Swaziland HIV Incidence Measurement Survey (SHIMS)

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Principal Investigators:

Rejoice Nkambule
Deputy Director of Health Services
Ministry of Health
PO Box 5
Mbabane, Swaziland
Phone +268 760763219
Email: nkambulere@gov.sz

George Bicego, PhD
CDC Country Director
PEPFAR Program - US Embassy
Mbabane, Swaziland
Office phone: +268 404 1158
Cell phone: +268 602 8664
Fax: +268 404 2885
Email: bicegog@state.gov

Jessica Justman, M.D.
Columbia University
Mailman School of Public Health
722 West 168th Street
Room 714
New York, NY 10032
Phone: (212) 342-0537
Fax: (212) 342-1824
Email: jj2158@columbia.edu

Jason Reed, M.D., M.P.H.
Global AIDS Program, HIV Prevention Branch
Centers for Disease Control and Prevention
1600 Clifton Rd. NE, MS-E04
Atlanta, GA 30333
Office: (404) 639-6269
Fax: (404) 639-8114
Email: jreed1@cdc.gov

Swaziland HIV Incidence Measurement Survey

TABLE OF CONTENTS

TABLE OF CONTENTS 2
LIST OF TABLES AND FIGURES 4
LIST OF ACRONYMS AND ABBREVIATIONS 5
PROTOCOL TEAM ROSTER 6
1.0 SCHEMA 8
2.0 INTRODUCTION 12
2.1 Literature Review/Background 12
2.2 Study Justification 16
2.3 General Study Approach 19
2.4 Audience and stakeholder participation 20
2.5 Rationale and intended use of study findings 21
2.6 Hypotheses 22
3.0 OBJECTIVES 22
3.1 Primary Objectives 22
3.2 Secondary Objectives 23
4.0 STUDY DESIGN 24
4.1 Study Timeline 30
4.2 How study design meets objectives and addresses research questions 30
4.3 Limitations to the study designs 31
4.4 Prevention effectiveness/cost benefit 34
5.0 STUDY POPULATION 35
5.1 Source of household-based study population 35
5.2 Case Definitions 35
5.3 Participant Inclusion/Exclusion Criteria 37
5.4 Justification for exclusion of sub-segments of the population 38
6.0 STUDY PROCEDURES 38
6.1 Pre-cohort Survey 38
6.2 Baseline and Post MC ASI Cohorts (A1 and A2) 41

6.3 Longitudinal high risk cohort ("B") 44
6.4 Participant Retention 47
6.5 Study Assessments 48
6.6 Study forms 49
6.7 Laboratory Procedures 53
6.8 Training of study personnel 54
7.0 STATISTICAL CONSIDERATIONS 55
7.1 Overview of Statistical Considerations 55
7.2 Sampling, including sample size and statistical power 56
8.0 DATA HANDLING AND ANALYSIS 60
8.1 Data Analysis Plan 63
8.2 Data Collection Methods 67
8.3 Information Management 67
8.4 Data Entry, Editing, and Management 68
8.5 Data Storage and Disposition 68
8.6 Data Ownership 68
8.7 Intermediate Reviews and Analysis 69
8.8 Quality Control/Assurance 69
8.9 Limitations of study 69
9.0 HUMAN SUBJECTS CONSIDERATIONS 70
9.1 Collaborating sites and institutional review 70
9.2 Consent process 70
9.3 Risks and Benefits 71
9.4 Confidentiality 73
9.5 Identifying, managing, and reporting adverse events (AEs) 74
9.6 Protocol Deviations/New & Unexpected findings/Changes to the study environment 75
10.0 DISSEMINATION, NOTIFICATION, AND REPORTING OF RESULTS 75
10.1 Notifying participants of their individual results 75
10.2 Notifying participants of study findings 75
10.3 Dissemination of study findings 76
11.0 REFERENCES 77
12.0 APPENDICES 80

Swaziland HIV Incidence Measurement Survey

LIST OF TABLES AND FIGURES

FIGURE 1: Modeled reductions in HIV incident infections averted among women following MC scale-up to 80% coverage of 15-49 year old men by year-end 2011 .....Page 16

FIGURE 2: Modeled reductions in HIV incident infections averted among men following MC scale-up to 80% coverage of 15-49 year old men by year-end 2011 .....Page 17

FIGURE 3: Short-term cohorts.....Page 23

FIGURE 4: Long-term prospective cohorts.....Page 24

FIGURE 5: All three cohorts.....Page 25

FIGURE 6: Cross-sectional survey .....Page 28

TABLE 1: Effective person years required to detect 30-60% difference between infection rates with 6 month follow-up in first cohort, 12 month follow-up in second cohort, for two sided alpha of 0.05, and 80% power. Wald test statistic for slope in Poisson Regression, under assumption of simple random sample.....Page 48

TABLE 2: Number of enrolled male participants, participating households and households in sample required to achieve the targeted effective number of person years. .....Page 49

TABLE 3: Number of enrolled female participants, given sample of 14,884 and 15,758 households in the pre- and post-MC ASI cohort.....Page 50

LIST OF ACRONYMS AND ABBREVIATIONS

AE	Adverse Event
AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral Therapy
ARV	Antiretroviral
ASI	Accelerated Saturation Initiative
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
CRF	Case Report Form
ECTIP	Emergency Care and Treatment Implementation Plan
EIA	Enzyme Immuno-Assay
GCLP	Good Clinical Laboratory Practices
GKOS	Government of the Kingdom of Swaziland
GPS	Global Positioning Systems
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
ICAP	International Center for AIDS Care and Treatment Programs
IRB/EC	Institutional Review Board/Ethics Committee
MC	Male Circumcision
MoH	Ministry of Health
MSM	Marginal Structural Models
NAT	Nucleic Acid Detection Test
PEP	Post-exposure Prophylaxes
PEPFAR	President's Emergency Plan for AIDS Relief
PITC	Provider-initiated opt-out HIV testing and counseling
QA/QC	Quality/Assurance/Quality Control
RCT	Randomized Clinical Trial
RT-PCR	Reverse transcription-polymerase chain reaction
SOP	Standard Operating Procedure
STI	Sexually Transmitted Infection
USAID	United States Agency for International Development
USG	United States Government
UNAIDS	United Nations Joint Programme on HIV/AIDS
VCT	Voluntary Counseling and Testing
WHO	World Health Organization

Swaziland HIV Incidence Measurement Survey

PROTOCOL TEAM ROSTER

	George Bicego, PhD CDC Country Director PEPFAR Program - US Embassy Mbabane, Swaziland Office phone: +268 404 1158 Cell phone: +268 602 8664 Fax: +268 404 2885 Email: bicegog@state.gov
Naomi Bock, M.D. Global AIDS Program, HIV Prevention Branch Centers for Disease Control and Prevention 1600 Clifton Rd. NE, MS-E04 Atlanta, GA 30333 Phone: (404)639-8134 Fax: (404)639-8114 Email: neb2@cdc.gov	**Muhle Dlamini, M.Sc, BSc** Prevention Coordinator (SNAP) Ministry of Health PO Box 1119 Mbabane, Swaziland Phone +268 76052293 Email: muhledlamini@gmail.com
Deborah Donnell, PhD SCHARP, Vaccine and Infectious Disease Institute Fred Hutchinson Cancer Research Center 1100 Fairview Ave. N., Mailstop: M2-C200 , P.O. Box 19024 Seattle, WA 98109-1024 Phone: (206) 667-5661 Email: deborah@scharp.org	**Dennis Ellenberger** Global AIDS Program, International Lab Branch Centers for Disease Control and Prevention 1600 Clifton Rd. NE, MS Atlanta, GA 30333 Phone: (404) 639-1016 Email: dxe1@cdc.gov
Tedd V. Ellerbrock, M.D., FACOG Global AIDS Program, NCHHSTP U.S. Centers for Disease Control and Prevention 1600 Clifton Rd. NE, MS-E04 Atlanta, GA 30333 Phone: (404) 639-8944 Email: tve1@cdc.gov	**Wafaa El-Sadr, M.D., M.P.H.** Columbia University and Harlem Hospital Mailman School of Public Health 722 West 168th Street Room 715 New York, NY 10032 Phone: (212) 342-0532 or (212) 939-2936 Fax: (212) 342-1824 or (212) 939-2968 Email: wme1@mail.cumc.columbia.edu
Jessica Justman, M.D. Columbia University Mailman School of Public Health 722 West 168th StreetRoom 714 New York, NY 10032 Phone: (212) 342-0537 Fax: (212) 342-1824 Email: jj2158@columbia.edu	**Amy Medley, PhD** Global AIDS Program, HIV Prevention Branch Centers for Disease Control and Prevention 1600 Clifton Rd. NE, MS-E04 Atlanta, GA 30333 Office: (404) 718-8601 Fax: (404) 639-8114 Cell: (404) 455-7923 Email: igm8@cdc.gov
Jan Moore, PhD ranch	**Emmanuel Njeuhmeli, M.D., M.P.H, MBA** USAID Global Health Fellows Program

Centers for Disease Control and Prevention 1600 Clifton Rd. NE, MS-E04 Atlanta, GA 30333 Office: (404) 639-8954 Fax: (404) 639-8114 Cell: (404) 455-7923 Email: jym3@cdc.gov	GH/OHA/TLR, Rm 5.10-013 1300 Pennsylvania Avenue, NW Washington, DC 20523 Phone: (202) 216-3409 Email: enjeuhmeli@usaid.gov
Ingrid Peterson, PhD ICAP, Columbia University Lot 192 Somhlolo Road Mbabane, Swaziland Phone: +268 2404-5797 Fax: +268 2404 8085 Email: idp2@columbia.edu	**Jason Reed, M.D., M.P.H.** Global AIDS Program, HIV Prevention Branch Centers for Disease Control and Prevention 1600 Clifton Rd. NE, MS-E04 Atlanta, GA 30333 Office: (404) 639-6269 Fax: (404) 639-8114 Cell: (404) 455-7923 Email: jreed1@cdc.gov
Rejoice Nkambule Deputy Director of Health Services Ministry of Health PO Box 5 Mbabane, Swaziland Phone +268 76078909 Email: nkambulere@gov.sz	**Jonathan Grund, M.A., M.P.H.** Global AIDS Program, HIV Prevention Branch Centers for Disease Control and Prevention 1600 Clifton Rd. NE, MS-E04 Atlanta, GA 30333 Fax: (404) 639-8114 Email: iha3@cdc.gov

Swaziland HIV Incidence Measurement Survey

1.0 SCHEMA

Title:	Swaziland HIV Incidence Measurement Survey (SHIMS)
Short Title:	HIV Incidence in Swaziland
Protocol Co-Chairs:	George Bicego, Jessica Justman, Rejoice Nkambule, and Jason Reed
Background:	The Government of the Kingdom of Swaziland plans to scale-up HIV prevention, care and treatment public health programs over a twelve-month period, including the Male Circumcision Accelerated Saturation Initiative (MC ASI) campaign. The MC ASI *program* will track the number of MC procedures but will not evaluate the impact of the campaign on the HIV epidemic. This research study will neither provide nor promote male circumcision services.
Purpose:	To determine HIV incidence at a population level before and after the independent public health program--Male Circumcision Accelerated Saturation Initiative (MC ASI)—and concurrent with the expansion of other HIV prevention, care and treatment programs in Swaziland
Design/ Duration:	Two separate cross-sectional household surveys, one before and one after MC ASI, in order to identify three prospective, longitudinal observational cohorts Two independent separate short term cohorts representative samples of HIV-uninfected adult men and women before and after completion of the MC ASI. (Cohort "A1" before ASI, and "A2" after ASI). Cohort A1 is followed for 6 months; Cohort A2 is followed for 12 months. One long-term cohort (Cohort "B") comprised of a subset of the highest risk age groups of men and women from Cohort A2. Cohort B is followed for an additional 6-18 months (beyond the 12 months of Cohort A2). One cross-sectional observational survey (Cross-Sectional Cohort "C") comprised of a subset of HIV-infected men and women who participated in Pre-Cohort A1. Cohort C participants are visited approximately 6 to 12 months after the initial A1 visit.
Sample Size:	A total of approximately 34,421 individuals from a sample of approximately 30,645 households over a 3 1/2-year period will be included in the study. Of the 34,421, a subset of 26,618 individuals

will participate in either Cohort A1, Cohort A2, or in A2 and B, as follows:

1) Two cross-sectional household surveys: 14,884 households for A1 and 15,758 households for A2.
2) Two separate, nationally representative, HIV-uninfected longitudinal cohorts of 5,823 men and 7,106 women (Cohort A1) and 6,165 men and 7,524 women (Cohort A2)
3) One longitudinal sub-cohort consisting of a subset of HIV-uninfected individuals from Cohort A2, with 1,541 men ages 25-34 and 2,182 women ages 18-24 (Cohort B)
4) One cross-sectional survey (Cohort C) comprised of a subset of 1,000 HIV-infected men and women ages 18-49 who participated in the Pre-Cohort A1

Study Objectives:

Primary Objectives

1a) To estimate HIV incidence rates in a household-based, nationally representative sample of men ages 18-49 before and after completion of the MC ASI.

1b) To estimate HIV incidence rates in a household-based, nationally representative sample of women ages 18-49 before and after completion of the MC ASI.

2a) To estimate HIV incidence rates in a highest risk age subset of the household-based, nationally representative sample, men ages 25-34, for an additional 6-18 months after the MC ASI completion.

2b) To estimate HIV incidence rates in a highest risk age subset of the household-based, nationally representative sample, women ages 18-24, for an additional 6-18 months after the MC ASI completion.

3) To estimate HIV incidence rates of circumcised men as compared to uncircumcised men in a household-based, nationally representative sample of men after completion of the MC ASI.

Secondary Objectives

1) To examine the association of baseline demographic characteristics and HIV incidence and prevalence in a household-based representative sample of men and women before and after completion of the MC ASI.

2) To determine the prevalence of circumcision among a

household-based representative sample of men before and after completion of the MC ASI.

3) To estimate HIV prevalence rates among men and women in a household-based representative sample of men and women before and after completion of the MC ASI.

4a) To describe sexual risk behavior and exposure to sexual risk reduction campaigns in a household-based representative sample of men and women before and after completion of the MC ASI.

4b) To assess sexual risk behaviors in a highest risk age subset of the household-based, nationally representative sample, men ages 25-34, for an additional 6-18 months after the MC ASI completion.

4c) To assess sexual risk behaviors in a highest risk age subset of the household-based, nationally representative sample, women ages 18-24, for an additional 6-18 months after the MC ASI completion.

5) To describe the proportion of HIV-infected men and women in a household-based representative sample with undetectable viral loads before and after completion of the MC ASI.

6) To measure the proportion of HIV-infected men and women in a household-based representative sample who report engagement in HIV care and ART use before and after completion of the MC ASI.

7) To estimate the annual number of HIV infections averted among men due to MC.

8) To describe the concurrent CD4 count and viral load distributions in a household-based, nationally representative sample of HIV-infected men and women ages 18-49, within the context of ART use.

9) To estimate the proportion of HIV-infected men and women ages 18-49 who have a CD4 count <350 cells/ mm^3 and who report no current ART use.

Study Outcomes:

Primary outcomes

- HIV incidence rates in men and women
- HIV incidence in circumcised and uncircumcised men

Secondary outcomes

- HIV prevalence rates in men and women
- Sexual risk behaviors
- Viral load in HIV-infected men and women
- Rates of engagement in HIV care
- Rates of ART use
- Prevalence of circumcision in men
- Exposure to other HIV prevention intervention
- CD4 count in HIV-infected men and women
- Proportion of HIV-infected men and women who report no current ART use

2.0 INTRODUCTION

2.1 Literature Review/Background

The HIV/AIDS epidemic in sub-Saharan Africa and Swaziland

Approximately 33.2 million (30.6-36.1 million) adults and children were living with HIV worldwide in 2007, and an additional estimated 2.5 million (1.8-4.1 million) new HIV infections occurred that year alone [UNAIDS, 2010]. Young adults (ages 15-24) have the highest HIV incidence and account for 45% of all new HIV infections. Sub-Saharan Africa continues to be the most heavily affected region accounting for approximately 68% and 90% of the world's adult and childhood HIV prevalent cases, respectively [UNAIDS, 2010]. Despite HIV prevention efforts, incidence rates in the region remain high with an estimated 1.9 million (1.6-2.1 million) adults and children newly infected with HIV in 2007. These high incidence rates, combined with increased survival rates due to expanded HIV care and treatment programs, contribute to the high HIV prevalence rates observed in the region.

Swaziland, a small landlocked country in southern Africa, has the highest HIV prevalence in the world. With approximately 1.1 million people, HIV prevalence is estimated at 26% for men and women ages 15-49 years with only modest differences in urban (31%) and rural (24%) regions [UNAIDS, 2010]. HIV prevalence differs significantly by sex with rates higher in women than men (31% v 20%). In addition, for women, prevalence peaks at age 25-29 (49%), while prevalence peaks at age 35-39 (45%) for men. HIV incidence rates are also high among both men and women. Among females 15-24 and males 25-34 years the annual HIV incidence is estimated to be 6% and 5%, respectively. The primary driver of the HIV epidemic in Swaziland, as in the rest of sub-Saharan Africa, is heterosexual sex [UNAIDS, 2010].

The Government of the Kingdom of Swaziland (GKOS) has provided antiretroviral therapy (ART) to persons with advanced HIV infection since 2003. In line with international guidance, the GKOS 2006 national treatment guidelines recommended ART for adults with a CD4 count below 200 cells/mm^3 [Ministry of Health, 2006]. National guidelines were recently revised to reflect 2010 WHO guidance, and recommend ART at a CD4 threshold of 350 cells/mm^3 [Ministry of Health, 2010].

HIV testing coverage is relatively low, with 17% and 36% of men and women 15-49 years reporting having ever been tested and receiving test results [CSO, 2008.] In contrast, projections of ART coverage, based upon modeling of national health systems data, indicate widespread coverage. In 2009, approximately 45,748 HIV-infected adults were estimated to be on ART, accounting for 93% of the population projected to have a CD4 count less than 200 and 59% of the population modeled to have a CD4 count less than 350 [UNAIDS, 2010]. A more recent estimate from the 2011 MOH Quarterly Service Coverage Report places ART coverage using a CD4 threshold of 350 at 79.5% (MOH QSCR, 2011). National goals aim for 85% coverage of all ART-eligible adults (i.e. CD4 count below 350) by 2014 [NERCHA, 2009].

HIV prevention efforts in Swaziland have primarily focused on behavioral change strategies such as delaying sexual debut, encouraging partner reduction and/or fidelity to one partner, and consistent and correct condom use at every sexual encounter. Unfortunately, the success in reducing HIV incidence from these initiatives has fallen far short of the goals established by the Swazi government and the President's Emergency Plan for AIDS Relief (PEPFAR) strategies.

Lack of male circumcision (MC) has been identified as one of the key drivers of the Swazi HIV epidemic [Swaziland, 2009]. Only 8% of men reported being circumcised during the 2007 Demographic and Health Survey (DHS) [CSO, 2008]. Based on strong scientific evidence that male circumcision is efficacious at reducing HIV acquisition among men, the Swazi government developed an HIV prevention strategy with a focus on the rapid scale-up of male circumcision services nationwide.

