate of CM treatment costs, data will be also collected at inpatient wards in CRICS-affiliated hospitals. Cost data will be collected from the programmatic perspective, including all sources of financial or in-kind support to provide the services to allow an accurate assessment of resource needs.

While all costs at the study site will be captured for analysis in this study, some resources will not be captured:

- Indirect costs associated with HIV and CM-related morbidity and mortality, as well as those stemming from AEs associated with care and treatment.
- Costs borne by the health system to increase the number of people who start and are retained on treatment.
- Higher-level overhead costs borne by government agencies to support the intervention.
- Out-of-pocket costs associated with care and treatment incurred by patients and their families.

In addition, descriptive data will be collected at each study site to determine if any site characteristics may affect costs. These data include:

- Site location
- Maturity (duration of operations for CrAg screening and CrAg+ treatment)
- Program model, encompassing aspects such as:
    - Staffing structure (e.g., nurses, doctors, administrative staff, etc.)
    - Capacity utilization
    - Comprehensiveness of other services provided at the study site

The cost study will be led by a local health economist who will be supported by a CDC health economist on data collection instruments and economic evaluation method. Data collection will be dictated by the data format and storage methods at the NHTD and at each site.

Data collection will be done by program activity and input type (Tables 5 and 6). As required, background calculations will be performed by the data collector to modify data into a standardized format so that they may be entered into the study instruments. Background calculations include but are not limited to: calculating annualized costs for capital investments and adjustments for mid-year salary changes. Data collection will be conducted by the local consultant and CRICS clinical research associates. Data entry will be performed at CRICS project office by data entry clerks or during fieldwork where appropriate. The data collection team will maintain control of hard copy and electronic versions of primary data, completed data collection forms, and undertake initial data cleaning and data quality control, in order to allow for rapid follow-up with site management and accounting staff for clarification where apparent errors, omissions or inconsistencies are found. Electronic data folders will be compiled for each site in preparation for analysis and will be integrated into the data collection for the rest of the CRICS project. These data will be used to develop a final data set that contains the final cleaned data. (Appendix 21: Cost study data collection tool).

Table 6. Programmatic Activity Categories
Training and Mentoring CrAg screening-specific training, mentoring and consultancies, continuing education of health care workers, personnel to perform CrAg screening.
Clinical Care Provision of CM (diagnosed and non-diagnosed) care and treatment services.
Lab Monitoring Provision of laboratory monitoring tests for CM negative and positive patients.
Monitoring through the information and reporting system (if applicable) Entering monitoring results in the system of information.
General Administration and Operations (if applicable) Management, administrative and maintenance activities at the facility.

Table 7. Input Type Categories

Recurrent Costs
Personnel The full cost of personnel employed for activities related to CrAg screening and CrAg+ diagnosis and treatment. This includes salaries or wages; employer share of taxes and fringe benefits; housing, transportation, and relocation support; signing and contract-fulfillment bonuses, and all other staffing expenses not captured in other categories.
Travel The cost of transport, accommodation, per diems, and other incidental expenses of staff travel for activities related to CrAg screening and CrAg+ treatment.
Drugs and Commodities The costs of medicines and other health commodities provided to patients as part of CrAg+ treatment services.
LFA Test Kits The costs of LFA test kits provided to patients as part of CrAg screening services.
Laboratory Supplies The costs of laboratory supplies expended as part of CrAg screening and CrAg+ treatment services.
Other Supplies The costs of other supplies expended as part of CrAg screening and CrAg+ treatment services.
Contracted Services (if applicable) The costs of activities contracted out to external service providers and not otherwise captured by other input type categories.
Existing Buildings The cost of using existing buildings for providing CrAg screening and CrAg+ treatment services.
Utilities The cost of utilities (power, water, electricity, etc.) expended for providing CrAg screening and CrAg+ treatment services.
Investment Costs

Training and Mentoring[2] CrAg and CM-specific training, mentoring, and continuing education of health care workers and other treatment facility personnel to support CrAg screening and CrAg+ treatment services.
Laboratory Equipment The cost of laboratory equipment of significant value with a useful life exceeding one year acquired for use in providing CrAg screening and CrAg+ treatment services.
Other Equipment The cost of other equipment of significant value with a useful life exceeding one year acquired for use in providing CrAg screening and CrAg+ treatment services.
New Construction and Renovation (if applicable) The cost of new construction or renovation of buildings for use in CrAg screening and CrAg+ treatment services.

