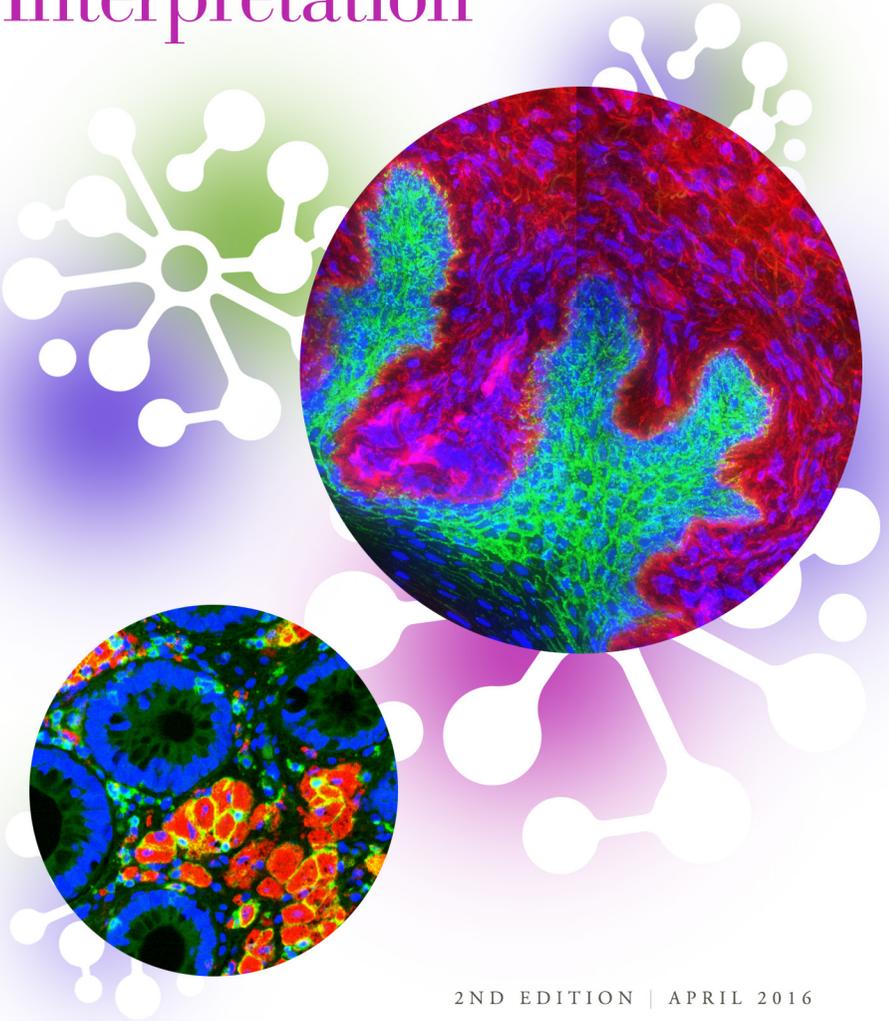


---

A GUIDE FOR HIV INVESTIGATORS

# Capturing Participant Data *for* Mucosal Sample Interpretation



2ND EDITION | APRIL 2016

---

---

Cover photographs of mucosal tissues kindly provided by:

- Ann Marie Carias and Thomas J. Hope (Northwestern University), US (top right, ectocervical tissue)
- Kimberly Smythe and Nicole Frahm (HVTN/FHCRC) and Kim Melton (FHCRC), US (bottom left, rectal tissue)

Back cover photographs kindly provided by:

- Kimberly Smythe and Nicole Frahm (HVTN/FHCRC), US (top right, rectal tissue)
- Ann Marie Carias and Thomas J. Hope (Northwestern University), US (bottom left, vaginal tissue)

How to cite this Guide:

*Capturing Participant Data for Mucosal Sample Interpretation:  
A Guide for HIV Investigators.*

Published by the Global HIV Vaccine Enterprise with the support of the National Institute of Allergy and Infectious Diseases (NIAID) and the HIV Mucosal Immunology Group (MIG). Second edition, March 2016. Organizing Committee: Mary Gross (Fred Hutchinson Cancer Research Center, FHCRC); Patricia D'Souza (NIAID); Amapola Manrique & Yegor Voronin (Global HIV Vaccine Enterprise).

To order copies:

Global HIV Enterprise

64 Beaver street, # 352

New York, NY 10004, US

Email: [timelytopics@vaccineenterprise.org](mailto:timelytopics@vaccineenterprise.org)

The online version is available at:

[www.vaccineenterprise.org/mucosal-sampling-guide](http://www.vaccineenterprise.org/mucosal-sampling-guide)



Global HIV Vaccine  
Enterprise



---

# Capturing Participant Data *for* Mucosal Sample Interpretation: A Guide for HIV Investigators

2ND EDITION | APRIL 2016



Global HIV Vaccine  
Enterprise



---

## *Acknowledgments*

The Global HIV Vaccine Enterprise, the National Institute of Allergy and Infectious Diseases (NIAID), and the HIV Mucosal Immunology Group (MIG) gratefully acknowledge the contributions of the many experts who responded to requests for concepts, content, references, and comments. We would like to thank them for the practical and essential feedback they provided on prior drafts of the document.