Male Circumcision for HIV prevention

For at least a decade, multiple cross-sectional, prospective and population-level ecologic studies have identified lack of MC as a risk factor for HIV infection [Siegfried et al., 2005; Weiss et al., 2000]. In 2000, a systematic review and meta-analysis of HIV in Africa, which included 19 cross-sectional studies, five case-control studies, and one partner study, noted a substantial protective effect of MC on risk for HIV acquisition for men [Weiss et al., 2000]. In population-based studies that adjusted for confounding factors, the relative risk for HIV infection was reduced by 58% in circumcised men. For men at high risk of HIV, such as those seeking treatment for sexually transmitted infections (STI), the association was stronger (adjusted relative risk 0.29, 95% confidence interval (CI) 0.20 - 0.41). A Cochrane Review from 2003 of 35 observational studies, including many of the same studies used in the 2000 meta-analysis, found inconsistent results in 14 cross-sectional and one prospective study in the general population [Siegfried et al., 2003]. However, the single cohort study included in the meta-analysis showed a significant difference in HIV acquisition rates (odds ratio 0.58, 95% CI 0.36 - 0.96 for circumcised men). As with the earlier meta-analysis, results from the subset of 19 studies among high-risk populations found a consistent, substantial protective effect.

Three randomized controlled clinical trials (RCTs) were conducted in Africa to address the possibility that the observational findings described above were due to confounding, and to determine whether medically-performed circumcision of adult males would reduce their risk of HIV acquisition. The first study, conducted in South Africa, was stopped in 2005, and subsequent studies in Kenya and Uganda were stopped in 2006 after interim analyses from each found that medical circumcision significantly reduced male participants' risk of acquiring HIV infection [Auvert et al., 2005; Bailey et al., 2007; Gray et al., 2007]. Men who had been representatively assigned to the circumcision group had a 60% (South Africa), 53% (Kenya), and 51% (Uganda) lower incidence of HIV infection compared to men assigned to the control group to be circumcised at the end of the trials. More recent data collected through 42 months of follow-up from the Kenyan trial demonstrated an even greater protective efficacy. HIV seroincidence was 0.77/100 person years among circumcised males compared to

2.37/100 person years among control group males who did not seek circumcision after the trial ended, equating to a 68% reduction in risk of HIV acquisition [Bailey et al., 2008]. Consistent with the Kenyan data, follow-up of men in the Ugandan trial at approximately 5 years post-circumcision also revealed a 68% reduction in HIV acquisition risk among circumcised males (compared to control group males who had not undergone circumcision after the trial ended) [Kong et al., 2011].

In March 2007, WHO and UNAIDS published normative guidance on policy and program implication resulting from the weight of the evidence for MC and HIV prevention [WHO/UNAIDS, 2007]. Key conclusions and recommendations included:

- MC should now be recognized as an additional important intervention to reduce the risk of heterosexually acquired HIV infection in men;

- MC should always be considered as part of a combination HIV prevention package, which includes the provision of HIV testing and counseling services; treatment for STI; the promotion of safer sex practices, such as abstinence from penetrative sex, reduction in the number of sexual partners, and delay in the onset of sexual relations; and the provision of male and female condoms, and promotion of their correct and consistent use;

- MC should be provided with full adherence to medical ethics and human rights principles, including informed consent, confidentiality, and absence of coercion;

- Countries with high prevalence, generalized heterosexual HIV epidemics and low rates of MC should consider urgently scaling up access to MC services and that additional resources be mobilized to support this; and

- Promoting circumcision for HIV-infected men is not recommended.

The Accelerated Saturation Initiative (ASI) for Male Circumcision in Swaziland

Based upon the convincing evidence for the protective effect of male circumcision, the Government of the Kingdom of Swaziland (GKOS) convened a National MC Task Force in 2006 to guide policy and strategy development. His Majesty King Mswati III, and high level government officials including the Prime Minister, Ministerial Cabinet, and Minister of Health are all proponents of MC for HIV prevention.

Swaziland's National MC Task Force created an initial MC strategy to circumcise 80% of 15-24 year old males within 5 years, and implementation of this strategy began in 2008. In the interim, the Government of Kenya launched a national month-long MC campaign, and 36,000 boys and men were circumcised in 30 working days. Based upon Kenya's success, PEPFAR's MC Technical Working Group suggested to Swaziland that an acceleration of their strategy from 5 years to 1 year might be feasible, and PEPFAR mobilized resources to assist.

Modeling conducted by the Future's Group in support of the accelerated approach projected that circumcising 80% of 15-49 year old men in one year could avert 88,000 new HIV infections by 2025, and that 1 HIV infection could be prevented for every 3-4 circumcisions performed. Annual HIV incidence under this accelerated scenario could be reduced by approximately 70% by 2025. Consequent HIV care and treatment cost savings could total over US$650 million over the same time period. Thus, in early 2010, the GKOS outlined an unprecedented national MC program—the MC Accelerated Saturation Initiative (ASI)—to provide voluntary medical MC to 80% of HIV-uninfected males aged 15-49 years by the end of 2011. There are approximately 240,000 15-49 year old males in the country; 92% are estimated to be uncircumcised, 80% are estimated to be HIV-negative. This equates to approximately 176,000 males that are uncircumcised and HIV-negative, 80% of which (the ASI target) equals approximately 140,000 males. Depending on the number of HIV-infected males who also seek MC after counseling regarding the lack of HIV prevention benefit, as many as 175,000 boys and men are anticipated to voluntarily receive MC services.

Program Description

The GKOS plans to launch the MC ASI in January 2011. Services delivered will include the following activities in addition to voluntary medical MC:

- information and education for both men and women on MC as an HIV prevention strategy;
- pre-operative provider-initiated HIV testing and counseling (PITC);
- individualized and partner HIV risk reduction counseling;
- syndromic screening and treatment for STIs;
- promotion and provision of male and female condoms; and
- either the offer of voluntary HIV testing and counseling (VCT) for sex partners of surgical candidates or couples HIV testing and counseling for sex partners who accompany surgical candidates to MC facilities.

For surgical candidates identified as HIV-infected during PITC, the following services will be provided, as is customary for all persons newly diagnosed with HIV in Swaziland:

- the recommendation and availability of VCT for sex partners;
- counseling regarding the lack of HIV prevention benefit from MC; and,
- referrals for HIV prevention, care, and treatment services

Efforts are underway to align MC service delivery locations with existing clinical staging and HAART-initiation centers to facilitate linkages between the MC program and HIV care and treatment program.

Approximately 35FTE service delivery teams during peak times will be required to meet the program target of reaching up to 175,000 boys and men with a comprehensive package of MC services in the 12-month period from January to December 2011. To minimize strain on existing health systems, the GKOS plans to utilize international human resources and mobile MC services to accomplish the campaign's strategic goals. It is planned that entire surgical teams—physician surgeons, nurses, surgical assistants,

cleaners, counselors—will be transported along with all necessary equipment and supplies to designated locations throughout the country. They will provide services in temporarily erected minor surgical theaters or in existing health facilities, schools or other buildings outfitted for short-term provision of MC services.

A national male circumcision performance measure—annual percentage of 15-49 year old males circumcised—is already reportable to the Swaziland National AIDS Program (SNAP), in accordance with Swaziland's Health Sector Strategic Plan 2008-2013 (HSSP). The ASI Detailed Action Plan calls for the development of additional performance measures—Strategic Operational Objective Indicators—as ASI implementation planning progresses. PEPFAR funded partners in all countries, including Swaziland, receiving support for male circumcision must already track and report performance indicators, including the total number of males circumcised annually, and the specific number circumcised using PEPFAR funds. Disaggregations by age and HIV status are included. Existing monitoring and evaluation (M&E) activities in support of these reporting requirements will be utilized, and additional resources have been allocated (separate from this study) to reinforce the timeliness, accuracy, completeness, and uniformity of M&E activities, given the extraordinary pace and scale of the ASI campaign and the need to align reporting with the HSSP format and across multiple service providers. Though our study is independent of the ASI campaign and is thus not at all responsible for collecting ASI performance indicators, we will liaise with implementing partners and SNAP to remain apprised of progress toward the targets. Evaluation of the performance indicators will also reveal whether uptake of male circumcision services is differential by age, HIV status, and region. As appropriate, the investigators may propose modifications of the study timeline and analyses, if targets are achieved in a timeframe that varies widely from projected and/or if uptake of male circumcision is non-uniform, according to the performance indicators.

2.2 Study Justification

The GKOS and the USG partners have developed plans to examine the reduction of new infections at the population level coinciding with the implementation of the MC ASI program beginning in January 2011. In addition to providing important programmatic information to the country, the evaluation of this program is of great value to prevention researchers and public health experts for a variety of reasons including:

- Although the body of scientific evidence and international guidance in support of MC for HIV prevention are irrefutable, the degree to which efficacy demonstrated in controlled clinical trial settings will translate to effectiveness in less controlled non-research, 'real-world' scale-up scenarios is unknown. Generally, this is because participants in clinical trials often encounter conditions that would not be expected when interventions are implemented at a population-level, and such differences may decrease or increase the efficacy observed in the clinical trials. For example, participants in the MC clinical trials may have altered their behavior as a result of the randomization process or may have behaved differently consequent to the frequent and rigorous counseling given during the multiple follow-ups over the course of the study. Also, men who sought

participation in the clinical trials may have been different from men in the general population in ways that relate to risk of HIV acquisition. These types of differences may not have been fully measured—or measured at all—and adjusted estimates of efficacy from the RCTs may not reflect any confounding that might have occurred as a result.

- Risk compensation refers to the phenomenon whereby individuals may engage in increased risk behavior due to the perception that measures taken to safeguard their health confer greater protection than is actually the case [Cassell et al., 2006]. There was little evidence of pre- to post-surgical risk compensation among men in the three MC clinical trials [Auvert et al., 2005; Bailey et al., 2007; Gray et al., 2007]. However, this lack of risk compensation among RCT participants may not reflect what may be observed in non-research implementation settings. It is also important to note that for participants in these clinical trials, the efficacy of MC for HIV prevention was yet to be determined with certainty. As mentioned previously, MC RCT participants experienced a frequency and intensity of counseling and follow-up that was different from what will occur in non-research settings. Though modeling studies suggest that risk compensation limited to men undergoing MC would not appreciably reduce the protection from HIV acquisition conferred by circumcision, these same studies suggest a potential attenuation of benefit if risk compensation were to occur among women and uncircumcised men in communities undergoing MC scale-up [Hallett et al., 2008]. Thus, risk compensation outside of research settings needs to be measured and characterized among both circumcised and uncircumcised men as well as women.

- Mathematical models have proposed an indirect protection for women following MC scale-up, based upon declining probabilities over time of encountering HIV infected male partners. The models suggest that these indirect effects that accrue for women lag behind the more immediate direct effects for men by several years. (See Figures 1 & 2). Except for the Swazi MC program, national MC strategies in sub-Saharan Africa are generally 5-10 years in length, and any ability to measure HIV incidence reductions in women as a result of MC are correspondingly delayed. Thus, the rapid nature of the MC program, coupled with Swaziland's very high HIV incidence rates among girls and women, makes the ASI well-suited to demonstrate HIV incidence reductions in women. In addition, Swaziland's MC program may result in as many as 34,000 men being newly diagnosed with HIV infection, and an estimated 4,500 of those with an initial CD4 count of <350 initiated on HAART. Such marked increases in new diagnoses, treatment, and viral suppression—as a consequence of the MC ASI—have the potential to synergistically reduce HIV incidence among women.

Figure 1. Modeled reductions in HIV incident infections averted among women following MC scale-up to 80% coverage of 15-49 year old men by year-end 2011

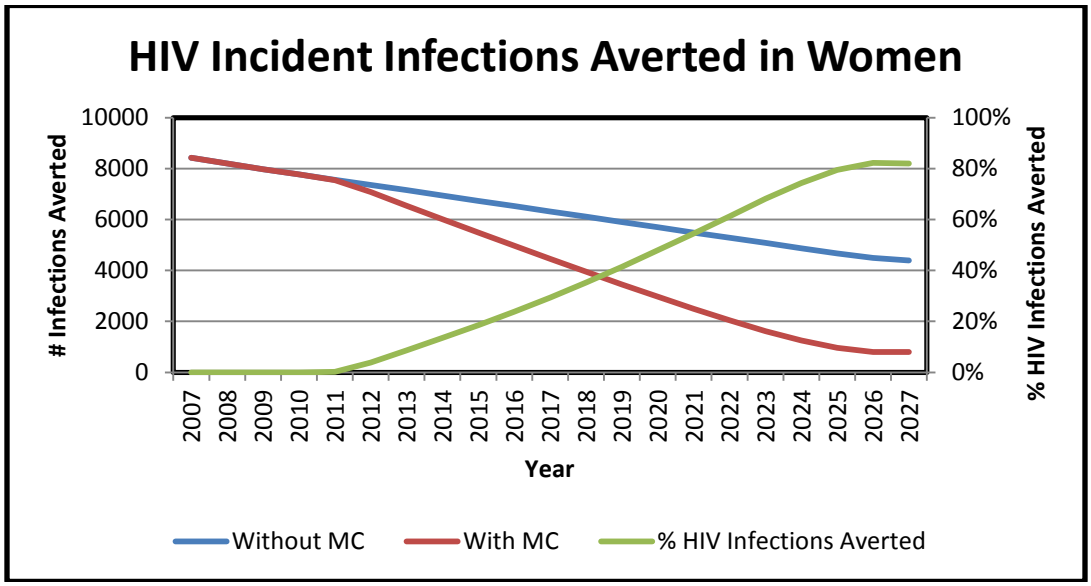
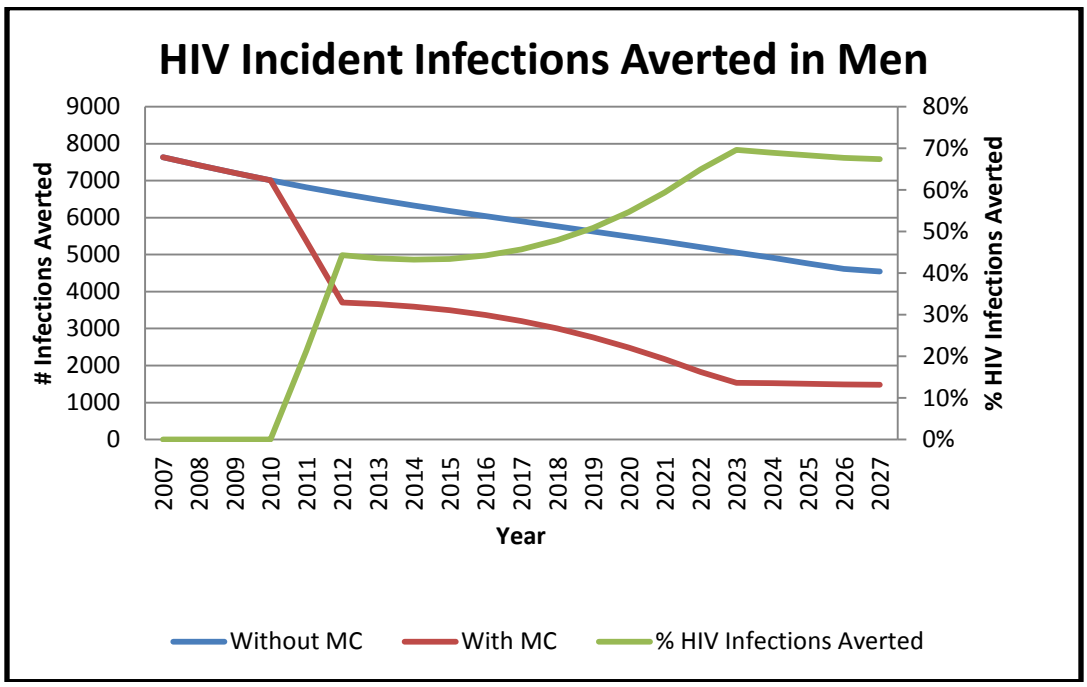


Figure 2. Modeled reductions in HIV incident infections averted among men following MC scale-up to 80% coverage of 15-49 year old men by year-end 2011



- Ongoing modeling exercises enable policy makers and other stakeholder throughout the region to make informed decisions about MC policies and implementation strategies. It is expected that the assumptions used in the

modeling exercises will be continuously refined based upon experiences from maturing MC programs, and projections should become more precise and useful over time. Notably, the 2009 UNAIDS/USAID model, disseminated widely as country-specific 'Issue Briefs', assumed that the effectiveness of MC delivered in non-research settings would be 60%, the same as the efficacy observed in the three RCTs at reducing HIV acquisition in men. To the extent that MC delivered outside of the research setting has an effectiveness greater or less than 60%, the modeled impacts are over or underestimating the true epidemiologic and financial benefits of MC scale-up. Demonstrating the equivalence or difference in MC effectiveness from efficacy will help inform future modeling exercises and enable more precise impact projections for decision makers.

- The framework already in place for SHIMS will readily permit an estimate of the immune status (CD4 counts) at the community level in a high HIV-prevalence setting. CD4 count distribution is an important indicator of a local HIV epidemic but is usually only available from clinical cohorts, where individuals tend to have more advanced disease and therefore lower CD4 counts. Obtaining a household-based, nationally representative CD4 distribution among HIV-positive persons in Swaziland will be useful for assessing the reach of ART scale-up and will help target public health programming to increase access and quality of HIV/AIDS care and treatment in Swaziland. Additionally, identifying the HIV viral load distribution for this nationally representative group of HIV-positive persons will assess the prevalence of viral load suppression across the population-level range of CD4 counts. The viral load distribution will likely differ according to ART eligibility and to reported current ART use and will inform program planners on the needs such as stronger ART adherence support programs, more effective linkages between HIV testing and HIV care services, or increased procurement of second line antiretroviral medications.

2.3 General Study Approach

The intent of the SHIMS study is to measure population level HIV incidence before the MC ASI and after its completion. The study relies on a combination of methodologies. A longitudinal cohort of men and women in the highest incidence age groups will be followed for HIV sero-conversion for an extra 6-18 months following the MC ASI completion. In addition, using a novel approach of measuring HIV incidence in sequential short-term cohorts of HIV-uninfected individuals selected from a representative sample of households, HIV incidence prior to and after completion of the MC ASI will be measured. The study will assess the effect of MC ASI in the context of other prevention activities in Swaziland on HIV incidence. The SHIMS study will not follow participants in the MC ASI program but instead will follow population-level changes in HIV incidence that coincide with the MC ASI program rollout.

The SHIMS study will not allow sole attribution of any observed changes in HIV incidence to the MC ASI for several reasons. First, since 2003, Swaziland's Emergency Care and Treatment Implementation Plan (ECTIP) has dramatically expanded access to HIV testing and ARV treatment. The GKOS has further extended treatment coverage

by revising the current policy for ARV initiation from < 200 CD4 cells to < 350 CD4 cells. ARV treatment scale-up may also impact population-level HIV incidence as HIV infected people may decrease behaviors that transmit HIV once they are aware of their HIV status [Rietmeijer et al., 1996; Gibson et al., 1999; Cleary et al., 1991] and ART-induced viral suppression reduces the risk of HIV transmission to sex partners [Granich et al., 2010; Wagner and Blower 2010, Quinn et al., 2000; Attia et al., 2009; Donnell et al., 2010; Sullivan et al., 2009; Reynolds et al., 2009; Cohen et al., 2011]. In fact, mathematical models indicate that the 50, 767 Swazi adults provided with free ARV treatment between 2006-2009 by the ECTIP prevented 10,770 new adults infections, assuming a one-year treatment retention of approximately 85%. This is approximately 18% of infections that would have occurred in the absence of the program. Furthermore, the MC ASI will result in more individuals identified as HIV-positive who will be linked to HIV care and treatment services. The anticipated changes in risk behavior and viral suppression may also affect transmissibility and reduce HIV incidence, but this is unlikely to occur within the timeframe of this study.

In summary, Swaziland is taking a lead role in implementing a combination of prevention interventions at an unprecedented pace. It is vital to describe changes in the rate of new HIV infections resulting from implementation of these interventions. The study described in this protocol seeks to document changes in HIV incidence and examine factors that may contribute to this change so that other countries and programs can develop and implement HIV prevention strategies that will lead to documented reductions in HIV incidence.