In addition, cost data collected will be categorized by source of financial support (Table 8).

Table 8. Source of Support Categories
Vietnam Government Expenditures made by the Vietnamese government related to the CrAg screening and CrAg+ treatment services.
United States. Government Funding provided by the United States government for project implementation.

The data on beneficiary volume will be collected through the CRICS project. The data will include de-identified aggregate data by study site on:

- Number of patients screened for CrAg
- Number of CrAg+ patients
- Number of CrAg+ patients in treatment (classified by meningitis negative, meningitis positive, diagnosed CM and non-diagnosed CM)

[2]Training and Mentoring are considered as an input type as well as a programmatic activity in order to allow these costs to be clearly identified as investments.

In addition, we will use data from HRQOL collected in the CRICS project to estimate an incremental cost-effectiveness ratio.

Data management and ownership for cost data will follow the standard of operations for other components of CRICS project.

10.3. TmAg in Stored Samples (Sub-study)

Talaromyces (formerly *Penicillium*) *marneffei* is a dimorphic fungus that causes a life-threatening infection in immunocompromised individuals living and traveling in Southeast Asia, China, and India[24, 25]. Talaromycosis is a leading cause of AIDS-related deaths, trailing only TB and cryptococcosis, accounting for 4-15% of AIDS admissions in endemic regions[26-28]. Patients present very late in the course of illness, which has significant treatment morbidity and a mortality rate of up to 30%; treatment options remain limited[26-28]. The recent development of point-of-care cryptococcal LFA (such as CrAg®LFA) has allowed early diagnosis and prevention of CM by pre-emptive fluconazole therapy. The approach is shown to reduce HIV mortality[29], is cost effective in low- and middle-income countries[7, 10]and is in the treatment guidelines in 20 countries.

Stored samples from the CRICS study provides the perfect opportunity to retrospectively investigate the prevalence and clinical significance of TmAg, as the patient population at risk for cryptococcosis is also at risk for talaromycosis.

We will use a novel TmAg detection ELISA developed by the University of Hong Kong for screening[30]. The assay has been validated in the Oxford University Clinical Research Unit (OUCRU) laboratory in Vietnam in more than 600 HIV-infected patients with and without culture-confirmed talaromycosis. The ELISA has the sensitivity and specificity of above 90% and is more sensitive than blood culture in diagnosing talaromycosis (unpublished data). This assay was able to detect TmAg in 9.4% of more than 8,000 archived serum specimens from patients attending HIV clinics in Guangzhou, China[31]. Linked clinical data were not available in that study; therefore the clinical significance of TmAg could not be assessed. Mortality and other clinical outcome data from Phase 1 of the CRICS study will be used to assess clinical significance of antigenemia.

We hypothesize that the prevalence of TmAg in HIV-infected individuals with CD4 counts ≤100 cells/μL will be at least 5%, and that asymptomatic TmAg is associated with a higher all cause morbidity and mortality.

This study will provide further evidence for the MOH on the potential role of screening for TmAg (and pre-emptive itraconazole therapy for TmAg-positive patients), an approach that may substantially reduce the excess morbidity and mortality in patients starting ART in Tm endemic regions in Asia.

Nearly all patients enrolled in CRICS agreed to the storage of blood specimens for later use; stored samples are identified only by study ID number. All available specimens will be tested for TmAg by ELISA in the OUCRU laboratory in Vietnam. The assay requires 500 mcl plasma out of 500-1000 mcl stored samples.

The study team will match the specimen's ID with the patient's ID as much as possible to ensure that positive TmAg results are returned to the primary doctors who are responsible for the clinical care for the patients. Secondly, doctors in OPCs with positive TmAg patients will be notified about the number of positive tests among their enrolled patients so as to increase their index of suspicion for *T. marneffei* infection. We hope that this will encourage providers to conduct intensive screening for symptoms of Talaromycosis during subsequent patient visits.