*Notable contributors are listed here in alphabetic order:*

Maria L Alcaide (University of Miami Miller School of Medicine), Michele Andrasik (University of Washington, HIV Vaccine Trials Network (HVTN)); Peter Anton (University of California, Los Angeles); Chuka Anude (Henry M. Jackson Foundation (HJF), NIAID, Division of Acquired Immunodeficiency Syndrome (DAIDS)); Omu Anzala (Kenya AIDS Vaccine Initiative, University of Nairobi); Jared Baeten (University of Washington); Rahul Bakshi (Johns-Hopkins University, HIV Prevention Trials Network (HPTN), Microbicide Trial Network (MTN)); Kristina Broliden (Karolinska Institutet); Susan Cu-Uvin (Brown University, AIDS Clinical Trial Group (ACTG)); Stephen De Rosa (University of Washington); Charlene Dezzutti (University of Pittsburgh, MTN); Catherine Godfrey (NIAID); Raul Gomez Roman (Independent Consultant, Mexico City); Ruth Greenblatt (University of California, San Francisco); Carolina Herrera (Imperial College London); Florian Hladik (Fred Hutchinson Cancer Research Center (FHCRC), University of Washington); John Hural (FHCRC, HVTN); Nicos Karasavvas (Armed Forces Research Institute of Medical Sciences (AFRIMS)); Shelly Karuna (FHCRC, HVTN); Rupert Kaul (University of Toronto); Douglas Kwon (The Ragon Institute of Massachusetts General Hospital, Massachusetts Institute of Technology and Harvard); Alan Landay (Rush University Medical Center); Dagna Laufer (International AIDS Vaccine Initiative, IAVI); Maria Lemos (FHCRC, HVTN); Magda Moutaftsi (Bill & Melinda Gates Foundation); Kenneth Mayer (Fenway Health and Beth Israel Deaconess Medical Center, Harvard Medical School); Julie McElrath (FHCRC, HVTN); Ian McGowan (University of Pittsburgh, MTN); Richard Novak (University of Illinois); Pietro Pala (Uganda Virus Research Institute (UVRI)); Harriet Park (International AIDS Vaccine Initiative (IAVI));

---

Jo-Ann Passmore (University of Cape Town); Jeanna Piper (DAIDS, MTN); Kristen Porter (Westfield State University); Steven Safren (Massachusetts General Hospital, Harvard Medical School); Barbara Shacklett (University of California, Davis); Arthur Sekiziyivu (Makerere University Walter Reed Project); Devika Singh (University of Washington, HPTN, MTN); Alexandra Schuetz (AFRIMS); Hans Spiegel (Henry M. Jackson Foundation (HJF), NIAID, Division of Acquired Immunodeficiency Syndrome (DAIDS)), Natasa Strbo (University of Miami), Georgia Tomaras (Duke University); Sandhya Vasan (HJF, AFRIMS); Otto Yang (David Geffen School of Medicine at UCLA).



---

# Table of Contents

Acknowledgments.....	i
Introduction.....	1
About the Guide.....	1
Categories of Participant Characteristics to Consider.....	5
1. Demographics.....	7
1.1. Age.....	7
1.2. Race/Ethnicity/Tribe.....	9
1.3. Sex at Birth/Self-identified Gender.....	10
1.4. Relationship Status.....	11
1.5. Education, Employment Status, Income.....	12
2. Reproductive History.....	15
2.1. Menstrual History.....	15
2.2. Pregnancy Status and History.....	17
2.3. Contraception.....	18
2.4. Menopausal Status.....	21
2.5. Female Reproductive Tract Procedures/Surgeries.....	22
2.6. Male Reproductive Tract Procedures/Surgeries.....	23
2.7. Sexually-transmitted and Reproductive Tract Infections (STIs/RTIs).....	25
2.8. Abnormal Cervical, Rectal, or Oral Cytology and Dysplasia.....	28
3. Medical History.....	31
3.1. Medical Conditions.....	31
3.2. HIV Status.....	33
3.3. Vaccinations.....	35
3.4. Medications.....	36
3.5. Allergies.....	37
3.6. Body Mass Index.....	38
3.7. Underexplored Areas and Other Factors.....	39

---

4.	Sexual History & Sexual Risk Behaviors	41
4.1.	Sexual Practices	41
4.2.	Use of Vaginal or Rectal Products or Devices	43
4.3.	Sexual Risk Behaviors	45
4.4.	Risk Behaviors of Partner	46
4.5.	HIV and Other STI Status of Partner(s)	47
5.	Other Risk Behaviors	49
5.1.	Drug Use	49
5.2.	Smoking	51
5.3.	Alcohol Consumption	52
6.	Symptoms	55
6.1.	Constitutional Symptoms	55
6.2.	Vaginal Discharge	57
6.3.	Rectal Discharge	58
6.4.	Pelvic Pain	59
6.5.	Oral, Respiratory, or other GI Symptoms	60
	Endnote	61
	References	61
	Index of Cited Case Report Forms	72
	Glossary	73
	Appendix (checklist)	74



---

# Introduction

## About the Guide

### What is the purpose of the Guide?

Mucosal immune responses and mucosal sampling from gastrointestinal (GI) or genitourinary (GU) tracts are an increased focus for the research and development of an efficacious HIV vaccine, as well as of other HIV prevention and treatment strategies. This guide is intended as a resource to help HIV investigators identify key participant characteristics that can affect mucosal immunity and which are therefore important factors to consider for proper interpretation and potential cross-trial comparison of mucosal immunology data.

### Why was the Guide developed?

Collection of GI and GU tract samples during clinical studies is associated with significant operational challenges and expenses, as well as some risk and discomfort to study participants. It is therefore critical that appropriate clinical, behavioral, and demographic characteristics are collected from study participants so that factors that may influence GI or GU immunology, and thus the interpretation of assay data, are efficiently captured in parallel with mucosal specimens. Although many of the operational and policy elements for the conduct of clinical studies are well established through Networks or similar entities, adopting recommendations of “Capturing Participant Data for Mucosal Sample Interpretation: A Guide for HIV Investigators” (hereafter referred to as the Guide) provides an opportunity for investigators to use best practices and develop a participant questionnaire that best matches the study-specific objectives of clinical studies involving sampling from mucosa.