2.4 Audience and stakeholder participation

Stakeholders include the GKOS, non-governmental partners involved in male circumcision implementation, partners involved in HIV care and treatment in Swaziland, other donors, and the population of men and women in Swaziland.
The SHIMS study is a joint endeavor of the GKOS MoH, the US government (USG) PEPFAR program in Swaziland, the Centers for Disease Control and Prevention (CDC) in Swaziland, CDC Atlanta, the International Center for AIDS Care and Treatment programs (ICAP) in Swaziland, ICAP New York,, Columbia University, and the University of Washington. USG staff in Swaziland have been in consultation with the Swaziland MoH, ICAP Swaziland, and other national stakeholders during the conception and development of this evaluation. A National MC Impact Evaluation Steering Committee has been formed to guide the process of implementing this evaluation in country and will continue to meet regularly to provide continuous guidance to investigators. The Steering Committee includes staff from the MoH and facilities providing MC services.

A Swaziland MoH staff member serves as one of the four co-principal investigators, along with co-principal investigators from CDC Swaziland, ICAP New York, and CDC Atlanta. Other investigators will be included representing key individuals involved in this evaluation and Swaziland civil society.

At the end of the evaluation, findings will be communicated with key stakeholders and information disseminated as appropriate.

2.5 Rationale and intended use of study findings

This evaluation will provide important information about changes in HIV incidence over time at a population level, coinciding with the MC ASI program and associated expansion in HIV prevention, care and treatment programs. Also, comparisons of HIV incidence rates among men by circumcision status will better inform our understanding of the reduced risk of acquiring HIV for men who are circumcised in non-research settings. Such information will help refine projections of HIV infections that may be averted and the potential cost savings realized, compared to other prevention and treatment strategies. Results may also demonstrate the effects of newly diagnosing men and women on the proportion of HIV-positive individuals with an undetectable viral load.

Previous statistical studies have modeled CD4 count distribution at a population level (Williams et al, 2006) but no population level measurement has ever been conducted. Population-level measures of the CD4 distribution among HIV-infected and of current ART use among eligible persons would provide nationally representative estimates of ART-eligible persons on ART and in need of ART. As ART-induced viral suppression protects against HIV transmission, an estimate of the number of persons who have a CD4 count less than 350 and who report no current ART use will indicate the proportion of HIV-infected who would benefit from ART both as individuals and at the public health level.

Similarly, the proportion of persons on ART and who have an undetectable viral load has been assessed in randomized clinical trial settings and clinic-based cohorts, but not at the population level. Such information would be key for program planners to determine future ART scale-up and to assess the quality of the ART program, based upon the correlation of reported ART use and viral load. Study results may inform the need for stronger adherence support programs, greater availability of second line antiretroviral regimens, or stronger linkages between HIV testing and HIV care services. Additionally, study data will allow an assessment of the proportion of ART naïve individuals with low viral load of < 50 c/ml (Cao et al 1995, Blankson JN 2010). These individuals, or 'elite' controllers, may account for up to 5% of the HIV-infected population (Harrer et al 1996, Okulicz et al 2009). Such individuals may have differing CD4 counts and may impact the number of persons in need of ART and planning for ART services.

Swaziland, other partner country governments, and donors may benefit from the evaluation findings as they determine the best allocation of resources to achieve and balance their HIV prevention and treatment goals. Information about how levels of high risk sexual behaviors reported by circumcised and uncircumcised men and women change over time will not only inform the independent calculations of protection

conferred by MC, but will also guide communication campaigns to help ensure that the maximum benefits of MC scale-up are realized. More broadly, there is global skepticism about HIV prevention possibilities in the absence of an effective HIV vaccine. While Swaziland's HIV epidemic has some unique features, findings from this study will be generalizable to other countries in the region. Demonstrating that the ASI approach to MC is effective in reducing HIV incidence would most likely lead other countries in the region to try to replicate the saturation approach, and would refocus attention on the ability of biomedical and behavioral interventions to prevent incident infections when resourced and delivered in concert and at an optimal intensity.

2.6 Hypotheses

1) HIV incidence among men ages 18-49 in Swaziland will decrease over time, coinciding with national HIV prevention, care and treatment programs, including the MC ASI.

2) HIV incidence among women ages 18-49 in Swaziland will decrease over time, coinciding with national HIV prevention, care and treatment programs, including the MC ASI.

3) HIV incidence among men and women in the highest risk age groups in Swaziland, men ages 25-34 and women ages 18-24, observed longitudinally, will decrease over time, coinciding with national HIV prevention, care and treatment programs, including the MC ASI.

4) HIV incidence among men in Swaziland will differ by male circumcision status, measured after completion of the MC ASI.

5) The proportion of individuals with CD4 counts in each of the following categories will be approximately 25%: 0-200, 201-350, 351-500, 501 cells/mm^3 and above.

6) The proportion of HIV-infected men and women ages 18-49 who have a CD4 count <350 cells/mm^3 and who report no current ART use is 20-40%.

3.0 OBJECTIVES

3.1 Primary Objectives

1a) To estimate HIV incidence rates in a household-based, nationally representative sample of men ages 18-49 before and after completion of the MC ASI.

1b) To estimate HIV incidence rates in a household-based, nationally representative sample of women ages 18-49 before and after completion of the MC ASI.

2a) To estimate HIV incidence rates in a highest risk age subset of the household-based, nationally representative sample, namely men ages 25-34, for an additional 6-18 months after the MC ASI completion.

2b) To estimate HIV incidence rates in a highest risk age subset of the household-based, nationally representative sample, namely women ages 18-24, for an additional 6-18 months after the MC ASI completion.

3) To estimate HIV incidence rates of circumcised men as compared to uncircumcised men in a household-based, nationally representative sample of men after completion of the MC ASI.

3.2 Secondary Objectives

1) To examine the association of baseline demographic characteristics and HIV incidence and prevalence in a household-based representative sample of men and women before and after completion of the MC ASI.

2) To determine the prevalence of circumcision among a household-based representative sample of men before and after completion of the MC ASI.

3) To estimate HIV prevalence rates among men and women in a household-based representative sample of men and women before and after completion of the MC ASI.

4a) To describe sexual risk behavior and exposure to sexual risk reduction campaigns in a household-based representative sample of men and women before and after completion of the MC ASI.

4b) To assess sexual risk behaviors in a highest risk age subset of the household-based, nationally representative sample, men ages 25-34, for an additional 6-18 months after the MC ASI completion.

4c) To assess sexual risk behaviors in a highest risk age subset of the household-based, nationally representative sample, women ages 18-24, for an additional 6-18 months after the MC ASI completion.

5) To describe the proportion of HIV-infected men and women in a household-based representative sample with undetectable viral loads before and after completion of the MC ASI.

6) To measure the proportion of HIV-infected men and women in a household-based representative sample who report engagement in HIV care and ART use before and after completion of the MC ASI.

7) To estimate the annual number of HIV infections averted among men due to MC.

8) To describe the concurrent CD4 count and viral load distributions in a nationally representative sample of HIV-infected men and women ages 18-49, in the context of ART use.

9) To estimate the proportion of HIV-infected men and women ages 18-49 who have a CD4 count <350 cells/mm^3 and who report no current ART use.

4.0 STUDY DESIGN

The study will utilize a combination of methods to achieve the objectives listed above in section 3.0. For all primary objectives and secondary objectives 1 through 7, three cohorts will be recruited and followed as part of this evaluation. Two short term cohorts of household-based representative samples of HIV-uninfected individuals will be recruited and followed: the first (Cohort A1), enrolled approximately six months before the MC ASI will be followed for six months; the second (Cohort A2), enrolled 12 months after the conclusion of MC ASI will be followed for 12 months. Cohorts A1 and A2 are selected independently. The third longitudinal cohort ("Cohort B") will follow a subset of Cohort A2 participants who are HIV-uninfected individuals in the highest-risk age groups for HIV acquisition (18-24 years of age for women and 25-34 years of age for men), for an additional 6-18 months (beyond the 12 months of Cohort A2).

- *Primary Objectives 1a & 1b: To estimate HIV incidence rates in a household-based, nationally representative sample of men and women ages 18-49 years before and after completion of the MC ASI*

- *Primary Objective 2a & 2b: To estimate HIV incidence rates in the highest risk age subsets of the household-based, nationally representative sample, namely men ages 25-34 years and women ages 18-24 years, for an additional 6-18 months after the MC ASI completion.*

HIV incidence will be determined by following separate sequential household-based representative samples of HIV-uninfected men and women for six and 12 months, before and after MC ASI, respectively, to directly measure new HIV infections. These two sequential longitudinal cohorts (independently selected), separated in time by approximately 24 months, will be used to measure baseline (before the MC ASI) and post-MC ASI program incidence rates. See Figure 3. (Note: In the Figures 3-5, month 0 is January 2011, the time of the launch of MC ASI. Thus pre-MC ASI baseline begins before Month 0.)

Men and women from Cohort A2 in the highest-risk age groups for HIV acquisition who are HIV-uninfected at Month 36 are eligible to remain in a longer-term longitudinal

cohort (Cohort B). These individuals will be retested for HIV at approximately Month 42. See Figure 4. Thus changes in HIV incidence among men and women in the high-risk age groups can be compared with changes in HIV incidence from the sequential longitudinal cohorts, and the overall impact of MC ASI on new HIV infections will be observed. See Figure 5.

Additionally, for secondary objective 8 and 9, a cross-sectional observational survey of a household-based, nationally representative sample of HIV-seropositive individuals will be identified from Pre-Cohort A1 participants. This subset of individuals will be recruited for participation in Cross-Sectional C. Recruitment will occur approximately six to twelve months after participation in Pre-Cohort A1.

Below is the time frame for each of the three cohorts and the additional cross-sectional measurement. Each is presented in a figure, and the study objectives for each cohort are described.

Cohort A1: Baseline (pre MC ASI) short-term cohort of household-based nationally representative sample of men and women 18 – 49 years of age

- Enrollment (Month -6)
- Follow-up (Month 0)

Cohort A2: Post MC ASI short-term cohort of household-based nationally representative sample of men and women 18 – 49 years of age

- Enrollment (Month 24)
- Follow-up (Month 36)

Cohort B: Longer-term prospective cohort of household-based nationally representative sample of men and women in the highest risk age groups (men 25-34 years, women 18-24 years)

- Enrollment (Month 36)
- Follow-up (Month 42)

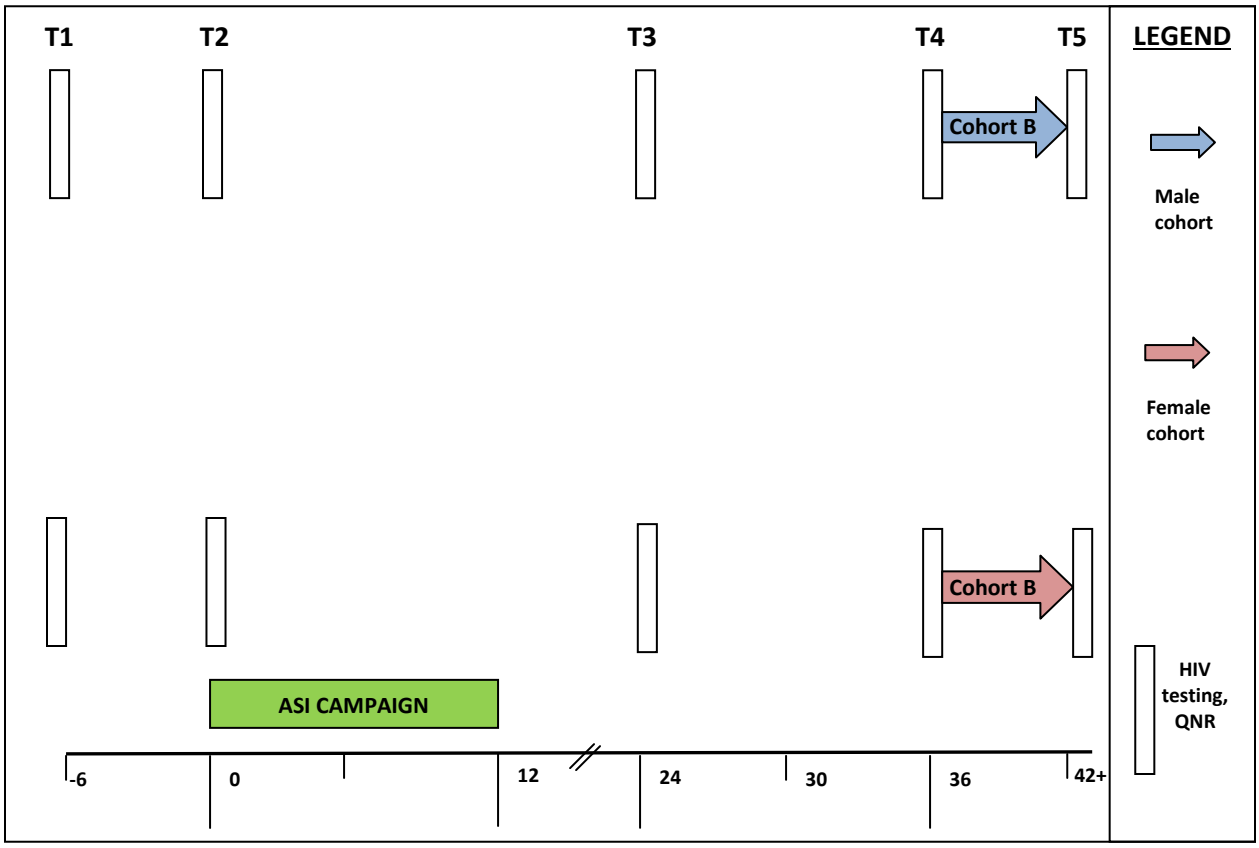
Cohort C: Cross-sectional, observational survey of a household-based, nationally representative sample of HIV-infected men and women 18-49 years of age

- Enrollment (Month 0)

Primary Objectives 1a & 1b and Secondary Objective 1
Data collected during enrollment and follow up of Cohort A1 and Cohort A2 will be used to estimate HIV incidence rates in a household-based representative sample of men and women 18-49 years old before and after completion of the MC ASI and to examine the association of baseline demographic characteristics with HIV incidence.

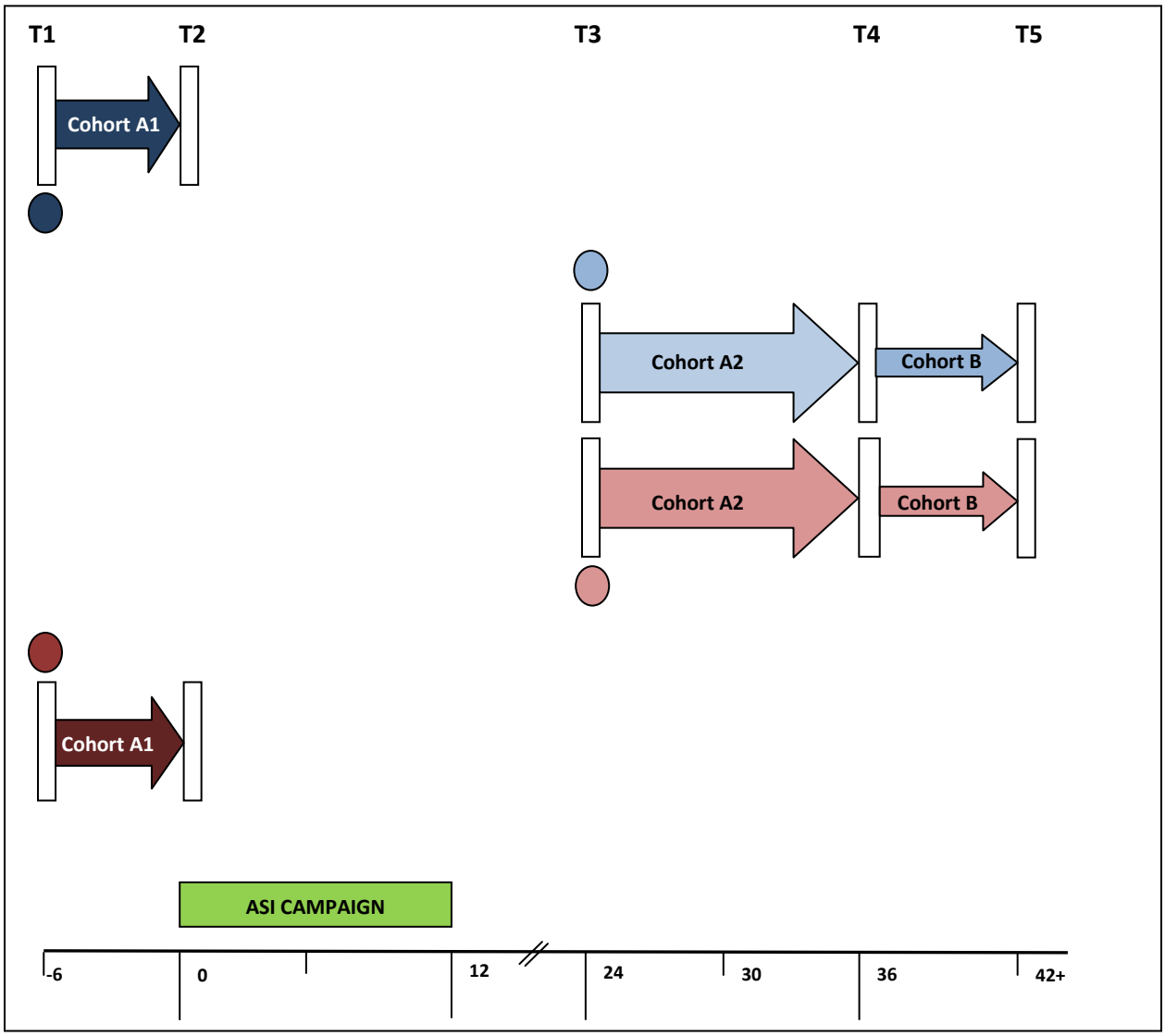
Secondary Objectives 1, 2, 3, 4a, 5, 6
Data collected during the eligibility screening and enrollment of Cohort A1 and A2 will be compared to examine changes in HIV prevalence, prevalence of circumcision among men, sexual risk behavior, exposure to sexual risk reduction campaigns; among those HIV infected, self-reported use of ART, engagement in care, and viral load level; and to examine the association of baseline demographic characteristics with HIV prevalence.