Because TmAg is not the nationally approved laboratory test for diagnosis of *T. marneffei* infection in Vietnam, there is no official recommendation for treating asymptomatic patients who are TmAg positive. The decision on antifungal treatment will be made by the primary physicians based on local practice. The treatment regimen for those who are symptomatic will be determined by the primary physician on a case-by-case basis and will follow the standard of care at OPCs in accordance with national guidelines. Per the national guideline the treatment includes amphotericin B (0.7 -1,5 mg/kg/day) for 2 weeks, then itraconazole 200 mg bid (in children 5-6 mg/kg bid) x 8- 10 weeks) or for mild cases or when Amphotericin B is unavailable, itraconazole 200 mg bid x 8 weeks. Maintenance therapy includes itraconazole 200 mg/day in adults and

Funding will be contributed by OUCRU to test specimens for TmAg.

10.4.Datastorage

CRFs will be used as a data collection tools. Source documents which are generated during the study by the clinical staff will be kept at the study sites. CRFs and other project documents

will be stored in a restricted-access room that only investigators and study staff can access. At NHTD, all project documents and copies of CRFs will also be protected in a secure room or locked cabinet; and personal data existing in non-digital format including CRFs or signed consent forms, containing names, addresses and signatures will be stored separately from data in a separated locked cabinet. After completion of the study, all study documents kept at study sites will be transferred back to NHTD. All essential documentation for all study patients will be maintained in original paper format by the investigators in a secured storage facility for a minimum of 3 years and as required by local regulations thereafter. All stored records will be kept secure and confidential.

10.5. Data transfer

Every 1-2 months, original completed CRFs from nearby study sites will be securely transported to NHTD for data entry and returned to the site afterwards for storage. CRFs from remote sites will be photocopied by the OPC POC and sent to NHTD for data entry. The original CRF will be maintained at the OPC. The copies of CRF or original CRFs will be secured in a locked box and protected by study staff during transferring.

Following data entry, data will be stored in a personal computer (PC) secured with a password. A data entry officer will be responsible for data entry into the PC, using data entry software. The PC will have firewall protection and security-related upgrades and patches to operating systems to avoid viruses and malicious code. Only designated NHTD staff will be approved for access to the PC containing study data. It's required to encrypt data containing personal information before they are stored. Confidential data such as those containing personal information will not be stored on servers or computers connected to an external network, particularly servers that host Internet services. Sending personal or confidential data via email or other file transfer means without encrypting is also not allowed.

Besides, to reduce risk as far as possible, backups will be made after every change to data. There are at least two different forms of storage, for example on hard drive and on CD in case of accidental loss of data. Data files will be kept in portable computers, non-network computers and home-based computers.

10.6 Data ownership, governance, sharing, dissemination

The database and samples arising from this study will remain the property of NHTD and investigators. NHTD also carries responsibilities to protect confidentiality and the privacy of research participants. Access to certain data sets will therefore be carefully managed and granted in a transparent manner to all appropriately qualified researchers. The final study database will be made available to and will be archived at NHTD. Investigators not affiliated with this study who are interested in analyzing the study data, will submit a brief proposal that will be reviewed by a study team that includes at least one study investigator from NHTD and one from CDC.

In terms of TmAg sub-study, all TmAg-related data will reside at NHTD but will also be accessible all co-investigators from NHTD, CDC and OCRU.

10.7 Data quality assurance

Before sending to NHTD, the OPC POC will check the quality of all CRFs to ensure that all information has been properly collected and recorded. Data will be entered in an MS Access database. The MS Access sets up the tables before you can enter data and the data entry forms are incredibly flexible. There are different objects within the Access database file such as tables (which store the data), queries (which ask questions of the data), forms (used for data entry and editing) and reports (for summarizing data) – but there is just one single file (with the extension .mdb) so only one file to backup and/or pass to colleagues. Double data entry will be used to eliminate mistakes during data entry by checking for agreement with computer verification. The data manager at NHTD will provide feedback via phone calls and through coordinator's visits to the study sites regarding any errors or missing data on a monthly basis, but forms are only transferred to NHTD every 2 months. During data processing, the information will be checked again for completeness and consistency.

11. Data Analysis

Data analysis plan:

Primary objectives:

Proportions will be calculated and presented with associated 95% confidence interval (CI). Clustering effect will be taken into account when calculating 95% CI.

Secondary objectives:

Descriptive statistics (proportion, mean, standard deviations, median, and range) of all studied variables will be presented. Point estimates will be presented with associated 95% CIs. Chi square test will be used to compare proportions. T-test or ANOVA will be used to compare means. Logistic regression analysis will be used to examine factors associated with mortality.