### How was the Guide developed?

The Guide was developed by the Global HIV Vaccine Enterprise, NIAID, and the HIV Mucosal Immunology Group (MIG). The MIG was co-established by DAIDS and the HVTN in 2009, and was co-funded through the Bill & Melinda Gates Foundation’s Collaboration for AIDS Vaccine Discovery (CAVD) until 2016. It comprises more than 40 leading investigators in HIV mucosal immunology research who have pursued

collaborative studies to identify and standardize best practices for the collection, storage, and analysis of mucosal specimens from the GI or GU tracts. The idea for the Guide evolved during the 2012 MIG Annual Meeting. Because of its field-wide relevance, the development of the Guide was facilitated by the Global HIV Vaccine Enterprise as part of the Timely Topics in HIV Vaccines initiative. The organizing committee of the Guide comprises Patricia D’Souza (NIAID), Mary Gross (FHCRC), Amapola Manrique, Yegor Voronin, and Hélène Zinszner (Global HIV Vaccine Enterprise). The Guide was conceptualized and created with the support of many contributors (see acknowledgements at the beginning of the Guide).

### How should this guide be used?

The Guide is intended as a user-friendly resource and quick-reference index, listing many of the participant characteristics that may be collected during clinical trials involving mucosal sampling. It should be used *in conjunction* with Network procedures and policies, protocols, and Case Report Forms (CRFs), and *does not* replace existing clinical research policies and procedures, which ensure compliance with federal regulations including procedures to protect participants’ safety. Rather, it is anticipated that the Guide will serve as a tool to supplement and enhance the quality of clinical research conducted with mucosal specimens. Many research groups have used this Guide during protocol development and study design.

### How is this guide structured?

The Guide is divided into six major categories of participant information that are important to consider in clinical trials with a mucosal sampling component:

1) Demographics; 2) Reproductive History; 3) Medical History; 4) Sexual History & Sexual Risk Behaviors; 5) Other Risk Behaviors; and 6) Symptoms. Each of these categories is annotated with the following:

- Rationale (brief description of why the category is important for interpretation of mucosal data)
- Considerations (important subgroups of information to consider relevant to this category; precautions or potential issues to note)

---

Additionally, sections include symbols indicating whether they are relevant to males, females, or participants of both genders.

Selected references (relevant reviews, as well as specific literature cited in the sections above) are listed in the Endnote section. CRF examples are also provided for reference.

### Are there any general considerations or caveats to remember when using this guide?

The Guide was purposely written to be inclusive of participant factors that may affect data derived from mucosal samples, with the aim of minimizing the chance that key descriptors might be overlooked or forgotten during study design and protocol development. For that reason, some of the data categories suggested for consideration (e.g., demographics) include several routinely collected data elements regardless of whether a clinical trial involves a mucosal sampling component.

Furthermore, final decisions of what information is important to collect when the GI or GU mucosa are sampled in clinical studies may vary considerably as a function of the study objectives, the specific mucosal compartment and sample type being collected, operational feasibility of data collection or verification, or the particular participant population and investigational product under study. For example, depending on the phase of studies (e.g., phase 1 studies enrolling only participants at low risk for HIV infection vs. later-phase studies enrolling those at higher risk of HIV infection based upon baseline behaviors), participant questionnaires may be less or more detailed as a consequence. The specific study products undergoing testing (microbicides, vaccines, etc.) and their routes of administration may impact the details of information acquired, because the type of information critical in each of these categories may vary depending upon the intervention under study.

Three of the limitations of this Guide are: (1) it does not discuss all the listed mucosal topics in great depth; (2) it does not provide specific instructions on how to diagnose diseases or medical conditions; and (3) it does not provide specific instructions on how to collect mucosal specimens. In consultations with expert reviewers and users of the First Edition, a consensus was reached to preserve the broad, “checklist” nature of the

Guide, and to provide links to additional references and to more in-depth sources of information in this Second Edition. In addition, a separate guide for mucosal specimen collection and related protocols—which are beyond the scope and purpose of this Guide—may be developed in the future. In the meantime, please refer to the PLoS Journals Collection, [Advances in HIV Mucosal Immunology: Challenges and Opportunities](#), as a general resource, and for examples of some of the published methods for mucosal sample collection and processing.

### What is new about this edition?

This Second Edition has been reviewed by additional experts. Throughout the Guide, new references have been included to reflect recent and relevant observations in the field. The oral mucosa has now been considered in updating the Guide. In addition, a new checklist has now been included in the Appendix as a resource to guide the collection of data during clinical trials.

### Where can I get more information or how can I provide feedback?

This Guide is available online: [www.vaccineenterprise.org/mucosal-sampling-guide](http://www.vaccineenterprise.org/mucosal-sampling-guide). Your feedback is valuable to update the Guide in the future. Please send your questions, comments or suggestions to [mucosalguide@gmail.com](mailto:mucosalguide@gmail.com), or to [timelytopics@vaccineenterprise.org](mailto:timelytopics@vaccineenterprise.org).

# Categories of Participant Characteristics to Consider

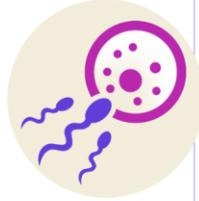
## 1. Demographics

- 1.1. Age
- 1.2. Race/Ethnicity/Tribe
- 1.3. Sex at Birth/  
Self-identified  
Gender
- 1.4. Relationship Status
- 1.5. Education,  
Employment Status,  
Income



## 2. Reproductive History

- 2.1. Menstrual History
- 2.2. Pregnancy Status  
and History
- 2.3. Contraception
- 2.4. Menopausal Status
- 2.5. Female Reproductive  
Tract Procedures/  
Surgeries
- 2.6. Male Reproductive Tract Procedures/Surgeries
- 2.7. Sexually-transmitted and Reproductive  
Tract Infections (STIs/RTIs)
- 2.8. Abnormal Cervical, Rectal, or  
Oral Cytology and Dysplasia