Figure 4. Longer-term prospective cohorts



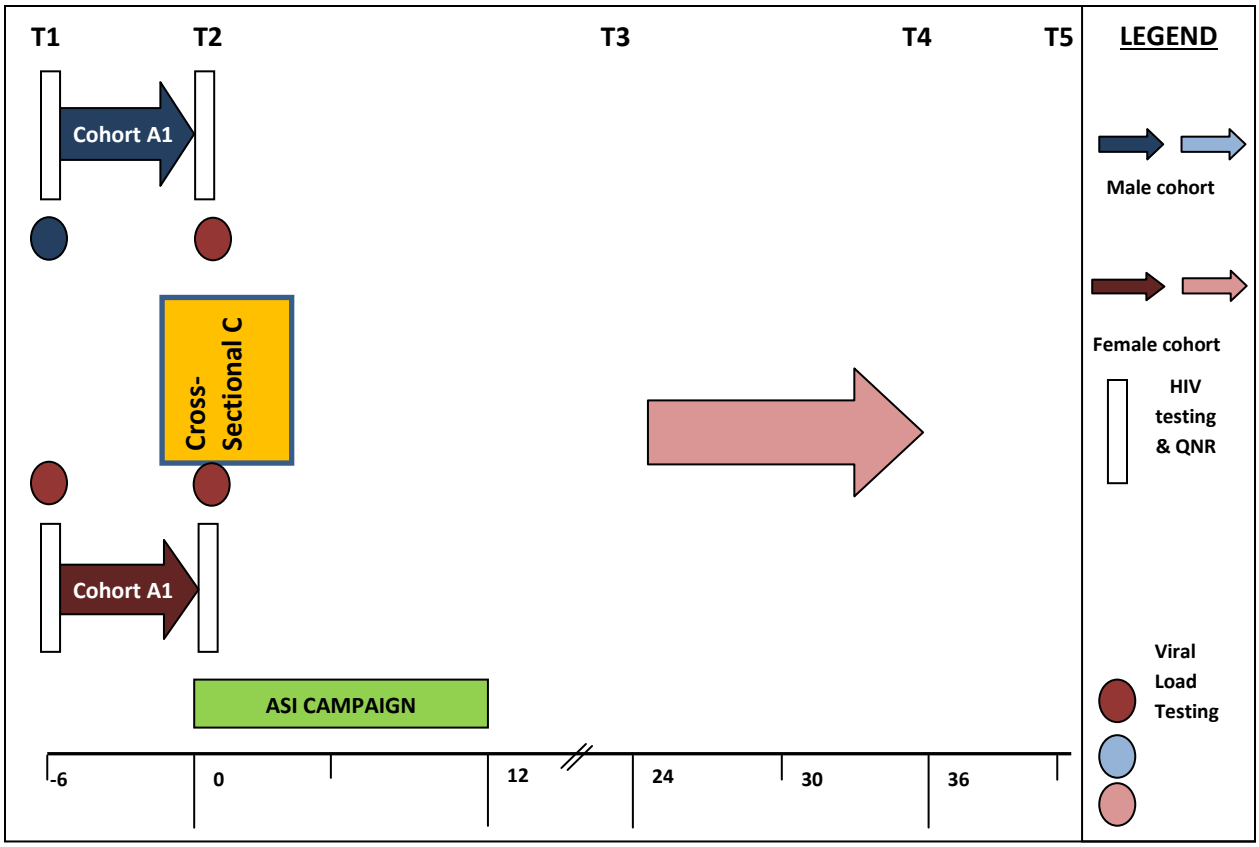
Primary Objective 2a & 2b and Secondary Objective 4b & 4c
Data collected during enrollment and follow up of men 25-34 years old and women 18-24 years old enrolled in Cohort B will be used to estimate HIV incidence rates and describe sexual risk behaviors for an additional 6-18 months after completion of MC ASI.

Figure 5. All three cohorts



Primary Objective 3 and Secondary Objective 7
HIV incidence rates in men in all cohorts described above will be compared by the circumcision status of the men. The annual number of HIV infections averted among men due to circumcision calculated.

Figure 6. Cross-Sectional Cohort C



Secondary Objective 8 and 9

Data collected from HIV-infected men and women will be used to estimate CD4 count distribution, viral load distribution, and the proportion who have a CD4 count <350 cells/mm^3 and who report no current ART use.

4.1 Study Timeline

YEAR	2010						2011			2012			2013			2014				2015	
Timeline – Months)	**J**	**A**	**S**	**O**	**N**	**D**															
2010 - 2015																					
Protocol development	**x**																				
Submit protocol for PHE review, CDC IRB, Swazi Ethics Review	**x**																				
Translate study instruments	**x**	**x**																			
Recruitment and training of research staff	**x**	**x**	**X**	**x**	**X**	**X**	**X**														
Procurement of supplies and Swaziland laboratory preparations	**x**	**x**	**X**	**x**	**X**	**X**	**X**														
Data collection (Cohort A1)				**x**	**x**	**x**	**X**	**x**	**X**												
Data collection (Cross Sectional C)									**x**	**x**											
Data collection (Cohort A2)													**x**	**x**	**x**	**X**	**X**				
Data collection (Cohort B)														**X**	**X**	**X**	**X**	**X**	**X**		
Laboratory processing				**x**	**x**	**x**	**X**	**x**	**x**				**x**	**x**	**x**	**x**	**x**	**X**	**X**		
Interim analysis and progress report								**x**			**x**			**x**							
Data analysis																**x**	**x**	**X**	**x**	**x**	**x**
Writing of reports																		**X**	**x**	**x**	**x**
Dissemination & Discussion of results with stakeholders																			**x**	**x**	**x**

4.2 How study design meets objectives and addresses research questions

The study design incorporates two different strategies for measuring the change in seroincidence. To measure the population effect of the MC ASI campaign on HIV infection in Swaziland, we plan two independent household based samples, one prior to the campaign, one 12 months after the completion of the campaign. Each gives an

independent population "snapshot" of the current rates of infection. The second strategy enrolls a cohort of men and women in the ages at highest risk prior to the MC ASI and measures that seroincidence in this group for an additional 6-18 months after the campaign's completion. There are several reasons for this hybrid approach. Two sequential short term cohorts provide two similar 'snapshots' of HIV incidence before and after the ASI campaign and coinciding with scale-up of other prevention programs. These two cohorts are representative of the national population and the HIV seroincidence estimates before and after ASI are therefore likely to represent actual changes in the epidemic. The use of a more traditional longitudinal cohort is conceptually familiar from randomized placebo controlled clinical trials; here it is used to follow HIV incidence among young adults in the highest incidence age groups over an additional 6-18 month period.

The comparison of HIV incidence rates between circumcised and uncircumcised men offers insight into the role played by MC in reducing HIV infection in the cohort and in the general population, if observed.

Blood specimens from persons identified as HIV-infected *during pre-cohort surveys* (A1 and A2) will be collected to measure viral load. These data will be used to determine the proportion of HIV infected persons with undetectable viral load in the community. The proportion of undetectable viral load at the baseline pre-cohort survey will be compared to the post-MC ASI pre-cohort survey to explore the potential effect of scale up of the ART program and viral suppression on reduced HIV incidence in the general population, if observed.

Blood specimens to measure CD4 count will be collected using a multistage clustered random sample of persons who were identified as HIV-seropositive at the pre-cohort survey for Cohort A1 (T1). These data will be used to estimate the CD4 count distribution, viral load distribution, and self-reported ART use among HIV-infected individuals at the population-level.

Surveys of sexual risk behavior and exposure to sexual risk reduction campaigns of men and women will be administered as part of the pre-cohort survey before and after the MC ASI. In addition, men and women in the longer term cohort (Cohort B) will provide sexual risk behavior information at all scheduled study visits. Repeated measures of sexual risk behaviors and exposures to sexual risk reduction campaigns over time, allows for examination of whether risk behavior changes may be coincident with MC ASI and reduced HIV incidence, if observed.

4.3 M&E Data from Programs

The ability to evaluate the stated hypotheses (Section 2.6) is predicated, in part, on achievement of the ASI targets and validity of the modeled HIV incidence reductions (Figures 1 and 2). If the ASI campaign effects a different level of MC coverage than 80%, achieves the targets in a different timeframe than one year, if the effectiveness and efficacy of MC differ, and/or if HIV incidence is increased or reduced as a result of

changes in other HIV preventative programs, then the anticipated reductions in HIV incidence—magnitude and time-horizon—will likewise be different. Knowledge of all of these factors is important, and in the 3 ½ -year context of SHIMS, awareness of MC coverage levels within specific time frames is critical.

An independent cooperative agreement was thus awarded to John Snow, Inc. to support the MOH and implementing partners in designing and implementing a real-time M&E system to keep stakeholders apprised of program performance. A hierarchical system of MC indicators is being designed, prioritized according to the time-frames within which data are needed. Not all indicators to be reported by the ASI M&E system are relevant to the SHIMS evaluation but all are presented:

<u>Daily information</u>

- Number of clients circumcised
    - Disaggregation
        - Service delivery team
- Number of clients with severe adverse event(s)
    - Disaggregation
        - Type of severe adverse event(s)
        - Timing /onset

<u>Weekly information</u>

- Weekly and cumulative number of clients circumcised
    - Disaggregation
        - Age (<1, 1-9, 10-14, 15-19, 20-24, 25-34, 35-49, 50+)
        - HIV Status (positive/documented, negative/documented, indeterminate, unknown/refused)
        - Region (1-4) of client residence
        - Urban/rural designation of client residence
        - Service delivery team
- Weekly and cumulative number of clients experiencing adverse event(s) of at least moderate severity
    - Disaggregation
        - Severity, maximum (moderate or severe)
        - Timing/onset
        - Type of adverse event
        - Service delivery team (dissemination of rates by team may be limited)
        - Surgeon (dissemination of rates by surgeon may be limited)

<u>Monthly information</u>

- Aggregation of all 'weekly' data to monthly
- Self-reported sexual activity by clients presenting for MC
- Number of clients provided with post-operative clinical care

- Monthly and cumulative number of persons newly diagnosed with HIV via ASI, by client self-report (regardless of whether MC surgery was performed subsequent to diagnosis)
    - Disaggregation
        - Surgical outcome (went for surgery or did not go for surgery, same day/facility)
- Monthly and cumulative number of persons provided with written referral to pre-ART services
    - Disaggregation
        - Region (1-4) of client residence
        - Urban/rural designation of client residence
        - Service delivery team
        - Pre-ART clinic on referral form

Quarterly information

- Aggregation of all 'monthly' data to quarterly
- Addition of Tinkhundla-specific residency information to regional disaggregations described above

JSI, Inc. already supports the MOH and pre-ART/ART implementing partners in the M&E system for HIV care and treatment programs. Because ART initiation may reduce the transmissibility of HIV between partners, knowledge of ART coverage levels (including any expansion resulting from ASI) is important. The MOH M&E unit presently prepares a quarterly report of treatment-related indicators, including HIV testing rates. Again, not all indicators reported by the care and treatment M&E system are relevant to the SHIMS evaluation but all are presented:

Quarterly information

- Number of persons tested for HIV
- Number of patients initiated on ART during the quarter.
- Number of patients initiated on ART since the beginning of the program (2004) by age ($\geq$15 vs. <15) and sex.
- Total number of patients currently on ART at the end of the quarter by age ($\geq$15 vs. <15) and sex.
- Number of patients currently on first-line ART at the end of the quarter by age ($\geq$15 vs. <15) and sex.
- Coverage of patients in need of ART (denominator based on most recent Spectrum projection. The numerator is "iii" above.)
- Retention on ART: Number of patients initiating ART considered still alive and on ART at 12 months. Although this is reported in each quarterly report, the retention data apply only to patients initiating ART throughout the previous year. Only by the end of the reporting year (e.g., 2009) does the quarterly report have complete

12-month cohort data for patients enrolled the previous year (e.g., 2008). Definition of LTFU = any patient who missed a scheduled appointment by more than 60 days (this is different from PEPFAR indicators).

- Retention on first-line regimens: Number of patients initiating ART considered still alive and on first-line ART at 12 months. (Details of calculation same as "vi" above but those patients not on 1st line regimens are no longer included in the retention numerator.)
- Total number of ART patients currently enrolled who are receiving nutritional support.

Individual-level information on exposure to HIV prevention programs will be collected from all participants (Appendices E, F, N). These data, along with information collected by NERCHA on national HIV prevention mass media and community-based prevention activities will be used to evaluate the role of non-MC prevention programs during the study period.

4.4 Limitations to the study designs

The accelerated nature of the MC ASI prevents the use of evaluation designs that allow causal inference to a particular intervention such as serial rollout of different interventions or of establishment of one intervention in certain geographic areas and not others. In addition, given the overwhelming evidence of the efficacy of MC at preventing HIV acquisition among men, it would be unethical to have a true comparison group, even if that group were wait-listed for services. Instead, multiple evaluation designs and data collection on other factors that could influence HIV incidence besides an increase in MC prevalence will be used in an attempt to distinguish the effect of each of these variables on HIV incidence. For example, data on HIV-related sexual risk behaviors will be collected to determine if changes HIV incidence coincide with change in risk behaviors, resulting from the MC ASI or other ongoing HIV prevention interventions. Additionally, data on prevalence of persons on ART and proportion of persons with undetectable viral load will be collected to assist in understanding the potential effect of expanding care and treatment services on changes in HIV incidence. Cohort effects are a major threat to validity due to power reductions or bias from loss to follow-up and repeated measures (i.e. repeated counseling and interview sessions with staff reduces HIV acquisition risk among cohort participants over time). The two short term cohorts, with their one-time 6 or 12 month follow up will minimize cohort effects. The hybrid approach with addition of a longer term cohort with multiple follow-up observations will allow comparison of measurements from the different methodologies.

4.5 Prevention effectiveness/cost benefit

By combining: 1) the baseline HIV incidence rate among men; 2) the estimated HIV acquisition risk reduction (if observed) and; 3) the estimated number of males circumcised between baseline and post-MC ASI surveys, we will project and annualize the number of HIV infections averted due to MC among men in the population (as a proxy for population-level 'impact' of MC in men).

Via a separate study, cost-effectiveness and other economic analyses of the MC ASI will be conducted.

5.0 STUDY POPULATION

5.1 Source of household-based study population

To measure variables being evaluated and establish the baseline (Cohort A1) and post-MC ASI (Cohort A2) cohorts, pre-cohort surveys of households followed by cohort formation will take place. Separate, independent samples of enumeration areas (sampling points defined by the 2007 Swaziland Population and Housing Census), households, and individuals will be selected at baseline and post-MC ASI. The final number of households drawn, approximately 14,884 for Cohort A1 and 15,758 for Cohort A2, is derived from the sample size calculation (see Section 7.0).

Pre-cohort Survey
Prior to implementation, a representative sample of enumeration areas will be identified and an exhaustive listing of households within each sampled enumeration area will subsequently occur. A random sample of households within each enumeration area will be drawn in order to select approximately 15,000 households. All men and women, age 18-49, within sampled households may participate in the pre-cohort survey.

Formation of Cohorts
From among individuals completing the pre-cohort survey, the subset of those who are HIV-negative and who satisfy criteria for inclusion in the male and female longitudinal cohorts, will be followed for 6 months (Cohort A1) and 12 months (Cohort A2). From among individuals completing follow-up in Cohort A2, the subset of those who are HIV-negative and who meet criteria for inclusion in the male and female highest risk age group longitudinal cohort, will be followed for an additional 6-18 months (Cohort B). From among individuals completing the pre-cohort survey for Cohort A1, a cluster sampled subset of those who are HIV-infected and who satisfy criteria for inclusion/exclusion in Cross-Sectional Cohort C, will be recruited to participate in a cross-sectional observational survey (Cross-Sectional Cohort C).

5.2 Case Definitions

The following case definitions will be used throughout the study to establish participant study eligibility and outcomes.

5.2.1 HIV status

During each contact with participants, consenting adults will be tested for HIV for this study using a serial algorithm for rapid tests approved by the Swaziland National Reference Laboratory. This algorithm includes the use of the 4th generation Determine rapid test kits that are capable of detecting HIV antigens. Samples from persons that are

HIV antigen reactive will subsequently be tested using both enzyme immunoassay (EIA) and nucleic acid detection tests (NAT) in the laboratory (Appendix A). NAT are HIV-discriminatory assays approved by the FDA to detect HIV-1 protein/antigens, which are produced by the body prior to the production of antibodies. NAT will be used in this protocol to identify people who were very recently HIV-infected who might not have had their HIV infection detected if only rapid HIV tests that detect antibodies were being used. The use of HIV antigen detection tests is to avoid enrollment of acutely HIV-infected individuals into the cohorts.

1. Confirmed HIV-negative: Negative per the serial algorithm for rapid HIV testing that includes 4th generation Determine, approved for this study. See Appendix A.

2. Confirmed HIV-positive: Positive per the serial algorithm for rapid HIV testing that includes 4th generation Determine, approved for this study. See Appendix A.

   Sub-categories defining duration of HIV infection among confirmed HIV-positive persons are as follows:
   - HIV-positive Incident Infection: identified by direct observation of HIV seroconversion during the period of participation in the cohort, i.e. a person was confirmed HIV-negative at cohort enrollment and confirmed HIV-positive at cohort follow-up.
   - HIV-positive Non-recent/Prevalent Infection: identified as HIV-positive as part of the pre-cohort survey based upon the serial algorithm for rapid testing approved for this study. See Appendix A.

3. HIV indeterminate: Indeterminate per the serial algorithm for rapid HIV testing that includes 4th generation Determine, approved for this study. See Appendix A.

5.2.2 Male circumcision status

Circumcision status of males will be determined by participant self-report. Male participants will be shown illustrations on laminated cards of a circumcised and uncircumcised penis and given supportive instructions from the interviewer. Men will be asked to identify which of the illustrations they most resemble. See Appendix R.

5.2.3 Household

A household will be defined as a group of people who share a physical structure such as a compound or homestead and who consume or make some contribution to food and other shared household resources. Households will be eligible for participation in this study if they are within the predefined study area.

5.2.4 Household resident

A household resident is defined as an individual who:

1. has been sharing a physical structure such as a compound or homestead and who has been consuming or making some contribution to food and other

shared household resources; guests who stayed at the household the night before will also be offered participation (if they are 18-49 years of age) ; and

2. is listed by the head of household as being a household resident or overnight guest the prior night on the Household Composition Form(Appendix B)

5.2.5 Head of household

The head of household is defined as the person who is recognized within the household as being the head.

5.3 Participant Inclusion/Exclusion Criteria

5.3.1 Pre-cohort surveys

- Inclusion criteria
  - Resides in selected household
  - Reports age between 18-49 years
  - Agrees to study procedures
  - Able to provide answers to the pre-cohort survey questions administered in either English or siSwati
  - Able to provide consent in either English or siSwati

5.3.2 Short-term Longitudinal cohorts (Cohorts A1 and A2)

- Inclusion criteria
  - Meets eligibility criteria for pre-cohort survey (listed above)
  - Confirmed HIV-negative by Swazi rapid HIV test algorithm
  - Willing to adhere and undergo all study visits and procedures
  - Able to provide answers to the study questionnaires administered in either English or siSwati
  - Able to provide consent in either English or siSwati

- Exclusion criteria
  - Stated intent to leave Swaziland indefinitely for work or any other reason in the next 6 months (baseline cohort, Cohort A1) or 12 months (post-MC ASI cohort, Cohort A2)

5.3.3 Longer-term Longitudinal cohort (Cohort B)

- Inclusion criteria
  - Completed 12- month follow-up in Cohort A2
  - For males, reported age between 25 – 34 years; for female, reported age between 18 – 24 years
  - Confirmed HIV-negative by Swazi rapid HIV test algorithm
  - Willing to adhere and undergo all study visits and procedures
  - Able to provide answers to the study questionnaires administered in either English or siSwati
  - Able to provide consent in either English or siSwati

- Exclusion criteria
  - Stated intent to leave Swaziland indefinitely for work or any other reason in the next 6 months

5.3.4 Cross-sectional survey (Cohort C)

- Inclusion criteria
  - Completed the pre-cohort A1
  - Confirmed HIV-positive during the pre-cohort A1 by SHIMS testing algorithm
  - Provided written informed consent to be contacted for future research
  - Willing to adhere and undergo all study visits and procedures
  - Able to provide answers to the study questionnaires administered in either English or siSwati
  - Able to provide consent in either English or siSwati

- Exclusion criteria
  - Refused HIV test results during pre-cohort survey
  - Expired consent to be contacted for future research (i.e., more than 12 months have passed since the individual's pre-cohort survey visit occurred)

5.4 Justification for exclusion of sub-segments of the population

Except for the exclusion criteria described above, sub-segments of the population will not be excluded from participation. Persons < 18 years of age will be excluded from study participation, because the age of consent for research participation in Swaziland is 18 years and because HIV incidence in men, the primary population under study, is estimated to increase significantly after 18 years of age. Persons with a stated intention of leaving Swaziland indefinitely may be more easily lost-to-follow-up. Substantial attrition from these persons could reduce the power of the study, and ultimately jeopardize the ability of the study to detect differences in the key study outcomes. During the pre-cohort survey visit, willing participants provided written informed consent to be contact for future research for up to 12 months. For this reason, the cross-sectional survey (Cohort C) excludes participation by those whose consent for future research has expired.