In addition, proportional hazards analysis will be used to study time to deaths and to compare survival between HIV-infected patients with CD4 ≤100 cells/μL enrolled in this study and who test CrAg+ and a historical cohort of patients enrolled in prior studies who were not screened for cryptococcal disease at the time of enrollment, but who test CrAg+ on stored specimens. Clustering effect will be taken into account in all analyses. Stata version 13.0 (Stata Corporation, College Station, TX) will be used for all statistical analyses.

Cost and cost-effectiveness analysis

The prices of some inputs may need adjustments for inflation if purchase occurred in earlier time periods, and all costs will be converted to a common currency, United States dollars (USD) for analysis (final results will be reported as both USD and Vietnam Dong). The cost data collection described in the previous sections allow the calculation of the total cost of the study intervention described by programmatic activity, input type, and source of funding. The analysis will evaluate the full and incremental costs of CrAg screening and CrAg+ treatment services.

Donated or subsidized inputs are valued at their market value, and buildings are estimated as the amount of rent equivalent space in the local market. Likely shared costs are: buildings and lab equipment. Building costs would be shared based on an estimate of floor space. Lab equipment utilization data exist based on share of laboratory test runs by disease type. The cost analysis will be performed to evaluate the economic and financial costs. From the economic standpoint, the costs of capital investments (laboratory equipment and other equipment and civil/construction renovation) will be depreciated over the estimated cost of each item life, with a discount rate of 3%. Laboratory equipment will be assumed to have a lifetime of 7 years, while construction will have a lifetime of 20 years

Clean and adjusted data on program costs will be analyzed to reveal:

- Average cost per patient.

- Distribution of costs between the three categories described in Tables 5-7.
- Differences in cost per patient between locations, OPCs and lab facility types and how this may or may not be explained by facility characteristic data.

Specifically, programmatic activity costs will be aligned with patient data to calculate the following:

- Cost per person screened and subsequently tested and treated.

The incremental cost-effectiveness ratio (ICER) for each outcome (number of CM deaths averted and QALY) will also be calculated. The ICER adds value to the analysis by comparing the additional health benefit gained relative to the additional costs incurred when comparing the various interventions. The cost-effectiveness analysis is designed to answer the questions: "Is CrAg screening and associated CrAg+ treatment a cost-effective intervention to avert CM deaths and improve quality of life of HIV+ patients in Vietnam compared to SOC?"

For example, the ICER of intervention A compared to intervention B is calculated as the difference in total costs between the two modalities divided by the difference in effectiveness:

$$ICER_{(Intervention\ A|B)} = \frac{(Total\ Cost_A - Total\ Cost_B)}{(Total\ Effectiveness_A - Total\ Effectiveness_B)}$$

The ICER indicates the change in cost for one unit of outcome (i.e. the effectiveness measure) for different interventions. In this evaluation, the interpretation of the ICER will be the additional cost in dollars of CrAg screening and CrAg+ treatment compared to the SOC per CM death averted and QALY. The cost-effectiveness analysis will adopt the provider perspective.

Sensitivity analysis will be performed on key parameters in the model to assess the robustness of the results to changes in base values of effectiveness measures or intervention costs.

The cost data analysis, including the identification of cost drivers, sensitivity analysis or cost data, cost projection (financial resources requirements), and potential cost savings will be performed in STATA. The cost-effectiveness analysis will build on the exiting model developed by Smith et al.

12. Expanding implementation of CrAg screening: Feasibility

To contribute to future roll-out of CrAg screening, lessons learned and challenges encountered during study implementation will be captured as they occur. Key aspects will include documentation of the following:

- Training needs and materials for OPC staff
- Laboratory infrastructure for CrAg screening and diagnosis of meningitis
- Stability of CD4 testing during transition from international donor financing to Vietnamese sources and from CD4 monitoring to viral load monitoring
- Sample transportation
- Time for receipt of CrAg results
- Patient referrals
- Patient return for results
- Patient flow
- Patient acceptance of and adherence to fluconazole

13. Ethical considerations

13.1 Ethical review and informed consent

The invitation to participate in this Cryptococcal Antigen Screening Program will be sent to all sites (see Appendix 13: Invitation to participate in the Cryptococcal Antigen Screening Program). Ethical approval for this program rollout and evaluation will be obtained from the NHTD Ethical Review board and from the CDC IRB. The protocol, consent forms and other study materials will be submitted. Final permission to conduct the study will be obtained from the MOH.