## 3. Medical History

- 3.1. Medical Conditions
- 3.2. HIV Status
- 3.3. Vaccinations
- 3.4. Medications
- 3.5. Allergies
- 3.6. Body Mass Index
- 3.7. Underexplored Areas  
and Other Factors



## 4. Sexual History...

- 4.1. Sexual Practices
- 4.2. Use of Vaginal or  
Rectal Products  
or Devices
- 4.3. Sexual Risk  
Behaviors
- 4.4. Risk Behaviors  
of Partner
- 4.5. HIV and Other STI  
Status of Partner(s)



## 5. Other Risk Behaviors

- 5.1. Drug Use
- 5.2. Smoking
- 5.3. Alcohol  
Consumption



## 6. Symptoms

- 6.1. Constitutional  
Symptoms
- 6.2. Vaginal Discharge
- 6.3. Rectal Discharge
- 6.4. Pelvic Pain
- 6.5. Oral, Respiratory,  
or other GI Symptoms





---

# 1. Demographics



## 1.1. Age

### Rationale

Age is one factor that influences the structure and the function of the genital and GI mucosal environments. In reproductively mature adults, changes within the genital and GI mucosa, such as immune states (e.g., inflammation), sexually-transmitted infections (STIs), hormonal-changes, etc., have shown associations to HIV susceptibility (refer to Sections 2-4 of this Guide). However, less is known about how susceptibility to HIV acquisition may or may not be related to changes observed in the genital and GI mucosal environments in reproductively immature adolescents, in post-menopausal women, in middle-aged persons, and in the elderly.



During adolescence, the female reproductive tract (FRT) is marked by cervical ectopy, a high pH likely due to the heterogeneity of the vaginal microbiome [1], an immature vaginal epithelium, reduced cellular glycogen levels, thin levels of vaginal mucus, and reduced immune system education and response [2]. Substantive differences have been observed in several biomarkers of mucosal innate immunity measured in cervico-vaginal lavage samples from sexually active adolescents compared to adult females [3, 4].

Following menopause, the FRT resembles that of the pre-pubescent adolescent, with friable tissues, reduced estrogen, thinning of the vaginal mucus, and a heterogeneous microbiome [2]. Indeed, the vaginal microbiomes of prepubescent girls resemble those of post-menopausal women, who have a skin-type vaginal flora [5]. Immunologically, the post-menopausal FRT is characterized by a heightened inflammatory state, which correlates with enhanced HIV infection [6]. Changes in the reproductive tract brought about by menopause and peri-menopause are also important considerations for studies of mucosal immunity (see Section 2.4). Equivalent studies to evaluate the effects of hormonal changes in the GI tract are underway, but have not yet been reported.

Furthermore, a recently emerging field of study, termed immunosenescence, involves research into age-related changes in the immune system. Immunosenescence adversely impacts mucosal immunity and is associated with diminished protective immune responses to infectious diseases as well as reduced vaccine efficacy, among other reported factors [7, 8]. Moreover, the effects of aging may exacerbate the effects of HIV enteropathy on microbial translocation and immune activation in the gut [9].

## Considerations

Knowledge of birth date or age may vary in different countries; if date of birth is unknown, record best estimate of age.

For pre-teens and youth the pace of maturation varies widely for a given chronological age. In the case of delayed or precocious puberty, physical maturity assessment by Tanner staging and bone age needs to be considered and can provide a measure of biological age.

---

## 1.2. Race/Ethnicity/Tribe

### Rationale

Regional and racial disparities in HIV prevalence are known, some of which reflect biological components (e.g., CCR5 deletion and HLA class I types), as well as socio-behavioral factors [10, 11].



### Considerations

Inquiring about race/ethnicity/tribe may be considered politically incorrect in some regions. Furthermore, sense of ethnicity may vary in different countries/regions; consider country-specific ethnicity groups. For example, investigators have found that questioning about “tribe” can be a sensitive issue in Rwanda and is therefore often omitted in questionnaires in that country (Park, H. Personal communication, IAVI).

Race/ethnicity/tribe may be difficult to identify/self-identify: individuals frequently do not self-identify with one of the categories and thus are classified as “other.” In addition, while some individuals of mixed race/ethnicity/tribe may be able to identify their contributing races/tribes, others may just claim their maternal or paternal race/tribe. If the intended study requires precision in population subgroup, genetic methods for determination of racial makeup may be helpful.

## 1.3. Sex at Birth/Self-identified Gender

### Rationale

Women and men respond differently to many infectious diseases and have a different incidence of autoimmune conditions including gut-associated inflammatory and autoimmune disorders. Sexual hormones affect the number and function of immune cells [12, 13]. The mechanistic underpinnings of gender differences in mucosal immune responses are, however, not well understood. Studies of gender-specific immune responses in the GI and GU tracts are starting to emerge, indicating that immune activation and inflammation-associated gene expression in gut mucosal samples are gender specific [14]. This finding may underscore the importance of enrolling both men and women in studies that examine mucosal immune responses in the gut. In addition, there is evidence that gender bias may be affected and/or reinforced by the host's microbiome [15].



Transgender individuals are an important focus for HIV prevention research, as they have some of the highest incidence rates of HIV infection. Transgender communities have complex patterns of sexual identity and expression and it is important to acknowledge this diversity during the collection of sexual behavior information.

### Considerations

Important sex/gender variables to consider may include:

- Sex at birth
- Self-identified gender
- Transgender (male-to-female and female-to-male). Stage of transition:
  - Gender reassignment surgery
  - Use of exogenous sex hormones
    - Testosterone in female to male transitions
    - Estrogen in male to female transitions
  - Use of high doses oral contraception

---

## 1.4. Relationship Status

### Rationale

Characterization of relationship status should go beyond just a record of “marital status,” as domestic and sexual partners may exert influence on receptiveness or adherence to study product.