6.0 STUDY PROCEDURES

6.1 Pre-cohort Survey

A size-proportional, representative sample of enumeration areas will be selected. An exhaustive listing of households within each sampled enumeration area will

subsequently occur, and a random sample of households within each enumeration area will be drawn. All eligible persons within sampled households may participate.

6.1.1 Recruitment

Study staff will approach all households included in the sample and make appropriate introductions, provide information about the study, identify the head of household, and ask him or her to identify potentially eligible adults, using the Household Composition Form (Appendix B).

Designated heads of households will be identified and told briefly about the survey as an invitation to learn more. Those who are willing will be asked to provide the age and sex of all usual household members and overnight visitors as well as basic socio-demographic profile of the household. This information will be entered on the Household Composition Form (Appendix B). From this information, household members and those who stayed in the household the previous night who are 18-49 will be identified as eligible to participate.

Those individuals who meet the eligibility criteria will be asked to participate in the pre-cohort survey. Those who decline will be thanked for their time and asked to provide basic information about their reason for declining via the Refusal: Pre-cohort Survey Form (Appendix D) to assist in characterizing the impact of refusal on study outcomes.

Those who agree to participate will be asked to designate a relatively private location either inside or outside their home where the remainder of activities may be conducted with as much privacy as possible, as appropriate. From this chosen area, the participant will be asked to first provide written informed consent for the pre-cohort survey using the procedures described in Section 9.0. See Appendix C.

6.1.2 Enrollment

After providing written informed consent, individuals participating in the pre-cohort survey will receive:

- An interviewer-administered questionnaire that assesses demographic, behavioral characteristics, self-reported MC status and HIV testing history.

  Participants who report 2 or more sexual partners in the last 6 months will be asked additional questions regarding multiple and concurrent partnerships.

  These questions comprise the Case Report Form: Routine Survey (Appendix E)
- Approximately 500 men and 500 women will be randomly selected to receive a more detailed, extended questionnaire, estimated to take an additional 15-

20 minutes to administer. These questions comprise the Case Report Form: Extended Survey. See Appendix F.

- Standardized pre- and post-test HIV risk reduction counseling and HIV testing using the serial algorithm for rapid HIV testing that is approved for this study (Appendix A).
- Counseling regarding importance of HIV testing for partners and referral to HIV testing sites
- Blood draw of approximately 7-10mL

Once HIV testing is completed in the home, study staff will follow different procedures based on the participant's HIV test result(s). (Note: If after consenting to participate in the survey a participant then refuses the rapid HIV test, then he or she will be re-classified as refusing to participate in the study, and no further study procedures will be conducted. These individuals will be asked to provide basic information about their reason for declining via the Refusal: Pre-cohort Survey Form (Appendix D).)

6.1.2.1 Study procedures for persons who test HIV-positive

- Individuals who test HIV-positive in the home during the pre-cohort surveys will not be eligible to participate in the short-term longitudinal cohorts, Cohort A1 and A2. Likewise, men 25-34 years of age and women 18-24 years of age who test HIV-positive at the follow-up visit for Cohort A2 will not be eligible for enrollment in Cohort B.
- Counseling about the benefits of HIV care and treatment services and information to help such persons link to these services will be provided.
- Counseling regarding the importance of HIV testing of partners and referral to HIV testing sites will be provided.
- Persons who test HIV-positive in the laboratory (following an indeterminate test result in the home) will have their result returned to them in their home within 2 weeks, and the same considerations around cohort eligibility and care and treatment outlined in the three bullet points immediately above will be made.

6.1.2.2 Study procedures for persons who test HIV-negative

- Individuals who test HIV-negative in the home during the pre-cohort surveys will be recruited for participation in the short- term longitudinal cohorts, Cohort A1 and A2. Men 25-34 years of age and women 18-24 years of age who test HIV-negative at the follow-up visit for Cohort A2 will be offered enrollment in Cohort B.
- Persons who test HIV-negative in the laboratory (following an indeterminate test result in the home) will have their result returned to them in their home within 2 weeks, and the same consideration regarding cohort eligibility outlined in the bullet point immediately above will be made.

6.1.2.3 Study procedures for persons with an indeterminate HIV status

- Blood specimens from persons with an indeterminate HIV status according to the results of the rapid tests performed in the home will undergo additional testing at the National Reference Laboratory (Appendix A). The testing algorithm-defined results (Appendix A) from the additional lab test(s) at NRL will determine subsequent activities and timelines:
    - HIV-positive by EIA testing in the lab: Staff will return to the participant's home within approximately two weeks of the initial specimen collection date and inform the participant of his/her HIV-positive test result. Individuals whose blood specimen tests HIV-positive by EIA in the lab and who are not currently enrolled in HIV care and treatment services, will be counseled about the benefits of HIV care and treatment services and provided with information to help them link to these services. Such individuals will also be counseled regarding the importance of HIV testing of partners and referral to HIV testing sites will be provided
    - HIV-negative by EIA/NAT testing in the lab: Staff will return to the participant's home within approximately two weeks of the initial specimen collection date and inform the participant of his/her HIV-negative test result. Individuals whose blood specimen tests HIV-negative in the lab as part of testing during the pre-cohort surveys will be recruited for participation in the short- term longitudinal cohorts, Cohort A1 and A2. Men 25-34 years of age and women 18-24 years of age whose blood specimen tests HIV-negative in the lab at the follow-up visit for Cohort A2 will be offered enrollment in Cohort B.
    - HIV status indeterminate by EIA/NAT testing in the lab: Staff will return to the participant's home within six weeks of the initial specimen collection date and inform the participant of the inconclusive HIV test result. The participant will be encouraged to have blood drawn at that time so that the rapid testing algorithm may be repeated in the home. Results of the repeat, out-of-cycle rapid testing will determine subsequent activities, as outlined in sections 6.1.2.1 – 6.1.2.3 above.
        - Individual participants with indeterminate test results in the lab by EIA/NAT on two separate blood specimens collected 6 weeks apart will be adjudicated on a case-by-case basis.
- A total of 4 attempts over a period of 4 weeks will be made to locate participants to notify them of their HIV status based upon EIA/NAT testing at NRL (and provide repeat HIV testing, if appropriate). If after 4 attempts, the participant is not found, they will be considered lost-to-follow-up.

<u>6.2 Baseline and Post MC ASI Cohorts (A1 and A2)</u>

6.2.1 Recruitment

Participants completing the pre-cohort survey who test HIV-negative will be invited to participate in the baseline or post MC ASI cohorts (A1 or A2). Possible candidates will be provided with a brief description of the cohort study to determine their interest in the study.

Participants who are willing to participate will be asked to provide a separate (different from the pre-cohort survey informed consent) written informed consent using the procedures described Section 9.0. See Appendix H (baseline MC ASI or Cohort A1 ICF) and O (post MC ASI or Cohort A2 ICF).

Eligible men and women who enroll in the baseline cohort (A1) will be followed-up 6 months later. Those who enroll in the post-MC ASI cohort (A2) will be followed-up 12 months later.

Those persons who are eligible but decline enrollment in the cohort will be thanked for their time and asked to provide basic information, using the Refusal: Short Term Cohort Form, to assess reasons for refusal and to assist in characterization of the impact of refusal on study outcomes. See Appendix J.

6.2.2 Enrollment

After providing informed consent for cohort participation, cohort participants will be asked to provide additional locator information to assist with future contact. Telephone numbers, usual hangouts, names of family members/friends who can be contacted for tracing participants with information as to whether the participant agrees to such contact by study staff will be requested. Locator information questions comprise the Locator Information Form. See Appendix I.

6.2.3 Interim Telephone calls

Study staff will attempt to contact cohort participants at 3 month intervals by telephone after enrollment to update locator information and, if needed, approximately two weeks before their scheduled follow-up visit. Cohort participants will be provided with a study telephone number to call for updating their information or for seeking information regarding the study assessments and anticipated scheduled visits. Cohort participants determined to have moved within Swaziland will be visited at their current residence at the time of follow-up assessment (or alternate location of their choice).

6.2.4 Follow-up Visits

Cohort A1 and A2 participants will be visited again at their place of residence for a follow-up visit at 6 months (Cohort A1)) or 12 months (Cohort A2) after enrollment.

For each follow-up appointment at the home, at least 3 attempts will be made by study staff to establish contact in person and complete the follow-up visit activities. Visits will

ideally be completed within a specified ± 14 day window around target follow-up dates. The allowable follow-up dates, however, will extend to the date of study conclusion. Detailed information regarding visit windows will be thoroughly described in the SHIMS SOP on retention. The following procedures/assessments are to be performed at the cohort follow-up study visits:

- Verification of the participant's identity and participation in the study
- Selection of a relatively private location either inside or outside the participant's home, so that the follow-up visit may take place with as much privacy as possible, as appropriate review of the study goals and procedures
- For men, an interviewer-administered questionnaire to detect changes in self-reported MC status
- Participants who were uncircumcised at cohort enrollment but circumcised at cohort follow-up will be asked to provide information about the date and location of the MC surgery (Appendix K). Their medical records related to their MC surgery will be abstracted at the facility that performed the MC surgery to determine the date of surgery.
- Blood draw of approximately 7-10mL
  - HIV testing and counseling using the serial algorithm for rapid HIV testing that is approved for this study (Appendix A).
  - See procedures outlined in Section 6.1.2.1-6.1.2.3 for instructions on handling HIV test results.

Those who decline cohort follow-up will be thanked for their time and asked to provide basic information about their reason for refusal to assist in characterizing the impact of refusal on study outcomes. This information will be documented in the appropriate section of the Short/Long-term Cohort Follow-up CRF(s) (Appendices K-N).

6.2.4.1 Additional Study procedures for participants who test HIV-positive at the follow-up visit

- Interviewer administered questions to assess whether the participant has previously tested HIV-positive and whether HIV care and treatment has been sought.
- Counseling about the benefits of HIV care and treatment services and provision of information to help them link to these services.
- Counseling regarding importance of HIV testing of partners and referral to HIV testing sites.
- Male participants identified as newly circumcised ***and*** newly HIV-infected at follow-up (were uncircumcised at cohort enrollment) will be asked to provide information about the date and location of the MC surgery, whether they had an HIV test the day of the MC surgery, and the HIV test result, to help determine HIV status at the time of circumcision. Their medical records related to their MC surgery will be abstracted at the facility that performed the MC surgery to determine whether an HIV test was conducted pre-operatively and the results of the test, if done.

6.2.5 Cross-Sectional Cohort C

6.2.5.1 Recruitment

A cluster sampled subset of pre-cohort A1 survey participants who test HIV-positive will be invited to participate in the cross-sectional survey (Cross-Sectional Cohort C) at month 0, approximately 6 to 12 months after their initial pre-cohort A1 visit. Only those who consented to be contacted for future research in the pre-cohort A1 Informed Consent Form will be offered participation. Upon first contact with potentially eligible participants, verification of the person's identity and participation in the pre-cohort A1 survey will be conducted according to existing SHIMS SOPs. Procedures will ensure that the potentially eligible participant's HIV-positive status (as determined during the pre-cohort A1 survey) is not revealed until after identity verification.

After identity verification, potential participants will be asked to designate a relatively private location either inside or outside their home. From this chosen area, the potential participant will be provided with a brief description of the cross-sectional survey. Those who agree to participate will be asked to provide written informed consent for the cross-sectional survey using the procedures described in Section 9.0. See Appendix U.

Those persons who are eligible but decline participation in the Cross-Sectional Cohort C sub-study will be thanked for their time and asked to provide basic information, using the Refusal: Cross-Sectional Survey C Form, to assess reasons for refusal and to assist in characterization of the impact of refusal on study outcomes. See Appendix V.

6.2.5.2 Enrollment Procedures

After participants have given written informed consent, the following procedures/assessments will be performed at the cross-sectional survey visit:

- An interviewer-administered questionnaire with questions on demographic characteristics, current ART use, and locator information to be used for returning CD4 test results to the participant (see Appendix W and Section 6.2.5.3).
- Review of the participant's HIV clinic card or PMTCT/ANC card, if available
- Blood draw of approximately 7-10 mL
    - See procedures outlined in Section 6.2.5.3 for instructions on handling of CD4 test results.
- Counsel about the benefits of HIV care and treatment services and provision of information to help them link to these services.
- Issue a referral to appropriate health facility if applicable.

6.2.5.3 Additional study procedures

All Cross-Sectional Cohort C participants who consent to have their CD4 result returned to them will be visited again at their place of residence approximately 1-28 days after enrollment. As often as possible, the same field worker who enrolled the participant into Cohort C will return the CD4 results. All field workers have signed a statement of confidentiality and have been trained on the importance of protecting participant confidentiality. .At least 3 in-person visit attempts will be made by study staff to establish contact and to return the CD4 result to the participant. Details regarding the return of CD4 results are described in SHIMS SOPs.

The following procedures are to be performed at the return visit:

- Verification of the participant's identity and participation in the study
- Selection of a relatively private location either inside or outside the participant's home
- Provide the participant with a paper copy of their CD4 test result. This will be accompanied by information to the provider to explain the context of participant CD4 testing and return of results. See Appendix Y.
- Encourage the participant to discuss the CD4 test result with a health care provider. Study staff will not provide clinical interpretation of the CD4 test.
- Counsel about the benefits of HIV care and treatment services and provision of information to help them link to these services.
- Counsel regarding importance of HIV testing of partners and referral to HIV testing sites.

Among all Cohort C participants who consent to storage of their blood specimen, viral load testing will be conducted.

6.3 Longitudinal high risk cohort ("B")

6.3.1 Recruitment

A subset of Cohort A2 participants who test HIV-negative at the 12-month follow-up visit and who are eligible for the longitudinal high risk cohort (Cohort B) will be invited to participate. Possible candidates will be provided with a brief description of the cohort study and receive pre-screening to determine their interest in the study and potential eligibility.

Participants who are willing to participate will be asked to provide a separate (different from the pre-cohort or post MC ASI cohort informed consents) written informed consent using the procedures described Section 9.0. See Appendix M.

Eligible men and women who enroll in this longitudinal, high risk cohort (Cohort B) will be followed-up for an additional6-18 months.

Those persons who are eligible but decline enrollment in the cohort will be thanked for their time and asked to provide basic information, using the Refusal: Long Term Cohort

Form, to assess reasons for refusal and to assist in characterization of the impact of refusal on study outcomes. See Appendix J.

6.3.2 Enrollment

After providing informed consent for Cohort B participation, participants will be asked to provide additional locator information to assist with future contact. Telephone numbers, usual hangouts, names of family members/friends who can be contacted for tracing participants with information as to whether the participant agrees to such contact by study staff will be requested. Locator information questions comprise the Locator Information Form. See Appendix I.

6.3.3 Interim Telephone calls

Study staff will attempt to contact Cohort B participants at approximately 3 month intervals by telephone after enrollment to update locator information and, if needed, two weeks before their scheduled follow-up visit. Cohort participants will be provided with a study telephone number to call for updating their information or for seeking information regarding the study assessments and anticipated scheduled visits. Cohort B participants determined to have moved within Swaziland will be visited at their current residence at the time of follow-up assessment (or alternate location of their choice).

6.3.4 Follow-up Visits

Longitudinal cohort participants will be visited again at their place of residence for a follow-up visit at 6-18 months after enrollment. For the follow-up appointment at the home, at least 3 attempts will be made by study staff to establish contact in person and complete the follow-up visit activities. Visits will ideally be completed within a specified ± 14 day window around target follow-up dates. The allowable follow-up dates, however, will extend to the date of study conclusion. Detailed information regarding visit windows will be thoroughly described in the SHIMS SOP on retention.
The following procedures/assessments are to be performed at the cohort follow-up study visits:

- Verification of the participant's identity and participation in the study
- Selection of a relatively private location either inside or outside the participant's home, so that the follow-up visit may take place with as much privacy as possible, as appropriate
- Review of the study goals and procedures
- For men, an interviewer-administered questionnaire to detect changes in self-reported MC status
    - Participants who were uncircumcised at cohort enrollment but circumcised at cohort follow-up will be asked to provide information about the date and location of the MC surgery (Appendix N). Their medical records related to their MC surgery will be abstracted at the facility that performed the MC surgery to determine the date of surgery.

- An interviewer-administered questionnaire that assesses demographic, behavioral characteristics, self-reported MC status, HIV testing history and experience with binge drinking.
- Blood draw of approximately 7-10mL
- HIV testing and counseling using the serial algorithm for rapid HIV testing that is approved for this study.
    - See procedures outlined in Section 6.1.2.1.-6.1.2.3. for instructions on handling HIV test results.

Those who decline cohort follow-up will be thanked for their time and asked to provide basic information about their reason for refusal, to assist in characterizing the impact of refusal on study outcomes. This information will be documented on the Refusal: Cohort Participation Form (Appendix J).

6.3.4.1 Additional Study procedures for participants who test HIV-positive at the follow-up visit

- Interviewer administered survey to assess whether the participant has previously tested HIV-positive and whether HIV care and treatment has been sought.
- Counseling about the benefits of HIV care and treatment services and provision of information to help them link to these services.
- Counseling regarding importance of HIV testing of partners and referral to HIV testing sites. Male participants identified as newly circumcised ***and*** newly HIV-infected at follow-up (were uncircumcised at cohort enrollment) will be asked to provide information about the date and location of the MC surgery, whether they had an HIV test the day of the MC surgery, and the HIV test result, to help determine HIV status at the time of circumcision. Their medical records related to their MC surgery will be abstracted at the facility that performed the MC surgery to determine whether an HIV test was conducted pre-operatively and the results of the test, if done.

6.4 Participant Retention for Cohorts A1, A2 and B

Participant follow-up visits may take place in a location that assures adequate privacy and confidentiality, such within or near the participant's home provided there is adequate privacy, a community-based organization or a tent. Once a participant has enrolled in the study, the study staff will make every reasonable effort to retain them for the entire study period. Every effort will be made to maintain lost- to- follow-up at minimum. Approximately 10 and 12% per year lost-to-follow-up of enrolled female and male participants, respectively, during participation in each cohort is anticipated based on the rates of lost-to-follow-up from previous trials. Study enrollment and retention will be closely monitored by the protocol team and the SHIMS Steering Committee.

Study staff are responsible for developing and implementing local standard operating procedures (SOPs) to achieve high levels of follow-up, the following procedures are examples of locator devices and retention techniques that will be implemented during the study:

- Thorough description of the study visit schedule and procedural requirements during the informed consent process and re-emphasis at each study visit.
- Explanation of the importance of adhering to study visits to the overall success of the study.
- Completion of Locator Information Form (Appendix I) with multiple means to contact participants and to include place of residence and important landmarks, if use of postal address is not feasible) to be updated at every study contact.
- Assisting with childcare and/or transportation services whenever possible.
- Flexibility in scheduling time and location of follow-up visits
- Use of appropriate and timely visit reminder mechanisms.
- Immediate and multifaceted follow-up of missed visits.
- Mobilization of trained outreach workers or "tracers" to complete in-person contact with participants at their homes and/or other community locations.
- Obtain advance permission using the Locator Information Forms to contact participants who have missed visits during the study by mail, phone, and if possible, in person.
- Regular review of data on scheduled contacts/visits as well as missed contacts/visits to ensure prompt reconnection to participants.
- Community education to increase awareness about HIV/AIDS and importance of prevention.
- Garnering support of community advisory boards, advocacy groups and others in support of the study.

For each participant, study staff will obtain confidential contact information using the locator forms. This information will be updated during each study visit (both in-person and via phone). In the event that a participant misses a scheduled appointment, study staff will try to establish communication with the participants through all agreed upon means relevant to specific participant (e.g., telephone, text messages, toll-free lines, e-mail, mail contact, and home or workplace visits). The importance of maintaining phone contact and scheduled visits will be emphasized to all study participants.