Reflex CrAg screening on remnant plasma of all patients with CD4 ≤100 cells/µL: This testing is considered SOC as screening patients for plasma CrAg is included in the Vietnamese national guidelines and is also recommended by WHO[11].

- **Informed consent for prospective cohort (Phase 1):** Patients who return to the OPC for the CD4 result, will be explained and offered enrollment in the study if they meet

eligibility criteria (including CD4 ≤100cells/μL), regardless of CrAg result. The study staff will give the patient/legal representative a copy of the Informed Consent Form and explain the details of the study including the study procedures (including requirement for CrAg-positive women or reproductive age for pregnancy testing at enrollment and at any follow-up visits occurring >5 weeks after LNMP), risks and benefits, financial and confidentiality considerations, and how to obtain more information. The Consent Form will be in Vietnamese and will be translated and back-translated prior to study initiation to ensure the adequacy of the translation.

- Study staff will invite the patient to ask questions and will endeavor to ensure that he/she understands the information given. The study staff will then ask the patient to consider study participation. The consent will cover 1) recruitment of eligible patients into the prospective cohort; 2) abstraction of relevant information from patients' medical records at enrollment and during 12 months of follow-up; 3) permission to contact for reminder phone calls and tracking for those who miss routine appointments and 4) permission to store remnant specimen for future testing.

Both CrAg-positive and negative patients will be enrolled for study participation. One Consent Form and Appendix 3B: Inform Consent Form (Vietnamese)) will be used for consenting CrAg-positive and negative patients. Patients who provide written informed consent to participate in the study will sign and date two copies of the informed consent form. The study staff will also sign and date the two copies. One copy will be given to the participant; the other will be retained and stored securely (see Confidentiality below) at the clinic.

If at any point in the consent process the patient is judged by the staff to be unable to give written informed consent because of altered mental state or unconsciousness, a relative or legal representative will approached and told about the study. If the patient/representative is illiterate, a witness who is not a member of the study staff will be present during the informed consent discussion. The Informed Consent Form will be read to the patient/representative in the presence of the witness. If the patient/representative agrees to participate, the form will be thumb printed by the patient/representative and signed and dated by the witness in case the patient is unable to sign.

Those who refuse to give written informed consent will continue to be treated, as per the standard care at the clinic, but no data will be collected from them.

13.2. Waiver of consent (specific items)

There are a number of routine procedures and quality improvement practices as a part of clinical care from which this study will draw aggregate, de-identified data. Regardless of CrAg screening results, supporting the adherence to therapy through the help of families, peer groups, healthcare workers are recommended by the MOH in accordance with decision No. 3003/QD-BYT [15]. These items for which a waiver of consent is requested include:

- A home visit from a health or a community worker and phone contact if a patient misses his/her appointment is a part of routine practice at study sites. This occurs for patients who have missed an appointment and also for patients lost to follow-up to encourage the patient to return for care and to determine whether the patient has transferred to another clinic, defaulted on treatment, or died. Initially, the patients are to be contacted by phone or, if that fails, a home visit is to be made. We believe that these routine procedures are beneficial to all patients at the sites, not only study participating patients.
- Abstracting CD4 count and CrAg testing results in affiliated labs. The recommendation for CrAg screening was issued by the MOH in decision No. 3047/QD-BYT [16]. We believe that the routine monitoring of baseline CD4 to identify patients with advanced disease and of CrAg results will be beneficial to understand the prevalence of CrAg in select provinces and inform allocation of resources for differentiated care, including management of cryptococcal disease, for patients with advanced disease.

13.3Confidentiality

No names will be recorded on CRFs; CRFs will contain both study IDs and the client number from the OPC medical record. Data will be entered onto electronic databases on password-protected computers, which will only be accessible to study personnel. Only study ID and not the participant's OPC medical record number or name will be entered into the database.