Relationship status plays a critical role in vulnerability to acquisition of STIs including HIV. For example, the VOICE (MTN-003) Pre-exposure prophylaxis (PrEP) study of oral and topical tenofovir observed an HIV incidence of 8.8% for unmarried women younger than 25 compared to 0.8% for older married women [16]. Marital status has also been associated with disease progression and mortality from HIV/AIDS, with significantly higher risk for single or never-married men across ethnicities [17]. These observations may reflect less stable sexual networks or lower social integration in unattached individuals, among other factors. Investigators should aim to characterize and record partnership status, while keeping in mind that it is a potentially unreliable, rapidly changing, or not fully disclosed variable.

### Considerations

Important Relationship Status variables to consider may include:

- Length of relationship(s)
- Cohabitation status
- Number and gender of sexual partners (see Sections 4.1 and 5.4)
- Types of partners, number of primary and non-primary partners (see Section 4.1)

## 1.5. Education, Employment Status, Income

### Rationale

Education, employment status, and source of income are useful predictors or analytical variables for factors such as retention, missed visits, long-term follow-up, adherence to study visit schedule, and HIV risk behavior. Some studies may record the length of time in the area for work and frequency of work-related travel (e.g., fishermen around Lake Victoria in East Africa; miners in South Africa) because migration can play a large role in HIV risk [18, 19] Terms reflecting level of education may require adjustment for the educational system in the study locale. Some studies may require indirect socioeconomic status (SES) data, such as data on stability of housing, which can impact hygiene practices, and history of food insecurity. In addition, maternal educational attainment can be a useful indicator of SES in childhood.



### Considerations

Important education/employment/income variables to consider may include:

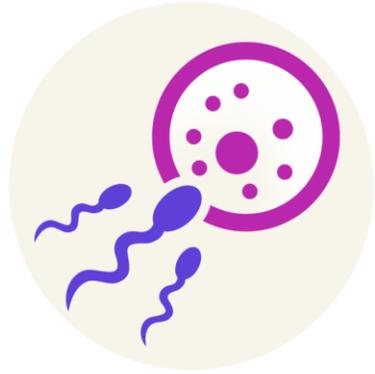
- Highest level of education:
  - No formal education
  - Some primary school
  - Completed primary school
  - Some high school
  - Graduated high school
  - Some college
  - Graduated college
  - Graduate school or professional degree
  - Don't know

- 
- Current employment status or source of income:
    - Formal employment (full-time vs. part-time)
    - Temporary work (seasonal work/work study)
    - Not working but actively looking vs. not actively looking
    - Temporarily laid off
    - Informal salaried workers (e.g., housekeeper, day laborer, shopkeeper's assistant, etc.)
    - Other informal employment (e.g., recycling cans/bottles, selling drugs or sex, or begging)
    - Self-employment
    - Other
  - Travel to the area from home for work purposes
    - Frequency and length of travel
    - Length of time in current area
  - Leisure travel
    - Frequency and length of travel
    - Length of time in travel area
  - Indirect SES indicators
    - Type and stability of housing
    - Maternal level of education



---

## 2. Reproductive History



### 2.1. Menstrual History

#### Rationale

Hormonal fluctuations during the natural menstrual cycle influence immune homeostasis in the female genital and gastrointestinal tract, as well as potential susceptibility to HIV infection [20, 21]. Therefore, clinical studies that evaluate mucosal immune responses in women who are not on hormonal contraception should document the phase of the menstrual cycle at which female reproductive tract samples or gut samples are being collected, and whenever possible try to standardize the phase at which to collect specimens. It is critical to establish cycle phase because tissues in the female reproductive tract are hormonally responsive, which can alter the volume and content of genital tract secretions, among other mucosal effects.



Normal values for humoral and cellular indices of mucosal immunity at different phases of the menstrual cycle are only beginning to be established [22], but for women with normal menstrual cycles who are not on hormonal contraception, there is already strong evidence that cervico-vaginal concentrations of numerous soluble mediators with immuno-modulatory or HIV-inhibitory effects vary during the course of the cycle [23, 24]. There is also growing evidence of the relationship between reproductive hormones and adaptive and innate mucosal immunity [25, 26].

### Considerations

Knowledge of the menstrual cycle can also inform the timing of mucosal sample collection. Collection of cervico-vaginal mucus should be avoided during menstruation, as blood contamination of mucosal samples will provide antibody specificities and concentration that are not actually present in mucosal secretions but in blood (systemic compartment). In women with regular cycles and no hormonal contraception, knowing the first day of the last menstrual period and typical cycle length can be helpful to roughly estimate the phase of the menstrual cycle, other than two days at midcycle. In women with irregular cycles, however, the date of last menstrual period, by itself, is very inaccurate to ascertain phase of the menstrual cycle at which a mucosal sample was collected. In studies where it is critical to accurately define the menstrual cycle phase, consider performing confirmatory hormonal laboratory tests in addition to recording an in-depth menstrual history.

Depending on design and goals of a study, in many cases a more extensive menstrual history is warranted, including data regarding:

- Current use of hormonal or non-hormonal contraception (see Section 2.4)
- Date of first day of last menstrual period
- Date of first day of menstrual period prior to the last one (to establish cycle length)
- Duration of last menstrual period, date of last day of last menstrual cycle
- Average interval between cycles, any recent changes
- Regularity
- The presence or absence of inter-menstrual bleeding

- 
- Intravaginal practices (e.g. douching frequency and tampon use) (see Section 2.5)
  - Breast feeding
  - Time since last pregnancy
  - Time since last childbirth
  - Time since last vaginal coitus

## 2.2. Pregnancy Status and History

### Rationale

There is evidence that pregnancy and the 12-month postpartum period are high-risk periods for HIV acquisition in women [27], and for HIV transmission from HIV-infected women to their male partners [28]. Both biological and behavioral changes have been hypothesized to explain these observations. Furthermore, hormonal changes that occur during pregnancy are associated with changes in immunoglobulin, cytokine, and antimicrobial peptide levels in genital secretions, and increased expression of CCR5 on CD4 T lymphocytes in the genital mucosa, suggesting a possible mechanism by which pregnancy may increase HIV levels in female genital secretions and HIV infectivity [29, 30]. Past pregnancies, miscarriages, and abortions, especially if recent, could also affect genital mucosal immune status through persistent hormonal effects.