6.5 Study Assessments

6.5.1 Laboratory Assessments

HIV status: At all study visits HIV status will be determined using a serial algorithm for rapid tests approved by the Swaziland National Reference Laboratory. (Appendix A). HIV rapid test result data will be recorded on the HIV Test Results Case Report Form. (Appendix G)

Viral load measurement: Men and women who test HIV-positive via an HIV rapid test during the pre-cohort surveys will have blood specimens tested for viral load. Men and women who participate in Cross Sectional Cohort C and who agree to have their blood specimen stored for future testing will have their specimens tested for viral load.

CD4 count: Blood specimens from participants in Cross-Sectional Cohort C will be analyzed for CD4 count.

6.5.2 Behavioral Assessments

Self-reported risk behaviors will be collected from all participants in the Pre-cohort Survey and at follow-up visits for all cohorts using structured questionnaires. Variables for sexual risk behaviors include number, type (regular/casual), and concurrency of sex partners, number and proportion of unprotected sex encounters, and knowledge of own and sex partner(s) HIV status. For those who report having known they were HIV-infected prior to testing for this study, information on date of diagnosis, linkages to HIV medical care, and ARV use will be collected.

6.5.3 Other assessments

- Circumcision status of males will be determined by participant self-report. Male participants will be shown illustrations on laminated cards of a circumcised and uncircumcised penis and given supportive instructions from the interviewer. Men will be asked to identify which of the illustrations they most resemble.

- Engagement in HIV care and use of ART: self-reported information about HIV care and treatment will be collected from all participants found to be HIV positive at any study visit.
    - In Cross-Sectional Cohort C, study staff will collect self-reported information about HIV care and treatment from participants. Study staff will also ask participants to show their HIV clinic card (and PMTCT/ANC card), if available, to study staff to collect information on HIV medical care and ART usage.

- Demographics including age, sex, marital status, occupation, and employment status will be collected. Information on knowledge about HIV transmission and treatment and history of exposure to HIV risk reduction campaigns will be collected.

6.6 Study forms

Data collected during the study through HIV testing, questionnaires will be recorded in the appropriate case report form (CRF). CRFs are divided into six sets: 1) Pre-Cohort Survey; 2) Short-Term Cohort (Cohort A1 and A2); 3) Short-Term Cohort Follow-up; 4) Longer-Term Cohort (Cohort B); 5) Longer-Term Cohort Follow-up; and 6) Cross-Sectional Cohort (Cohort C).

6.6.1 Pre-Cohort Survey

- *Household Composition Form*. The *Household Composition Form* will direct questions and answers between the staff and the potential participants in the household. See Appendix B.

- *Refusal: Pre-cohort Survey Form*. The *Refusal: Pre-cohort Survey Form* contains a single question about the reason an individual declined participation to help characterize how those who refuse may differ from those who agree to participate. See Appendix D.

- *Case Report Form: Routine Survey* (Appendix E). The *Case Report Form: Routine Survey* consists of interview-administered questions about the following topics:

    - *Demographic Information and Sexual Activity*. Questions about basic demographics, and behaviors that may be related to risk for HIV infection. Variables for sexual risk behaviors include number, type (regular/casual), and concurrency of sex partners, number and proportion of unprotected sex encounters, and knowledge of own and sex partner(s) HIV status. Demographic variables include age, sex, marital status, occupation, and employment status.

    - *Male Circumcision Status (for males only). P*rovides space to record the self-reported circumcision status of the participant.

    - *HIV Status Information*. Questions about HIV testing history, date of last HIV test, HIV results and current HIV treatment.

- *Case Report Form: Extended Survey* (Appendix F). The *Case Report Form: Extended Survey* consists of interview-administered questions about the following topics:

    - All topics listed in the *Case Report Form Routine Survey*.

    - *Experience with Binge Drinking*. Questions about participant's alcohol intake and habits.

- *Beliefs Regarding Male Circumcision*. Questions about circumcision and participant beliefs surrounding the health benefits of MC.

- *Case Report Form: HIV Test Results* (Appendix G). The *Case Report Form: HIV Results* provides space to record the results from the rapid HIV test(s) conducted in the home.

6.6.2 Short-Tem Cohort (Cohort A1 and A2) Recruitment/Enrollment

- *Locator Information Form* (Appendix I). The *Locator Information Form* includes interviewer-administered questions about telephone numbers, usual hangouts, and names of family members/friends who can be contacted for tracing participants with information as to whether the participant agrees to such contact by study.

- *Refusal: Cohort Participation Form* (Appendix J). The *Refusal: Cohort Participation Form* includes an interviewer-administered question about the reason the participant elected to not participate in the short-term cohort.

6.6.3 Short-Term Cohort Follow-up

Case Report Form: Short-Term Cohort Follow-up (Appendix K). The *Case Report Form: Short-Term Cohort Follow-up* consists of interview-administered questions about self-reported circumcision status, and date and location of circumcision if circumcised since the previous assessment.
- *Termination Form*. *The Termination Form notates the reasons the participant is not completing* the Short-Term Cohort Follow-up Visit.

6.6.4 Longer-Term Cohort (Cohort B)

- *Locator Information Form* (Appendix I). The *Locator Information: Long-Term Cohort Form* includes interviewer-administered questions about telephone numbers, usual hangouts, and names of family members/friends who can be contacted for tracing participants with information as to whether the participant agrees to such contact by study.

- *Refusal: Cohort Participation Form* (Appendix J). The *Refusal: Cohort Participation Form* includes an interviewer-administered question about the reason the participant elected to not participate in the long-term cohort.

- *Case Report Form: Longer-Term Cohort Baseline/Follow-up* (Appendix N). The *Case Report Form: Longer-Term Cohort Baseline/Follow-up* is comprised of the same interview-administered questions that are listed in the *Case Report Form: Extended Survey*. Topics include:

- *Demographic Information and Sexual Activity*. Questions about basic demographics, and behaviors that may be related to risk for HIV infection. Variables for sexual risk behaviors include number, type (regular/casual), and concurrency of sex partners, number and proportion of unprotected sex encounters, and knowledge of own and sex partner(s) HIV status. Demographic variables include age, sex, marital status, occupation, and employment status.

- *Male Circumcision Status (for males only). P*rovides space to record the self-reported circumcision status of the participant.

- *HIV Status Information.* Questions about HIV testing history, date of last HIV test, HIV results and current HIV treatment.

- *Experience with Binge Drinking*. Questions about participant's alcohol intake and habits.

- *Beliefs Regarding Male Circumcision.* Questions about circumcision and participant beliefs surrounding the health benefits of MC.

6.6.5 Longer-Term Cohort Follow-up (Month 42)

- *Locator Information: Long-Term Cohort Baseline/Follow-up Form* (Appendix N). See Section 6.6.4 for a detailed description of this section.

- *Case Report Form: Long-Term Cohort Circumcision Status Follow-up*. See Section 6.6.3 for a detailed description of this section.

- *Case Report Form: Long-Term Cohort HIV Test Results Follow-up*. See Section 6.6.3 for a detailed description of this section.

- *Termination Form*. The *Termination Form* notates the reasons the participant is not completing the Longer-Term Cohort Follow-up Visit.

6.6.6 Cross-Sectional C (Month 6-12)

- *Locator Information Form: Cross-Sectional Survey C* (Appendix W). The *Locator Information Form* includes interviewer-administered questions about telephone numbers and names of family members/friends who can be contacted for tracing participants with information as to whether the participant agrees to such contact.

- *Refusal: Cross-Sectional C*. The *Refusal: Cross-Sectional C Form* contains a single question about the reason an individual declined participation to help characterize how those who refuse may differ from those who agree to participate. See Appendix V.

- *Case Report Form: Cross-Sectional C Survey* (Appendix X). The *Case Report Form: Cross-Sectional C Survey* consists of interview-administered questions and data abstraction from participant client card about the following topic:

    - *ART Status Information*. Questions about HIV diagnosis, utilization of HIV clinical services and ART usage.

6.7 Laboratory Procedures

6.7.1 Sample collection, processing and storage

During the Pre-cohort Survey and all Cohort Follow-up visits, each participant will be asked to provide a sample of approximately 7-10 mL of blood, , collected via venipuncture. Sample transportation, processing and storage procedures are described in the SHIMS Study Operations Manual.

6.7.2 Testing

Rapid testing
Rapid testing will be done in the home following the serial algorithm for rapid HIV testing that includes 4th generation Determine, approved for this study. See Appendix A.

Viral load measurement
Viral load measurement will be done on all plasma samples from individuals who test HIV-positive during rapid testing in the home or by EIA in the lab at the time of the pre-cohort surveys. It will also be conducted among Cross sectional Cohort C participants who consent to have their blood specimen stored for future research. Specific details regarding the type of HIV viral load assays that will be used in this study are outlined in the Laboratory Standard Operating Procedures (SOPs).

EIA/NAT testing
All plasma samples from individuals with indeterminate HIV-test results will be tested within 7 days from the time that the sample was collected with an EIA or NAT according to the rapid HIV testing algorithm approved for this study by the NRL (Appendix A). Specific details regarding the type of assays that will be used in this study are outlined in the Laboratory Standard Operating Procedures (SOPs)

All participants with an indeterminate HIV status based upon EIA/NAT testing in the laboratory will receive repeat home-based rapid HIV testing within 6 weeks (see 6.1.2.3.).

CD4 count

CD4 enumeration will be done on blood samples from Cross-Sectional Cohort C participants. Specific details regarding the type of CD4 count assays that will be used in this study are outlined in the Laboratory Standard Operating Procedures (SOPs).

6.7.3 Quality assurance and quality control for laboratory testing

Good laboratory practices will be followed for all laboratory testing. Standard operating procedures will be used for all test and Quality Control procedures. Procedures will be in place for performing and documenting the quality of a specimen, including storage and transport conditions, monitoring of equipment and temperatures, and function indicators. At the Swazi laboratory, additional storage facilities consisting of refrigerators (4°C), freezers (-20°C), and ultra freezers (-70°C) will be available for the short term and long term storage of specimens. An effective QA/QC system will be maintained to ensure integrity of the specimens will be in place at the laboratory site. Each stored specimen will have a unique identifier which is unlinked from the study participant's name. The specimens will be stored for ten years.

6.8 Training of study personnel

Prior to initiation of the study, and again prior to the cohort follow-up assessments, all personnel that will have contact with participants will participate in a multi-day study-specific training. The curriculum of the training will cover, but will not be limited to the following: rationale, purpose and scientific objectives of the study; study design and methodology; conduct of study assessments, tracking of participants, completion of study forms, and data collection; staff responsibilities; locator and recruiting participants; procedures for enrolling participants into the study; universal precautions, communication skills, safety in the field, ethical guidelines for research including participants' rights; procedures for obtaining informed consent; and confidentiality requirements .

Study staff will receive a hands-on training that will include an introduction to data collection forms and procedures. Role playing and mock interviews will be an essential component of the training and protocol team members will act as both the trainer and the mock respondent. The trainer will take the staff through each step of the interviewing process, from enrolling participants to ending the interview and completing any necessary forms. Within the interview itself, the trainer will demonstrate both the interviewing task being required of them as well as the response task being required of the participant. Question-by-question instructions and the use of any visual or recall aids will be reviewed. Study staff will also be paired together with each taking a turn at interviewing their partner. This method more closely resembles an actual interview and the performance of each staff member can be more carefully monitored as the trainers walk around to observe and provide individual instruction. Study staff will be given a chance to practice both the English and siSwati versions of all the assessments in order to discuss and resolve any issues. Training for study staff is expected to take approximately 1-2 weeks per interviewer and additional follow-up training, as necessary.

Study staff responsible for providing HIV testing and counseling will receive additional training in these procedures. To ensure testing and counseling competency, study staff will complete the specific required training curriculum recognized by the GKOS and Ministry of Health. Staff will also be trained on the use and completion of all study forms related to these services. Mock participants will also be utilized for this component of the training. Study staff members who will collect blood samples and conduct laboratory analyses will receive training in universal precautions, sample collection and testing of study samples. Such staff will be trained in Good Clinical and Laboratory Practice (GCLP), quality control (QC) and quality assurance (QA), safety, post-exposure prophylaxes (PEP), methods of records keeping, and maintenance of laboratory related study files.For all study staff, there will be additional training days scheduled during the study for refresher training. During these refresher trainings, study staff will review study procedures and discuss any challenges encountered. In addition, all study staff will receive a Field Operations Procedures Manual which will serve as a procedural guide during actual data collection. Additionally, all staff who through the course of their work have knowledge of, or access to, personal information about participants will be required to sign a confidentiality agreement. (Appendix Q).

7.0 STATISTICAL CONSIDERATIONS

7.1 Overview of Statistical Considerations

The overall statistical approach follows from the goal of measuring the population effect of the MC ASI. This is achieved by estimating HIV incidence using two nationally representative, household-based samples of the Swaziland population, one before and one after the MC ASI (Cohorts A1 and A2). The study is powered for primary objective # 1a, with at least 80% power to detect a 45% reduction in the HIV seroincidence rate of HIV-uninfected men 18-49 in Swaziland from pre to post-MC ASI.

A longer longitudinal cohort (Cohort B) of the highest risk age group of HIV uninfected men and women from Cohort A2 will measure HIV incidence at an additional time point, at a greater period post- MC ASI campaign.

The two-stage cluster sampling scheme is designed to achieve a nationally representative sample of households. All consenting, eligible HIV uninfected men and women 18-49 will be followed to estimate HIV incidence. Weighted estimation will be used in order to achieve population based estimates of HIV seroincidence, using weights corresponding to the national household sample achieved.

The number of HIV uninfected men is chosen to achieve a nationally representative sample of men to have at least 80% power to detect 45% reduction in the HIV seroincidence rate of HIV uninfected men 18-49 in Swaziland from pre to post-MC ASI. The number of households approached will yield a sample of women from which we can estimate the reduction in HIV seroincidence rate of HIV uninfected women 18-49 in Swaziland from pre to post-MC ASI.

The longer-term longitudinal cohort, Cohort B, will be the subset of men aged 25-34 and women aged 18-25 from Cohort A2. Cohort B will be used to estimate the trend in

seroincidence following the MCASI campaign. Cohort B will also be used to assess the trend in sexual risk behaviors following the MC ASI campaign.

The pre vs. post MC ASI comparison of seroincidence rates does not allow causal interpretation of the MC ASI as the sole mechanism influencing observed change in HIV seroincidence rates. Likewise the assessment of trends in sexual behaviors over time does not allow causal interpretation of the MC ASI as the sole mechanism influencing observed change in levels of sexual risk behaviors. In this non-randomized design, other changes occurring over the same time period could also influence HIV seroincidence rates and sexual risk behaviors. The use of a nationally representative sample in each seroincidence cohort does allow a valid assessment of the population based change in HIV seroincidence in men and women 18-49 before and after the period in which the MC ASI occurred and sexual risk behaviors among the younger men (25-34) and women (18-24) at higher risk of HIV infection during and after the MC ASI.

7.2 Sampling, including sample size and statistical power

A nationally-representative sample of men and women will be identified for both the baseline and post-intervention surveys and cohorts via a two-stage cluster-based sampling of 'enumeration areas' and households. Enumeration areas are sampling points defined by the 2007 Swaziland Population and Housing Census. A total of approximately 575 enumerations areas will be drawn from the census sample frame of all 2000 enumeration areas with a probability of selection proportional to the number of households within the enumeration area. The enumeration areas in urban versus rural areas will be proportional to the urban versus rural distribution of the population. Between November 2010 and May 2011, staff will conduct an exhaustive listing of households within each sampled enumeration area. During household listing, study staff will use Global Positioning Systems (GPS) receiver to record the geographic coordinates of each cluster and household. In the pre-MCASI cohort, approximately 14,884 households will be sampled, and 12,301 households will participate in the study. From these households we expect to identify, recruit, screen, and enroll a sample size of 5,823 men and approximately 7,106 women in the first seroincidence cohort (Cohort A1). In the post-MC ASI cohort, approximately 15,758 households will be sampled, and 13.024 households will participate in the study. From these households we expect to identify, recruit, screen, and enroll a sample size of 6,165 men and approximately 7,524 women in the second seroincidence cohort (Cohort A2). From Cohort A2, we expect approximately 25% of the men will be 25-34 years old, and 29% of the women will be 18-24 years old, yielding the longer, 6 month longitudinal cohorts of 1,541 men and 2,182 women (Cohort B).

Statistical power calculations for primary objectives are included as Appendix P.

Primary Objective #1a:
To estimate HIV incidence in household-based representative sample of men at baseline and post MC ASI. (Cohort A1 vs A2)

The sample size for the men's Cohort A1 is 5,823 and for Cohort A2 is 6,165. Each person enrolled in Cohort A1 contributes 6 months of follow-up and in Cohort A2, each contributes 12 months of follow-up. The sample size is chosen to achieve sufficient "effective" person years to achieve 80% power to detect a 45% reduction in ratio of infection rates, assuming a Poisson distribution in each cohort of number of seroconversion events in total accumulated person years.

Note: Very similar sample sizes result from comparison of binomial probabilities of the proportion of the cohort infected in the pre vs. post MC ASI cohorts, provided exact methods are used. Poisson analysis is planned to accommodate varying lengths of follow-up time in the follow-up cohort.

- Men. The total number of person-years required to detect a 45% reduction in the HIV incidence rate from 2.0 per 100 person years (approximated baseline rate) to 1.1 per 100 person years (post-intervention) with a 1:2 ratio of person years in the two cohorts is 6,288. With a design effect of 1.25 and loss to follow-up of 10% and 15% in the pre- and post-MC ASI this yields a sample size of 5,823 men followed for 6 months and 6,165 men followed for 12 months in Cohort A1 and Cohort A2 respectively. (See Tables 1 and 2)

Table 1:

Effective person years required to detect 30-60% difference between infection rates with 6 month follow-up in Cohort A1, 12 month follow-up in Cohort A2, for two sided alpha of 0.05, and 80% power. Wald test statistic for slope in Poisson Regression, under assumption of simple random sample.

λ_1	λ_2	Ratio λ_2/λ_1	Total Effective[1] Person Years (Both serocohorts combined)
0.0200	0.0140	0.70	15865
0.0200	0.0130	0.65	11224
0.0200	0.0120	0.60	8272
0.0200	0.0110	0.55	6289
0.0200	0.0100	0.50	4901
0.0200	0.0090	0.45	3898
0.0200	0.0080	0.40	3155
0.0300	0.0210	0.70	10577
0.0300	0.0195	0.65	7483
0.0300	0.0180	0.60	5514
0.0300	0.0165	0.55	4192
0.0300	0.0150	0.50	3267
0.0300	0.0135	0.45	2599
0.0300	0.0120	0.40	2103

[1]Effective person years assume a simple random sample.

Number of households

The number of households required to achieve the effective sample size is shown in Table 2. The derivation of each of the adjustment factors is as follows:

- Design Effect: Design effect for HIV in men ages 15-49 of 1.25, and the DEFT of 1.32, are both based on the 2006-07 DHS.
- Household adjustment:
    - 13% will be vacant/non-contactable, 5% will refuse participation at the household level. Adjustment factor = 1/0.87x0.95 = 1.21
- Household individual adjustment
    - Men: 92% will have male residents within the eligible age range, 77%of men will be HIV uninfected, 99% will agree to cohort enrollment, 90% will be contactable, 95% will agree to pre-cohort survey. Adjustment factor = 1/(0.92x0.77x0.99x0.90x0.95)=2.11
- Loss to follow-up
    - Men: 90% and 85% of men will complete follow-up in the 6 and 12 month cohorts, respectively (accounting for loss-to-follow-up, interview/HIV test refusal at follow-up, data loss, and specimen loss totaling 10% and 15%, respectively). Lost to follow-up adjustment = 1.11 and 1.18

Table 2: Number of enrolled male participants, participating households and households in sample required to achieve the targeted effective number of person years.