13.4. Benefits/Risks

13.4.1 Potential Benefits to Patients

In 2011, WHO issued a conditional recommendation for routine screening for CrAg for PLHIV with CD4 ≤100 cells/μL in settings with a high prevalence of cryptococcal disease. Thus, the MOH aims to actively roll out routine CrAg screening in facilities providing HIV care. This evaluation will be implemented in the setting of program rollout. All patients with CD4 ≤100 cells/will be screened for CrAg and managed by their providers. Patients enrolled in this study will receive routine care recommended by their providers including high-dose fluconazole for those who are CrAg-positive and lack a diagnosis of CM. The fluconazole will be provided by the study for the duration of the study; and for any patients requiring fluconazole after the completion of the study, fluconazole will be provided by the MOH.

Although this study is being conducted in the setting of a program rollout under routine clinical conditions, enrolled participants may receive additional services such as reminder text messages and phone calls to ensure that they remain in care. This enhanced follow-up might reduce loss to follow-up and result in better care and outcomes for patients. If CrAg is detected, the patient will be treated with fluconazole, as recommended by WHO. The early detection opportunity can allow for earlier treatment of this infection, which is expected to improve the early mortality.

The participating patients will be reimbursed 65,000 Vietnam Dong [approximately US$3] for the extra time spent at the facility for study-related activities, including the time which was used to for answer the QALY questionnaire. Payment will occur at the time of enrollment (baseline visit), 6, and 12 months.

13.4.2 Potential benefits to the community

This project aims to evaluate CrAg screening in Vietnam. WHO recommends that screening for cryptococcal antigenemia be considered in areas with a high prevalence of cryptococcal disease, among high-risk patients (adults with CD4 counts ≤100 cells/μL) [11]. Information from this study will help inform decisions about expansion of CrAg screening throughout Vietnam.

13.5. Potential Risks to Patients

Risks from this program are minor. Plasma Crag-positive patients without CM will be receiving high-dose fluconazole as part of this study. Fluconazole, even at high doses (up to 1200mg/day) is well tolerated and safe. Common side effects include stomach upset, headache, and rash. Serious side effects such as liver toxicity are rare, and patients will be monitored closely for any AEs related to fluconazole.

13.6. Information dissemination

Programmatic and implementation lessons for individual sites will be shared with individual sites and with VAAC for purposes of quality improvement. Overall findings will be presented to the Vietnam MOH and relevant implementing partners to inform policy. The results from this research will be presented at local, regional, and international conferences and published in open source journals to reach a wider audience in countries with limited resources.

14. Safety monitoring and AE reporting

This is an implementation science research project investigating the operational issues with implementation of CrAg screening and pre-emptive therapy. No experimental procedures or medicines will be used in this evaluation. A slightly higher fluconazole dose than that recommended by WHO will be used in this study. However, higher doses have been used in previous studies. We will monitor AEs and report any that are severe. Patients will not be monitored prospectively during Phase 2. If the study team happens to learn of any unexpected SAEs that are related or possibly related to CrAg screening in Phase 2, they will be reported to the IRBs.

15. Sponsor Monitoring

As the study sponsor, the CDC may conduct monitoring or auditing of study activities to ensure the scientific integrity of the study and to ensure the rights and protection of study participants. Monitoring and auditing activities may be conducted by:

- CDC staff (“internal”)
- Authorized representatives of CDC (e.g., a contracted party considered to be “external”)
- Both internal and external parties

Monitoring or auditing will be performed by means of on-site visits to the Investigator's facilities or through other communications such as telephone calls or written correspondence. The visits will be scheduled at mutually agreeable times, and the frequency of visits will be at the discretion of CDC. During the visit, any study-related materials may be reviewed and the Investigator along with the study staff should be available for discussion of findings.

The study may also be subject to inspection by regulatory authorities (national or foreign) as well as the independent ethics committees and IRBs to review compliance and regulatory requirements.

AEs

An AE is any undesirable event that occurs to a study participant during the course of the study; that is, from the time of signing the Consent Form until study end (i.e., until the last follow-up visit at 12 months) whether or not that event is considered related to fluconazole or ART.

- Expected AEs:
  - Death due to CM
  - Symptoms of CM
  - Hospitalization for CM
  - Common ARV side effects
  - Death due to other OIs
- Unexpected AEs: Serious unexpected AEs that will be reported including potentially life-threatening reactions attributed to fluconazole and death (see Appendix 9: AE Reporting Form).