### Considerations

Important pregnancy status and history variables to consider may include:

- Currently pregnant, including gestational age or, at a minimum, which trimester
- Prior pregnancy/abortion/miscarriage
- Currently breastfeeding
- Undergoing fertility treatment
- Undergoing hormone therapy or treatments

## 2.3. Contraception

### Rationale

Biological and epidemiological evidence suggests that hormonal contraceptives, including daily oral pills and long-acting injectables, may influence HIV transmission and acquisition. Two recent reviews [31, 32] observed that most studies assessing the use of oral contraceptive or injectable norethisterone enanthate (NET-EN) showed no association with HIV acquisition, whereas contradictory or inconclusive results were found in studies assessing injectable depot medroxyprogesterone acetate (DMPA), suggesting differential impacts of these hormones. Data on the effect of implants, contraceptive patches, rings, or hormonal intrauterine devices on the risk of HIV acquisition is either non-existent or limited. Thus, documenting the use of oral, depot, or injectable hormonal contraceptive methods is important for proper interpretation of HIV prevention interventions.



Although progesterone induces changes in cervical mucus and in the uterine epithelium, such changes have not been conclusively shown to affect susceptibility to HIV infection. *In vitro* studies examining the effects of DMPA on cell population changes, activation status, or inflammatory mediators have yielded conflicting data that is sometimes, but not always, supported by *in vivo* observations. This is probably a result of different concentrations of DMPA used in experiments that do not necessarily reflect physiological MPA concentrations. For example, in a recent study, DMPA was shown to inhibit cytokine production by peripheral blood mononuclear cells (PBMCs), activated T cells, and plasmacytoid dendritic cells, and women on DMPA had lower interferon- $\alpha$  levels in genital secretions and plasma [33]. Although some *in vitro* studies suggest that DMPA also prevents CXCR4 and CCR5 down-regulation on the surfaces of T cells after activation and increases HIV replication in PBMC cultures [34, 35], no vaginal changes in HIV target cell densities or CCR5 expression were observed in a recent study of women on DMPA for one year [36]. In addition, duration of use of oral contraceptives is known to affect the degree of cervical ectopy, which in turn affects the levels of inflammatory and regulatory cytokines/chemokines present in cervico-vaginal secretions according to one study [37].

---

The serum concentration of progestin from long-acting injectable contraceptives (such as DMPA and NET-EN) varies over the two to three months for which they are active, spiking the first week after injection. Other hormonal contraceptive technologies such as long-term implants, intrauterine devices IUDs, or cervical rings may also produce varying contraceptive levels in serum and tissue over time. Thus, it is important to record the timing of their administration, as recent injection or insertion could lead to exacerbation of the alterations in laboratory measurement of various immune parameters.

The use of barrier contraceptive methods (condoms and diaphragms) in conjunction with spermicides or lubricants may affect the genital microbiome and consequently influence mucosal immunity [38-40]. Furthermore, when tested as a potential microbicide to prevent HIV acquisition, frequent use of spermicide nonoxynol-9 caused mucosal disruption that increased the incidence of HIV [41]. The effect of IUDs and implants, some of which are hormonally impregnated, on the local mucosal milieu and HIV risk in women is largely unknown.

## Considerations

Important contraception practice information to consider may include:

- Type and usage (brand and name) of any contraceptive (oral, patch, intrauterine device, implant hormonal) or topical agents (rings, condoms, other barrier devices, lubricants)
- Any method(s) of contraception/family planning the participant reports; practices can vary greatly within countries and communities (the rhythm method, withdrawal, use of local techniques like herbs, fruits, etc.)
- Frequency of use of oral hormonal contraception (and missed pills)
- Knowledge of competent use of condoms (see Section 4.3) and frequency of condomless sex (for consistency of condom use)
- Type of topical contraceptive, including product information and frequency of use, especially with respect to timing relative to collection of mucosal samples and/or product administration because of potential for inflammatory effects on exposed mucosa from spermicide or lubricant components (administered separately or present in coated condoms) or physical abrasion (e.g., from use of depot contraceptive devices such as vaginal ring)
- Timing of last administration of long-acting injectable contraceptive, implant, or hormone-intrauterine device (IUD)
  - Distinction between IUDs that do or do not contain hormones
  - Name of IUDs and devices, for checking the nature and concentration of hormones delivered
  - Time since placement of device
- Use of a combination of methods

---

## 2.4. Menopausal Status

### Rationale

Given the profound hormonal changes that accompany menopause, clinical studies that conduct mucosal sampling of the female genital tract should consider collecting information on menopausal status and may need to decide whether to control for it during enrollment, depending on study-specific objectives [42]. A recent report that estradiol directly reduces the susceptibility of target CD4+ T cells and macrophages to HIV infection in an *in vitro/ex vivo* viral challenge model using cervical or cervico-vaginal biopsies raises further questions regarding the relative risk of HIV acquisition in post-menopausal women [43]. While most women have used lubricants/vaginal moisturizers, lubricant use frequency increases with age due to vaginal dryness, dyspareunia, and dysuria [44]. These products are typically hyperosmolar and can affect the vaginal microenvironment [39, 45].