Effective number person years	Design effect	Loss to follow-up adjustment	Number Males enrolled	Male household adjustment	Number of households participate	Household adjustment	Number of households sampled
Pre-MC ASI Male Seroincidence Cohort (Cohort A1) – 6 month follow-up period							
2096	1.25	1.11	**5823**	2.11	**12301**	1.21	14884
Post-MC ASI Male Seroincidence Cohort(Cohort A2) – 12 month follow-up period							
4192	1.25	1.18	**6165**	2.11	**13023**	1.21	15758

The decision to power the evaluation to detect a 45% reduction in incidence in men is based upon a combination of factors: 1) mathematical modeling estimations of HIV incidence reduction anticipated in men following MC scale-up to 80% coverage in 2011; and 2) meta-analyses of efficacy results from the 3 MC RCTs (See Figure 2). The proportional hazard risk ratio (efficacy) from the 2009 Cochrane Review meta-analysis using case analyses from the three MC RCTs was 0.46 (95% CI, 0.34 – 0.62), which equates to a 54% risk reduction against HIV acquisition [Sigfried, 2009].

Primary Objective #1b:

To estimate HIV incidence in household-based representative sample of women at baseline and post MC ASI (Cohort A1 vs A2)

Mathematical modeling estimation of HIV incidence reduction anticipated in women following MC scale-up to 80% coverage in 2011 is expected to produce less than a 45% reduction in women (See Figure 1). Newly diagnosing approximately 35,000 men and

enrolled approximately 4,500 on ART may also contribute to reductions in HIV incidence.

The household sample of 14,884 and 15.758 in pre- and post- MC-ASI is expected to yield an enrolled cohort of 7106 women and 7,524 in the pre- and post-MC ASI cohorts respectively. (See Table 3)

Number of effective women-years accumulated-
The number of effective women years accumulated from the household sample of 14,884 and 15.758 in pre- and post- MS-ASI is shown in Table 3. The derivation of each of the adjustment factors is as follows:

- Design Effect: Design effect for HIV in women ages 15-49 of 1.25, and the DEFT of 1.32, are both based on the 2006-07 DHS.
- Household adjustment
    - 13% will be vacant/non-contactable, 5% will refuse participation at the household level. Adjustment factor = 1/0.87x0.95 = 1.21
- Household individual adjustment
    - Women: 105% will have female residents within the eligible age range, 65% will be HIV uninfected, 99% will agree to cohort enrollment, 90% will be contactable, 95% will agree to pre-cohort survey. Adjustment factor = 1/(1.05x0.65x0.99x0.90x0.95)=1.73
- Loss to follow-up

Women: 92% and 88% of women will complete follow-up in the 6 and 12 month cohorts, respectively (accounting for loss-to-follow-up, interview/HIV test refusal, data loss, and specimen loss totaling 8% and 12%, respectively). Adjustment factor = 1/0.92=1.09 and 1/0.88=1.14 respectively.

Table 3: Number of enrolled female participants, given sample of 14,884 and 15.758 households in the pre- and post-MC ASI cohort.

Effective number person years	Design effect	Loss to follow-up adjustment	Number Females enrolled	Female household adjustment	Number of households participating	Household adjustment	Number of households sampled
Pre-MC ASIFemale Seroincidence Cohort (Cohort A1) – 6 month follow-up period							
2615	1.25	1.09	7106	1.73	12,301	1.21	14884
Post-MC ASIFemale Seroincidence Cohort (Cohort A2) – 12 month follow-up period							
5297	1.25	1.14	**7524**	1.73	13,023	1.21	15758

With total effective person years of 7912 in the women, assuming a seroincidence rate of 3.0 per 100 person years, and a sample ratio of 1:2, we have __68% power to detect a 30% reduction in HIV seroincidence and __82% power to detect a 35% reduction in HIV seroincidence.

Primary Objective #2a

To estimate HIV incidence rates in the highest risk age subsets of men ages 25-34 in the household-based, nationally representative sample for an additional 6-18 months after the MC ASI completion (Cohort B).

From the sample of 5823 men in the pre-MC-ASI cohort (Cohort Aa), we expect 25% of the men to be aged 25-34, a sample of 1456. From the sample of 6,165 men in the post-ASI cohort (Cohort A2), we expect 25% of the men to be aged 25-34, a sample of 1,541. If we compare the seroincidence in the 26-34 year old men 6 months prior to MC ASI to the seroincidence in the 18 months period (24-42 months following the end of the pre-MC-ASI cohort), we would have _80% power to detect a 50% reduction in HIV seroincidence, assuming a pre-MC-ASI seroincidence of 5.0 per 100 person years, after accounting for lost to follow-up of 12% per year, and a design effect of 1.25 in each cohort

Primary Objective #2b

To estimate HIV incidence rates in the highest risk age subsets of women ages 18-24 in the household-based, nationally representative sample for an additional 6-18 months after the MC ASI completion (Cohort B).

From the sample of 7524 women in the post-ASI cohort (Cohort A2), we expect 29% of the women to be aged 18-24, a sample of approximately 2183. If we compare the seroincidence in the 6 months prior to MC ASI to the seroincidence at 24-42 months following MC ASI, we would have _80% power to detect a 40% reduction assuming a seroincidence of 6.0 per 100 person years, after accounting for lost to follow-up of 10% per year, and a design effect of 1.25 in each cohort

Primary Objective #3

To estimate HIV incidence of circumcised compared to uncircumcised men in a household-based representative sample of men post- MC ASI (Cohort A2).

The6165 men in the post-MC ASI cohort (A2) gives 71% power to detect a 55% decrease in the HIV incidence rate ratio between circumcised and uncircumcised men, assuming 80% of the men are circumcised and 20% are uncircumcised (i.e. a ratio of 4:1) and the seroincidence rate in the uncircumcised men is 2.5 per 100 person years, 12% loss to follow-up. If 2/3 of the men are circumcised, the power is increased to 83%. As the comparison will be within the cohort, no adjustment for design effect is assumed.

As the primary objectives capture the most important scientific objectives of importance, power consideration are only given for these objectives.

Secondary Objectives #8, and #9

8) To describe the concurrent CD4 count and viral load distributions in a nationally representative sample of HIV-infected men and women ages 18-49, in the context

of ART use.

9) To estimate the proportion of HIV-infected men and women ages 18-49 who have a CD4 count <350 and who report no current ART use.

A sample of 1,000 HIV-infected men and women ages 18-49 years will be randomly selected from a subset of pre-cohort A1 survey participants who tested HIV seropositive.

The sample size derivation is based on the following assumptions:

- The proportion of HIV-infected individuals with CD4 counts in each of the following categories will be 25%: 0-200, 201-350, 351-500 and 501 cells/mm^3 or above. Therefore, the proportion of individuals with a CD4 count less than or equal to 350 is 50%.
- The proportion of ART-eligible adults (CD4 < 350) not currently on ART is 40%.
- The proportion of HIV-infected adults who are ART-eligible and not currently on ART is 20%. (.50)(.40)=.20
- The design effect of the multistage clustered sampling will not be more than 3

As seen in Table 4 below, an observed prevalence of 20% among a sample of 1,000 individuals will yield a half-width of a 95% confidence interval less than 4.3% (i.e., a confidence interval [15.7%-24.3%]).

Table 4: Half-width of 95% confidence intervals for the prevalence of ART-eligible adults not currently on ART

Prevalence	N = 750		N=1000		N=1250		N=1500	
	Design effect = 2	Design effect = 3	Design effect = 2	Design effect = 3	Design effect = 2	Design effect = 3	Design effect = 2	Design effect = 3
5%	2.21%	2.70%	1.91%	2.34%	1.71%	2.10%	1.56%	1.91%
10%	3.04%	3.72%	2.63%	3.22%	2.35%	2.88%	2.15%	2.63%
15%	3.62%	4.43%	3.13%	3.83%	2.80%	3.43%	2.56%	3.14%
20%	4.04%	4.95%	3.51%	4.30%	3.14%	3.85%	2.86%	3.50%
25%	4.38%	5.37%	3.79%	4.64%	3.39%	4.16%	3.10%	3.79%
30%	4.64%	5.68%	4.02%	4.92%	3.59%	4.40%	3.28%	4.02%
35%	4.82%	5.91%	4.19%	5.13%	3.73%	4.57%	3.41%	4.17%
40%	4.96%	6.08%	4.30%	5.27%	3.85%	4.71%	3.51%	4.30%
45%	5.03%	6.17%	4.36%	5.33%	3.90%	4.78%	3.56%	4.36%
50%	5.06%	6.20%	4.38%	5.37%	2.53%	6.20%	6.20%	6.20%

Regarding viral load estimates, it is assumed that:

- The proportion of HIV-infected individuals with viral load counts in each of the following categories will be 25%: < 1000, 1000-50,000, 50,001-100,000, and > 100,000 copies/ml.
- The proportion of HIV-infected individuals with undetectable viral load and who are not currently on ART is 5%.
- The design effect of the multistage clustered sampling will not be more than 3

Per Table 4, an observed prevalence of 25% among a sample of 1,000 individuals will yield a half-width of a 95% confidence interval less than 4.64% (i.e., a confidence interval [20.36%-29.64%]).

Similarly, an observed prevalence of 5% among a sample of 1,000 individuals will yield a half-width of a 95% confidence interval less than 1.71% (i.e., a confidence interval [3.29%-6.71%]).

To reach a sample of 1,000 HIV-infected men and women ages 18-49 years, sampling will also take into consideration expected non-response rates and proportions of pre-cohort survey participants who will be ineligible due to inclusion/exclusion criteria.

7.3 Clustering and Sample Weighting in Analysis

As described in Section 7.2 the sample size calculations account for loss to follow-up, refusal, household size, the estimated proportion of the population in the age range, and non-contact (inability to contact). In addition, a design effect of 1.25 is used to ensure adequate power to detect differences in the presence of clustering in the outcome expected because of the two stage sampling design. As households are sampled at random from each enumeration area, we expect only a modest effect of clustering within enumeration areas. However clustering of new infections within a household may occur at a higher frequency than expected. A sensitivity analysis is planned to address the potential effect of clustering of incident infections within households on the primary population incidence comparison. Note that the analyses are independently conducted by gender, thus clustering of infection only impacts the estimates if two men or two women in the same household become HIV infected during the study. Bootstrap resampling methods will be implemented that randomly select one individual from each household to investigate the influence of clustering on the population estimates.

The use of samples weights is planned in analysis, as typically required to achieve population estimates from a two stage sampling design. All estimation of national rates will use appropriate sampling weights to achieve estimates representative of the Swaziland population aged 18-49. Three different types of weighting will be combined to define the sample weights: first-stage ratio adjustment; non-response adjustment; and post-stratification weighting. The two-stage sampling design provides for an equal probability weighting for selection of the enumeration areas in four regions and random

selection of households in each enumeration area. The sample selected will define first stage ratio adjustment weights that account for the probability of each respondent case being selected. Demographics information gathered about non-respondent households and non-respondent individuals will be used to compute sample weights that adjust for household and unit non-response. Post stratification weights may also be used to match the distribution of age and gender of the 2007 (or most recent) DHS.

Estimates from the baseline data of the cohort will use sample weights computed for the baseline cohort. Estimates from the 6 month follow-up will use sample weights computed for the cohort retained at 6 months, where the non-response weights will use information from both the non-response at baseline and the loss-to-follow-up non-response.

While sample weighting can be used to attempt to correct for potential bias arising from non-response and attrition, if the study achieves high response rates and high retention rates the need for non-response sample reweighting can be minimized .

8.0 DATA HANDLING AND ANALYSIS

8.1 Data Analysis Plan

8.1.1 Consideration of M&E/Program Performance Data

The MOH Permanent Secretary convened a SHIMS Steering Committee to ensure that the study's implementation is scientifically sound, ethical, and of high quality. The composition of the Committee makes it well-suited to continuously review the M&E data from programs and preliminary/intermediate study data (including HIV incidence estimates from Cohort A1). The Principal Investigators are represented on the Committee. The Committee meets bi-weekly, or more or less frequently if needed, and will include as a standing order of business the review of updated M&E data. Steering Committee teleconferences will occur quarterly with the participation of the all Principal Investigators and a majority of Co-investigators, specifically to review the ASI and ART program M&E data as relate to the SHIMS. Any necessary adjustments to the survey/cohort sample sizes, cohort follow-up durations, time period between surveys/cohorts, or other additions/modifications to the study will be at the discretion of the Principal Investigators.

8.1.2 Descriptive analyses

Descriptive analyses will include: a description of all individuals who are offered participation but refused in either the pre-cohort survey or longitudinal cohorts; characteristics of all individuals who consent to participate; and a description of all individuals who are enrolled in the longitudinal cohorts, including those lost to follow-up.

8.1.3 Analysis of primary objectives: Comparison of HIV incidence rates

Primary Objectives 1a & 1b and 2a & 2b
Comparisons of rates of seroincidence in the pre vs post MC ASI will compare incidence rate ratios assuming a Poisson distribution of seroconversions in the follow-up time in each cohort. The HIV seroincidence rates, computed as the total number of seroconversions divided by the total number of person years, adjusted by sampling weights to achieve a population, age, and gender based estimate of seroincidence will be compared in the pre vs post MC ASI in Cohorts A1 and A2. The statistic is the Wald test statistic for the slope of a Poisson regression with a binary covariate for pre vs post MC ASI. Cohorts A1 and A2 are based on independent samples. Each is intended to be representative of the contemporary Swaziland population, thus the sample weighting in each cohort will be based on current population estimates. s

For men in the baseline cohort (Cohort A1) who become circumcised between the time of cohort enrollment and follow-up, only person years prior to circumcision will be included in the analysis. All follow-up time from men who are circumcised prior to enrollment, and men who remain uncircumcised at follow-up, will be included. All follow-up time from women will be included.

To account for potential dependence of HIV seroincidence within households a sensitivity analysis using bootstrap resampling techniques will assess the effect of sampling multiple people from each household on the primary analysis.

Sensitivity analyses will be conducted to assess the impact of any population based demographic changes in the HIV-uninfected population between the pre and post MC ASI periods.

Primary Objective 3
Comparisons of rates of seroincidence in the circumcised vs. uncircumcised men in the post-MC ASI male cohorts will compare incidence rate ratios assuming a Poisson distribution of seroconversions in the follow-up time in each group. The HIV seroincidence rates, computed as the total number of seroconversions divided by the total number of person years in each group, adjusted by the cohort sampling weights to achieve a population age-based estimate of seroincidence will be compared in the circumcised vs. uncircumcised men. The statistic is the Wald test statistic for the slope of a Poisson regression with a binary covariate for circumcision.

Person years of men who are uncircumcised at the time of cohort enrollment and become circumcised during the 12-month follow-up period will be included in the uncircumcised follow-up until time of circumcision, and in the circumcised follow-up after the time of circumcision.

The exploration of factors that are significant effect modifiers and/or confounders of HIV seroincidence between these two groups will be used to further understand observed differences.

8.1.4 Analysis of secondary objectives

1) To examine the association of baseline demographic characteristics with HIV incidence and prevalence in a household-based representative sample of men and women before and after completion of the MC ASI. Within each cohort, logistic regression will be used to assess association of baseline demographic characteristics with HIV prevalence. Poisson, survival and logistic regression will be used as appropriate to assess associations with seroincidence and baseline demographics. Combined analysis of predictors of seroprevalence and seroincidence using both cohorts will assess effect modifiers associated with cohort (e.g. time) using test for interaction with time. Sample weights will be used in all analyses.

2) To determine the prevalence of circumcision among men before and after completion of MC ASI. Population-based circumcision prevalence rates will be computed using appropriate sample weights.

3) To estimate HIV prevalence rates among men and women before and after completion of the national male circumcision ASI. Population-based age and gender HIV prevalence rates will be computed using appropriate sample weights.

4) To describe sexual risk behavior in a household-based representative sample of men and women before and after completion of the MC ASI. This analysis will be conducted in both HIV-infected and HIV-uninfected participants. Sexual risk behaviors compared between the enrollment pre and post- MC ASI surveys will include the proportion of men and women reporting any unprotected sex and proportion reporting more than one partner. Logistic regression will be used to assess differences in proportions between the baseline cohorts, adjusted for baseline demographic factors, HIV status, and circumcision status (men).

   In the two longer longitudinal cohorts of younger HIV-uninfected men (25-34 yrs) and women (18-24 yrs), logistic regression will be used to compare change in behavior from baseline to follow-up. Factors predicting increase and decrease in risk behaviors will be assessed.

5) To describe the proportion of HIV-infected men and women in a household-based representative sample of men with undetectable viral loads before and after completion of MC ASI. Logistic regression will be used to assess differences in proportions of male participants with undetectable viral load.

6) To measure the proportion of HIV-infected men and women in a household-based representative sample of men and women who report engagement in HIV care and ART use before and after completion of the MC ASI. Logistic regression

will be used to compare any engagement in care and any ART use in the HIV infected persons identified in the household surveys.

7) To describe the CD4 count distribution in a nationally representative sample of HIV-infected men and women ages 18-49. Standard survey sampling approaches using sampling weights will be used to estimate proportions and 95% confidence limits.

8) To estimate the proportion of HIV-infected men and women ages 18-49 who have a CD4 count <350 cells/mm3 and who report no current ART use. Standard survey sampling approaches using sampling weights will be used to estimate proportions and 95% confidence limits.

9) To describe the viral load distribution in a nationally representative sample of HIV-infected men and women ages 18-49. Standard survey sampling approaches using sampling weights will be used to estimate proportions and 95% confidence limits.

8.1.5 Multivariate analyses

We will perform multiple regression analyses to examine the independent association of the primary outcome (HIV-incidence) using the covariates identified as significantly associated with the same in bivariate analyses.

8.1.6 Controlling for potential confounding variables

For all analyses of the primary outcome, we will control for potential confounding variables by either stratification or including the variable(s) as covariates in the multivariate analyses.

8.1.7 Alternative comparisons

Identification of a comparable group of uncircumcised men and unaffected women within the accelerated implementation context is challenging. It is acknowledged that men who choose to forego circumcision when it is widely available may differ in important characteristics (both observable and unobservable) from men who undergo MC. The most obvious possibility is that men who have riskier behaviors (and higher HIV risk) may have a greater propensity to seek circumcision than men who practice safer sex. If the decision to be/not be circumcised is correlated with HIV risk, then attempts to properly isolate the impact of MC on HIV may be limited in a simple comparison of men who have undergone and foregone circumcision. Although many measures of sexual risk can be ascertained from all men at baseline and follow-up surveys and controlled for in analyses, there may remain important immeasurable, undetected differences.

Short of randomization, for which equipoise has long since been reached, only second best analytical approaches can be used as a means of controlling for selectivity of

circumcision choice, such as non-experimental matching approaches. Matching estimators typically assume that the decision to undergo MC can be considered to be representative after conditioning on a set of observable characteristics. This approach compares the behavioral outcomes of men undergoing and foregoing MC, after controlling for differences in their observable characteristics. Because the set of observable characteristics is likely to be large, propensity score matching estimators can be used to reduce the matching problem to one dimension. This approach will be explored.

Marginal structural models (MSM) may also be useful in our analyses, as sexual risk behaviors may change over time relative to circumcision. MSM are utilized to mitigate biases introduced by time-dependent covariates for dichotomous outcomes, and aid in causal inference in observational evaluations [Robins, 2000].

8.2 Data Collection Methods

Data from participants: questions in the interview instruments will be read aloud to participants by the staff member and the participant's answers recorded on the appropriate paper form by the staff member. Participants will not complete the forms themselves.

Data from rapid HIV tests: are collected on paper forms and the relevant forms will be completed by study staff in the field.

Data from CD4 enumeration: will be entered electronically into the study database by the data management personnel.