Relatedness of AE to fluconazole

Any AE that occurs will be assessed by the team physician to determine the relationship between the AE and the fluconazole. This relationship will be graded as follows:

- Unrelated: clearly explained by another, documented cause
- Unlikely related: more likely explained by a cause other than fluconazole
- Possibly related: may be related to the drug or to another cause
- Probably related: more likely related to the drug than to another cause

- Definitely related: direct association with fluconazole

SAEs

In this study, an AE is a SAE if it results in any of the following outcomes:

- Death
- Life-threatening event – this means that the participant was at immediate risk of death and required immediate medical intervention. It does not refer to an event which hypothetically might have caused death, if it were more severe.
- Hospitalization, prolongation of existing hospitalization, or re-hospitalization once discharged.
- Persistent or significant disability/incapacity (a substantial disruption of a person's ability to conduct normal life functions),
- An important medical event that may not be immediately life-threatening or that result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above.
- Pregnancy in a CrAg-positive woman taking fluconazole
- A congenital abnormality or spontaneous abortion

Patient Management of AE

Once an SAE is known, the research team should ensure that the participant receives or is referred for appropriate care. The participant should be treated by the treating physician and followed by the investigator until the abnormal parameter or symptom has resolved or stabilised. The physician should perform any tests that are clinically indicated as standard practice. The treating physician can stop fluconazole if he/she determines that a SAE is definitely related to this drug and is severe. If the SAE is judged to be possibly or probably related to oral fluconazole, the treating physician should consider and discuss with the PI the risks and benefits of continuing or stopping it.

AE Recording

Only certain AEs will be recorded on the CRF, including:

- SAEs

- Unexpected AEs

Reporting of AEs

All SAEs and unexpected AEs must be reported to the study team within 2 working days. The study team will review all SAEs urgently to determine expectedness and relatedness.
It is the responsibility of the Investigator to request all necessary documentation (e.g., medical records, discharge summary, autopsy) in order to provide comprehensive safety information and to assess whether the risks of study participation have changed.

Those SAEs that meet all three criteria of Serious, Unexpected, and Related or Possibly Related must be reported to the study team as soon as the event is recognized; the initial report to the IRB will be generated by investigators within 2 working days of site-awareness of the AE (see Appendix 9: AE Reporting Form **and** Appendix 10A: Clinical Study SAE Reporting **).** The information on cases of death will also be captured in database using Appendix 10B: Clinical information for cause of death

As above, patients will not be monitored prospectively during Phase 2. If the study team happens to learn of any unexpected SAEs that are related or possibly related to CrAg screening in Phase 2, they will be reported to the IRBs.

16. Budget

Will be submitted separately

17. Implementation schedule

Table 9: Study timelines

	2014			2015[a]			2016			~2017		
Months	1-4	5-9	10-12	1-4	5-9	10-12	1-4	5-9	10-12	1-4	5-9	10-12
Phase 1												
Protocol development												
IRB clearance (Atlanta/Vietnam)												
Preparation for data collection												
Hiring staff												
Training staff												
CrAg screening starts at all sites												
Phase 2												
TmAg screening												
Data Collection												
Data Analysis												

[a]Recruitment will continue for at least 18 months or whenever the minimum sample size is accrued

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List of Appendices

Appendix 1: Screening Form

Appendix 2A: Script informing patients of CrAg screening (English)

Appendix 2B: Script informing patients of CrAg screening (Vietnamese)

Appendix 3A: Inform Consent Form (English)

Appendix 3B: Inform Consent Form (Vietnamese)

Appendix 4: Enrollment Form

Appendix 5: Starting ART Form

Appendix 6: Follow-up Form

Appendix 7: Hospitalization Form

Appendix 8: Late Attendance, Missed Appointment Form

Appendix 9: AE Reporting Form

Appendix 10A: Clinical Study SAE Reporting Form

Appendix 10B: Clinical information for cause of death

Appendix 11: Off Study Form

Appendix 12: Clinical scoring tools and WHO clinical stating of HIV AIDS

Appendix 13: Invitation to participate in the Cryptococcal Antigen Screening Program

Appendix 14A: Screening log for OPC (under Phase 1)

Appendix 14B: Enrollment Log for OPC

Appendix 15: CrAg Testing Request Form

Appendix 16: CrAg testing log for the lab

Appendix 17: Health-related Quality of Life Form

Appendix 18: Patient's visit logbook

Appendix 19: Fluconazole preemptive treatment log

Appendix 20: Script informing patients of reimbursement

Appendix 21: Cost study data collection tool