### Considerations

The peri-menopausal stage is more difficult to ascertain solely by clinical history; menopausal status can be confirmed by laboratory testing if the study warrants this level of specificity. However, it is not clear that such analysis of hormonal status is sufficient to ascertain peri-menopausal status and if this factor should be considered to limit study eligibility.

Important menopausal status variables to consider may include:

- Current (or estimated) menopausal phase:
  - Pre-menopausal (years leading up to the last menstrual period)
  - Peri-menopausal (6-10 year phase ending 12 months after the last menstrual flow)
  - Post-menopausal (no menstrual flow for a minimum of 12 months)
- Any peri/post-menopausal hormone replacement therapy
- Use of vaginal douches, moisturizers, or vaginal estrogen creams (see Section 2.5)

Consensus definitions established by the Stages of Reproductive Aging Workshop +10 are extremely informative for documentation of menopausal status in studies for which accurate staging of this participant characteristic is critical [46].

## 2.5. Female Reproductive Tract Procedures/Surgeries

### Rationale

Gynecological procedures such as total hysterectomies can radically alter the anatomy of the female genital tract and thus the mucosal surfaces available for sampling. Recent surgical procedures, such as biopsy, can place an individual at increased risk of acquisition of a sexually transmitted infection.



Female genital mutilation is associated with higher prevalence of genital herpes, leads to higher incidence of wounds and abrasions during sexual intercourse, and raises the likelihood of anal intercourse when vaginal penetration is impossible or difficult ([WHO, Female Genital Mutilation](#)).

### Considerations

Important female reproductive tract procedures/surgeries information to record may include the following, along with the time since procedure:

- Hysterectomy (with or without oophorectomy)
- Dilation and curettage (D&C)
- Biopsies
- Genital reassignment surgery for transgender individuals
  - If possible, include tissue types used on reassignment. For example, colon vaginoplasty for neovaginal reassignment may include antibodies that are present in colon with high IgA concentrations. Skin grafts may not contain such antibodies.
- Colposcopy/biopsy
  - and whether followed by endocervical curettage
- Cervical excisional treatment
- Drainage removal of tubal/ovarian abscess
- Tubal ligation

- 
- Ectopic pregnancy
  - Hysteroscopy/polypectomy/myomectomy/noninvasive treatment for uterine fibroids
  - Vaginal and rectal prolapse repair surgeries: cystoceles, rectoceles, or enteroceles
  - Female genital modifications

## 2.6. Male Reproductive Tract Procedures/Surgeries

### Rationale

Surgeries of the male reproductive tract can affect the quality of genital samples, and can have important consequences for STI risk. Three independent randomized controlled studies showed that circumcision reduced HIV infection risk by 51-60% [47-49]. As well, circumcision reduces the risk of herpes simplex virus type 2 (HSV)-2, human papillomaviruses (HPV), and genital ulcerative diseases, which are also associated with HIV infection risk and/or recruitment of HIV target cells [48, 50, 51]. Lastly, circumcision procedures cause alterations to the penile microbiome, which may affect inflammatory mediators at the foreskin [52].



Vasectomies and vasectomy reversal surgeries seem to alter the antibody composition of the seminal fluid of men who underwent surgery [53]. In the same manner, inflammatory markers and immune proteins in seminal fluid may be affected by prostatic and testicular procedures that can alter vascularization and urogenital tract inflammation. Vasectomies, however, do not seem to affect total HIV measurements in semen [54].

In healthy men, immunoglobulins in seminal fluid are believed to enter from blood plasma at the prostate and add to the local IgA production at the urethra [55, 56]. Most HIV in semen comes from distal genitourinary sources rather than the prostate [54, 57], but surgeries and trauma might facilitate communication between genital and systemic sources of HIV. Lastly, procedures for the diagnosis or treatment of benign prostatic hyperplasia, as well as cancer, cause variable changes in both erectile and

ejaculatory function, so they need to be examined individually to assess how likely are they to influence seminal HIV shedding and seminal antibody readouts [58, 59].

## Considerations

Important male reproductive tract procedures/surgeries information to record may include:

- Vasectomy and vasectomy reversal
- Circumcision
- Prostatic or testicular surgeries
- Any recent prostate biopsies that could result in inflammation or abscess
- Prostate radiation
- Hormonal therapy or orchiectomy for prostate cancer
- Genital reassignment surgery and hormone therapy for transgender individuals
- Rectal prolapse repair surgeries: rectoceles, enteroceles

---

## 2.7. Sexually-transmitted and Reproductive Tract Infections (STIs/RTIs)

### Rationale

Undiagnosed or untreated, chronic STIs/RTIs have adverse genital and reproductive sequelae and may confound the interpretation of mucosal immunology data. Even if not considered standard-of-care in some high-incidence regions (e.g., South Africa), the screening of STIs in asymptomatic study participants is a critical part of obtaining interpretable mucosal specimens. For example, among female participants of the CAPRISA trial in South Africa, only 11.8% of women with laboratory-diagnosed STIs had characteristic signs or symptoms of an STI [60]. Furthermore, cervicovaginal lavage samples from asymptomatic but STI-positive women showed elevated levels of inflammatory cytokines equivalent to levels in STI-positive women with a clinically evident vaginal discharge. This increase in inflammatory cytokine levels in genital tract secretions of both asymptomatic and symptomatic STI-positive women was highly significant when compared to healthy, STI-negative women [60, 61]. Similarly, a cross-sectional study among MSM in Cape Town showed that asymptomatic STI was common and would not have been detected using a syndromic management approach [62].