Data from viral load and EIA/NAT testing: will be entered electronically into the study database by the study personnel. Results of these tests will not be forwarded to the HIV care clinic to which the individual was referred, as viral load testing is not used clinically in Swaziland as criteria for HAART initiation and the EIA/NAT combination is only being used for the purposes of this research study.

8.3 Information Management

Study staff will collect study information on the paper forms (Appendix B, D-G, and I-L) and transport them to the central office for data capture and transmission. Data from the paper forms will be entered into one of two databases: 1) a Locator Information Database that includes information that will be used to contact participants again in the future, such as address and telephone number; and, 2) a SHIMS Database that includes all of the study variables for participants indexed by unique study ID number only. Until submitted to the SHIMS office, the forms will be kept in a locked portable file.

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8.4 Data Entry, Editing, and Management

8.4.1 Locator Information Database

The data from the Locator Information Forms (Appendix I) will be entered manually into a Microsoft Access database within 2 business days of receipt at the central office. Data in the locator database will be backed up daily, and a weekly backup to DVD will be created for offsite storage. Access to paper forms and the electronic data, including household GPS coordinates, will be restricted to senior data managers and a limited number of study staff who do not have repeated contact with study households. A list will be compiled to identify the above persons. Once all participant-related activities have ceased and all electronic data are deemed clean, all personal identifiers will be removed from the electronic files and all paper files destroyed. Study databases will be both encrypted and password protected at the database and network levels. All computers (notebooks, laptops, desktops, and servers) will store all data in encrypted format.

The registry linking unique study ID numbers with personally identifiable information ("link-log") will be securely maintained in a strong password protected file at the local data management center, with no electronic personally identifying information leaving the local site.

8.4.2 SHIMS Database

Due to the volume of study variables and the number of participants, data will be electronically uploaded into the SHIMS Study Database by optical scanning software (DataFax). Field staff will submit paper forms within 10 business days of completing the survey. Data cleaning, management, and report generation will be handled by the SCHARP data processing center in Seattle, Washington, USA, using the DataFax data management system. Daily, an identical version of the dataset will be available for download to key individuals identified by the SHIMS Steering committee, so that the most current data are in country.

8.5 Data Storage and Disposition

As noted above, all hard copy forms will be kept in portable locked files until transported to the central office. All hard copy forms will be kept in lockable rooms with locked filing cabinets once received at the central office. Access to these forms and the electronic locator information will be restricted to the data management staff, study coordinator, and principal investigators.

8.6 Data Ownership

The data in the SHIMS survey database and locator information database, the paper forms, and the faxed images, are the property of the GKOS. As described above, SCHARP will manage the data until staff from Swaziland have been trained on the DataFax data management software and data integrity is assured and there is no longer a need for data management support. Based upon guidance from the Swazi MOH, specific in-country staff will be designated to serve as custodians of the data.

8.7 Intermediate Reviews and Analysis

Study operations will be reviewed by a study monitoring committee as delegated by the SHIMS Steering Committee to assess study conduct, timelines and study quality. Reviews are planned every six months, with additional reviews as needed on an ad-hoc basis. There is no formal plan for intermediate analyses, as the MC ASI intervention is independent of this evaluation protocol. Thus intermediate analyses would not affect the pace or scope of the MC ASI implementation regardless of findings.

All abstracts and manuscripts developed in line with the study's Primary and Secondary Objectives for presentation at conferences and publication in peer-reviewed scientific journals will be in collaboration with investigators from Swaziland. Abstracts and manuscripts will be presented to the SHIMS Steering Committee prior to submission. At least one Swazi co-investigator, or other Swazi stakeholder as directed by the MOH SHIMS Steering Committee, will be included as a co-author on all manuscript and abstract submissions (unless co-authorship is declined in writing). Analysis of data to answer novel research questions will be appropriately supported by CDC, ICAP, and SCHARP.

8.8 Quality Control/Assurance

Data quality will be assured in the following ways: 1) paper forms will be reviewed for completeness before concluding visits with participants, and staff conducting interviews will check all the responses for missing or incorrect entries; 2) paper forms with errors will be presented during a weekly study staff meeting to review errors and provide additional training to mitigate continued mistakes; and, 3) data checks will be programmed to flag unlikely or inconsistent data values for inclusion in data query QC Reports sent to the site for resolution.

Quality improvement will also be supported by an SOP Manual to complement this protocol. The SOP Manual will outline in detail procedures for conducting home-based study recruitment, enrollment, informed consent, interviews, HIV testing and counseling, phlebotomy, handling of blood specimens, data collection, data handling, forms processing, data management, and other study operations.

8.9 Limitations of study

We propose a novel approach with population-based repeated short-term longitudinal cohort studies in addition to a longer-term longitudinal cohort to evaluate changes in HIV incidence before and after the MC ASI campaign. It is possible that other ongoing

factors occurring simultaneously may contribute to any observed changes (or lack of) in HIV incidence. To help infer the role of MC in the observed changes in HIV incidence, we will capture data from participants experiencing other interventions and account for these in analyses.

We anticipate an 8-12% annual lost-to-follow-up rate among cohort participants at follow-up due largely to movement within Swaziland or migration. There is a possibility that those who migrate and those who do not are different from each other in meaningful ways related to HIV infection risk. We have mitigated the effect of the migration on the evaluation by incorporating lost-to-follow-up into study calculations to ensure sufficient power is maintained.

Data on sexual behaviors (such as past/current number of partners, condom use, etc.) and history of STI symptoms will be self-reported and are thus subject to potential recall bias and social desirability bias. However, efforts will be made to ensure that the study staff recruiting, enrolling, interviewing and performing the blood sample collection will not be from within the community under evaluation. This should minimize concerns about stigma and disclosing personal information during data collection. There is also a possibility that self-reported circumcision status may be incorrect, despite the use of illustrations.

Persons who are in institutions such as prisons are not included in the study; persons who are transient and thus not at home during enrollment are also not captured.

Though every attempt will be made to determine HIV status at the time of circumcision for male participants in the post-intervention longitudinal cohorts identified as having become both circumcised and HIV infected since the prior assessment, it is possible that some men retrospectively determined to have been HIV-negative at the time of circumcision will in fact have been HIV-positive, and vice versa. A special questionnaire, review of surgical records, and laboratory assays will be performed.

9.0 HUMAN SUBJECTS CONSIDERATIONS

9.1 Collaborating sites and institutional review

This protocol will be submitted for ethical review to the Scientific and Ethics Committee (Mbabane, Swaziland), the Internal Review Board (IRB) at the Centers for Disease Control and Prevention (CDC; Atlanta, USA), University of Washington IRB and Columbia University Medical Center IRB. No study activities will begin until all necessary approvals have been obtained.

Subsequent to the initial review and approval, the responsible IRBs/ECs will review the protocol at least annually.

9.2 Consent process

Prior to being enrolled in the study, all participants will be required to give written informed consent. There are six consent forms that will be used in this study: 1) Informed Consent: Pre-cohort Survey, 2) Informed Consent: Short-term Cohort- 6 months, 3) Informed Consent: Short-term Cohort- 12 months; 4) Informed Consent: Long-term Cohort; 5) Informed Consent: Contact for Future Research Participation; 6) Informed Consent: Cross-Sectional Cohort C . All informed consent forms for this study can be found in Appendices C, H, M, O, T and U.

Informed Consent: Contact for Future Research will request consent from Cohort A1 participants to be contacted about potential participation in future HIV prevention studies. The consent requests permission to contact the participant for up to five years from the date of consent. All consent forms and case report forms will be translated from English into siSwati. Back translations will also be completed and reviewed by a bilingual independent source in order to ensure accuracy of translated information.

Before beginning the informed consent process, the potential participant will be asked to select a relatively private location either inside or outside their home, so that the study activities may be conducted in as much privacy as possible, as appropriate. The informed consent discussion will take place in either English or siSwati. Participants will be given the opportunity to choose their preferred language.

Prior to initiation of any study procedures, all potential participants will be given a printed copy of the consent form in either English or siSwati depending upon their preference. A staff member will then read the consent form aloud to the participant. At this time, potential participants will be informed that their participation in the study is voluntary and that they may withdraw at any time. Withdrawal from the study will have no affect on the participant's access to health facilities providing circumcision or HIV-related care and treatment. Further, participants will be informed that they do not have to answer questions that make them uncomfortable and that any information that they disclose during the course of the study will be considered confidential (i.e., no personal identifiers will be used and only summary information across all participants will be reported). Participants will have the potential risks and benefits of the study explained to them as well.

After the consent form has been read aloud, potential participants will be invited to ask questions about any aspect of the study and their participation. If they agree to participate in the study, literate participants will document their provision of informed consent by signing their name on the consent document. Non-literate participants will be asked to provide a thumbprint to indicate consent. Participants will be provided with a copy of their informed consent form.

Blind and deaf persons will be accommodated on case-by-case bases and all efforts will be made to include them in the evaluation if appropriate.

Detailed procedures describing such scenarios will be described in the SOPs.

9.3 Risks and Benefits

The study protocol is noninvasive and involves minimal risk to participants.

9.3.1 Potential Risks

As part of this study, participants will be asked to take part in HIV prevention activities, undergo HIV counseling and testing, and disclose personal information. Participants will be asked questions about sensitive topics, including sexual behavior, HIV, and male circumcision.

It is possible that some individuals may experience discomfort from taking part in these study activities. Study staff will be trained to address any potential stress or discomfort that may result from study participation and to help make participants feel comfortable. As part of the informed consent procedure, all potential participants will be instructed that they do not have to disclose personal information which they are uncomfortable sharing and that they can withdraw from the study at any time.

There is a potential risk for participants who are found to be HIV-infected to become distressed after receiving their test results. Study staff will be trained to provide HIV post-test counseling to persons newly diagnosed with HIV. Study staff will also be equipped to refer participants who need additional counseling and assistance to community resources, including referrals for HIV care and treatment.

There is a potential risk for participants to become distressed after receiving their CD4 test results. During the return visit, field staff will describe a CD4 test and will advise them to show the CD4 result to a health care provider to learn more about the test result and its implications for their health care. The participants will also be provided with the existing SHIMS hotline phone number to call if they have additional concerns. All study staff will be trained to direct participants to seek additional information from their medical provider after receiving the CD4 result.
There is also a slight risk of discomfort to participants associated with venipuncture. Feelings of discomfort could include feeling ill and/or having injection site complications such as slight bruising or tenderness. Study staff will be trained in how to deal with these complications and will refer participants to local health facilities for additional care, as needed.

Although every effort will be made to keep participant information confidential, complete confidentiality cannot be guaranteed. Participants will be informed of this potential breach of confidentiality as part of the informed consent process. Procedures for maintaining confidentiality are detailed in Section 9.4.

9.3.2 Potential benefits

Participants will receive several benefits from this study including learning their HIV status. If a participant tests HIV-positive, they will be counseled on the importance of linking to care and treatment; they will also be referred to closest HIV care site. In addition, they will be counseled regarding the importance of partner notification and testing, as well as provided with information regarding HIV testing sites.

For participants who receive CD4 testing, they will have the benefit of knowing this information related to their health status. This information will allow participants to seek care for HIV and to have the benefit of being referred for care and treatment at an earlier stage of illness. Earlier HIV care may offer individual and public health benefits.

Societal benefits of this study include gaining a better understanding of the effectiveness of male circumcision in reducing HIV incidence when provided at a population level in real-world settings. This information will help refine projections of HIV infections that may be averted from MC programs and the potential costs savings realized, compared to HIV care and treatment costs. The study will also contribute to the understanding of whether risk compensation is an unintended consequence of large-scale MC programs.

9.3.3 Incentives

Participants in both the short and long term longitudinal cohorts will be provided with allowances, primarily to facilitate ongoing communications and offset travel costs. Specific monetary amounts are in keeping with local customs and specified in the informed consent forms. These amounts are as follows (note $1 = 7.385 Emalangeni as of July 17, 2010):

- Household survey: Because household members may be eligible for future research and enrolled participants will be asked to frequently call the central office by telephone to provide updated locator/contact information, a card for cellular telephone airtime worth 25 Emalageni will be provided to participating households to offset future expenses incurred.
- Short term longitudinal cohorts (A1 and A2): 70-100 Emalangeni at Month 0 and Month 36 for Cohort A1 and A2 follow-up, respectively, depending upon transportation expenses, if incurred. Longer term longitudinal cohort (B): 80-110 Emalangeni at Month 42 for follow-up; depending upon transportation expenses, if incurred.
- Cross-sectional survey (Cohort C): 70 Emalangeni or equivalent voucher of airtime will be provided to participants completing the Cross-Sectional Cohort C.

9.3.4 Emergency Care

There are no expected harmful consequences as a result of participating in this evaluation. However, should an HIV-positive participant express a need for emergent medical care, study staff will attempt to locate immediate care at a nearby health facility.

9.4 Confidentiality

All study staff will receive training on procedures to protect participant confidentiality and Good Clinical Practices (GCP).

In order to protect confidentiality, each participant will be assigned a unique study ID so that their name is not linked to any of their personal data or lab results. That study ID will be written on all data collection forms. HIV test results and data collection forms will be matched only by this identification number, not by participants' names or other identifying information. A master list with each participant's name and their assigned identification number will be created. Only the Study Coordinator or designee will have access to this master list. The master list will be securely maintained in strong password protected file at the local data management center, with no electronic personally identifying information leaving the local site.

All study data, including lab results, will be stored securely in the study offices. All study data will be locked in a filing cabinet in a secure room in the office. All databases will be encrypted and password protected. Study data will be accessible only to study staff directly involved in this study (data manager, study coordinator and Principal Investigators). During transport, study data will be kept in a portable locked file.

Personal locator information, including participant's name, address and phone number, will be stored separately from study data in a filing cabinet in a secure room in the office.

All study consent forms will include the contact information of Principal Investigators and local IRB if participants have questions about the study; if they wish to withdraw themselves as a participant; if they have concerns about their rights as a study participant; or if they believe that have been harmed by the study.

All staff who through the course of their work have knowledge of, or access to, personal information about participants will be required to sign a confidentiality agreement. (Appendix Q).

The procedures for maintaining the confidentiality of participant data will be included in pre-established written procedures, such SOPs.

9.5 Identifying, managing, and reporting adverse events (AEs)

As SHIMS is an observational cohort study, standard adverse event (AE) reporting will not be undertaken; there are no anticipated adverse events. All unanticipated problems or AEs will be documented and immediately reported to the in-country study team who will then notify the MoH, CDC-Atlanta and ICAP Columbia University teams. These unanticipated problems/AEs will be discussed and a verbal and/or written action plan will be devised and implemented within 24 hours of the initial report. The study team will maintain written documentation on all events, including details of the action plan and event resolution. If necessary, a formal report will be sent to the appropriate IRBs using the CDC's Incident Report Form 1254. Reporting of unanticipated problems/AEs will be the responsibility of the Principal Investigators of this study.

The procedures for reporting unanticipated problems/ AEs will be included in staff trainings, the field operations manual, and SOPs.

9.6 Protocol Deviations/New & Unexpected findings/Changes to the study environment

All protocol deviations, new/unexpected findings and changes to the study environment will be documented and immediately reported to the in-country study team who will then notify MoH, CDC-Atlanta and ICAP Columbia University teams. If necessary, a formal report will be sent to the appropriate IRBs. Reporting of such incidents will be the responsibility of the Principal Investigators of this study.

Any discussions, issues, and complaints related to the study will be reviewed promptly to ensure close monitoring of the impact of the study on participants. Appropriate action will be taken to resolve or deal with all issues accordingly.

10.0 DISSEMINATION, NOTIFICATION, AND REPORTING OF RESULTS

10.1 Notifying participants of their individual results

Results from the HIV rapid test will be delivered to the participant on the same day that the test is conducted, unless the results are indeterminate, based upon the rapid HIV testing algorithm approved for this study (Appendix A). Pre-/post- test counseling will be performed by a member of study staff who has been formally trained in HIV counseling and testing techniques.

Results from NAT/EIA testing will be delivered as HIV-positive, HIV-negative, or indeterminate in accordance with the rapid HIV testing algorithm approved for this study (Appendix A). Participants with an indeterminate result based upon EIA/NAT testing at the NRL will be revisited approximately 6 weeks later for repeat rapid HIV testing in their home. Viral load test results from persons who test HIV-positive will not be forwarded to the HIV care clinic to which the individual was referred, as viral load testing is not used clinically in Swaziland as criteria for HAART initiation. Disclosure, counseling and re-testing with an HIV rapid test kit will be performed by a member of study staff who has been formally trained in HIV counseling and testing techniques.

Results from CD4 testing will be delivered to the participant at their residence. At the time of the return visit, field staff will provide the participant with their CD4 result and encourage the participant to discuss the CD4 test result with a health care provider.

Results from the viral load testing on Cohort C will not be returned to participants nor to their providers as viral load testing is not used clinically in Swaziland as criteria for HAART initiation.

10.2 Notifying participants of study findings

Written material summarizing the findings from this study will be made available to participants and study staff upon completion of the study.

10.3 Dissemination of study findings

The study findings will be disseminated through presentations and publications in peer-reviewed journals and other publications. Reports will be disseminated to an international audience, as well as to national and local HIV prevention planning groups who may use the findings to assist in the creation of HIV prevention activities. The CDC publications and presentations policy will be utilized.

In-country data and country-specific information will be made available to national policy-makers, organizations, and implementing partners as soon as possible. Study staff will be notified of the findings upon their presentation or publication. Any formal presentations at conferences or scientific publications will follow CDC procedures for publications and presentations.

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12.0 APPENDICES

Appendix A: **SHIMS HTC Guidelines and Algorithm**

Appendix B: **Household Composition Form**

Appendix C: **Informed Consent Form: Pre-cohort Survey**

Appendix D: **Refusal: Pre-cohort Survey**

Appendix E: **Case Report Form: Routine Survey**

Sections for Visit:
- Background Characteristics
- Sexual Activity
- HIV Testing History and Status
- Male Circumcision Status (self-report)
- HIV Rapid Testing

Appendix F: **Case Report Form: Extended Survey**

Sections for Visit:
- Background Characteristics
- Experience with Binge Drinking
- Male Circumcision Beliefs
- Sexual Activity
- HIV Testing History and Status
- Male Circumcision Status (self-report)
- HIV Rapid Testing

Appendix G: **Case Report Form: HIV Test Result**

Appendix H: **Informed Consent Form: Short-Term Cohort (A1) Pre-ASI**

Appendix I: **Locator Information Form**

Appendix J: **Refusal: Cohort Participation**

Appendix K: **Case Report Form: Short-Term Cohort Follow-up**

Sections for Visit:
- Background Characteristics
- HIV Testing History and Status
- Male Circumcision Status (self-report)
- HIV Rapid Testing

Appendix L: **Medical Records Abstraction Form**

Appendix M: **Informed Consent: Longer-Term Cohort (B)**

Appendix N: **Case Report Form: Longer-Term Cohort Baseline/Follow-up**

Sections for Visit:

- Background Characteristics
- Experience with Binge Drinking
- Male Circumcision Beliefs
- Sexual Activity
- HIV Testing History and Status
- Male Circumcision Status (self-report)
- HIV Rapid Testing

Appendix O: **Informed Consent Form: Short-Term Cohort (A2) Post-ASI**

Appendix P: **Sample Size Calculations**

Appendix Q: **Confidentiality Agreement**

Appendix R: **Penis Circumcision Illustrations**

Appendix S: **Alcoholic Beverages Illustrations**

Appendix T: **Informed Consent Form: Contact for Future Research Participation**

Appendix U: **Informed Consent Form: Cross-Sectional Survey C**

Appendix V: **Refusal: Cross-Sectional Survey C**

Appendix W: **Locator Information Form: Cross-Sectional Survey C**

Appendix X: **Case Report Form: Cross-Sectional Survey C**

Appendix Y: **Information for healthcare providers about SHIMS CD4 testing**

Appendices