Accordingly, screening for STIs (e.g., gonorrhea, chlamydia, syphilis, trichomoniasis, and herpes simplex virus) should be performed for all participants at each visit where mucosal samplings of reproductive tract secretions or tissues are scheduled, and should be budgeted in study funds, regardless of the local standard-of-care. The same recommendations apply with respect to mandatory STI screening for studies that conduct mucosal sampling of the GI tract (e.g., rectal swabs; rectal or colonic biopsies), given the prevalence of anal sex among both men who have sex with men and heterosexual female populations. The preferred test method for chlamydia, gonorrhea, and trichomonas is nucleic acid amplification testing (NAAT) using urine, genital, pharyngeal, or rectal swabs. Detailed methods for the screening and treatment of these and other STIs may be consulted in the [2015 STD Treatment Guidelines](#).

Because active GI or GU tract infections that cause local inflammatory responses in the mucosal compartment are such strong confounders for interpretation of mucosal immune responses, in general there should be mandatory exclusion of all symptomatic participants (those with genital or anal discharge, ulcer, etc.) as well as asymptomatic individuals who screen positive for STIs by laboratory testing in HIV prevention trials that collect samples from the GU or GI tracts. Exceptions to this approach may include clinical trials for which STIs are specifically the subject of study or study participants with vaginal discharge attributable to BV, a common RTI in many settings (see Section 6.2, Vaginal Discharge). Depending on the objectives and phase of a clinical trial, participants with STIs who have completed a confirmed course of full treatment may be considered for study enrollment, generally after re-screening. Furthermore, certain STI diagnostic data may be collected primarily for optimization of mucosal data (e.g., analysis of potential confounding factors), whether or not positivity is considered a study exclusion criterion.

Asymptomatic viral pathogens such as human papillomavirus (HPV) and herpes simplex virus types 1 and 2 (HSV1/2) can also cause immune cell activation and recruit immune cells to the mucosa [63]. While these pathogens are not typically screened for in participants unless clinical signs are present, they should be considered as viral replication can occur without overt symptoms. There is no unified diagnostic approach to this issue, and collection of samples should be tailored to the specific aims of the study.

Beyond study eligibility criteria, local inflammatory responses in the mucosal compartment caused by active GU or GI tract infections can predispose to other STIs including HIV.

---

## Considerations

Important STI/RTI information to record may include:

- History of a diagnosis and/or treatment for STIs in participant or sexual partners/contacts
  - Chlamydia, gonorrhea, syphilis, trichomoniasis, HSV
- Asymptomatic HPV/HSV genital and/or rectal shedding of HSV (see Section 2.8)
- Genital or rectal sores, ulcers, fistulas, fissures
- Ulcers or abscesses in the inguinal area
- Lumps, bumps, or warts
- Genital or rectal discharge
- Genital or rectal pain
- Skin rashes in the genital and rectal areas, and in general
- Participant reporting any unusual or foul odor in the genital area or genital secretions

Deferring to local public health standards for diagnosing STI/RTI may have limitations, as some remote sites do not test commonly, but treat empirically/syndromically using local, national, or WHO guidelines. In fact, some local public health authorities are reluctant to treat asymptomatic people except with clear laboratory diagnosis of an STI. Therefore, diagnostic testing capabilities must be offered.

Even if not considered standard-of-care in a region and if the participant is asymptomatic, positive results from a lab test performed under the standards of [Clinical Laboratory Improvement Amendments](#) (CLIA) equivalent need to be communicated to the patient and their health care provider should be informed. Participants should be informed in advance which study tests are CLIA equivalent with an obligation to report results to their health care provider for follow-up, and which tests (if any) are research tests that are not reported, but are nonetheless useful to help interpret the data obtained from a participant's mucosal sample.

Treatment decisions for a lab-diagnosed STI can be left to the local sites as different sites, countries, and regions use different treatment recommendations based on the local STI epidemiology and drug sensitivity. Asymptomatic participants with a lab diagnosis of chlamydia, gonorrhea, and syphilis should receive treatment regardless of local standards of care.

It is important to consider when or whether specific STI positivity results would be used post-hoc to inform analysis or would render a participant ineligible to enroll (entirely ineligible vs. eligible after treatment; is a test for cure necessary?). In regions with high STI burdens, inclusion or exclusion of participants based on their STI result and treatment criteria can increase or decrease the size of eligible participants, with an impact on the pace of enrolment. Accordingly, large efficacy trials may decide to screen, treat, and enroll, whereas smaller, earlier-phase trials may decide not to enroll post-cure if there are concerns that residual inflammation in the mucosal compartment being sampled may take an unknown interval to resolve.

## 2.8. Abnormal Cervical, Rectal, or Oral Cytology and Dysplasia

### Rationale

Cervical cancer is the third most common cancer among women, and the seventh overall, with an estimated 530,000 new cases in 2012 ([GLOBOCAN 2012](#)). More than 85% of the global burden occurs in developing countries, where it accounts for 13% of all cancers in women [64, 65]. Following infection with HPV, it is likely that changes in the mucosal adaptive immune system are critical to the control of HPV infection. Published work has demonstrated that women who clear HPV infection, as evidenced by a negative HPV PCR, had a preceding Th1 cytokine gene expression pattern, while those with persistent HPV lacked a Th1 response. It is known that persistent HPV infection with oncogenic HPV types is a critical step for the development of dysplasia, but the specific mucosal defects are yet to be elucidated [66]. Screening with regular gynecologic examinations and cytological testing (Pap smear or similar methods) with treatment of precancerous abnormalities decreases the incidence and mortality of HPV-induced cervical cancer.



---

Cytology screening programs have had a substantial impact on mortality in countries where access to regular screening is available and where there is an organized approach to cervical cancer prevention. HPV DNA testing may allow for better triage of women with the highest risk of clinical disease. Cervical cancer is the most common HPV-related cancer among HIV infected women.

Rectal sexual exposure also allows HPV infection of the anorectal region, which similarly can cause lesions, dysplasia, and rectal cancer that can be screened by anal Pap smear for effective preventive treatment of precancerous abnormalities. A strong association between HPV and anal cancer has been documented in the United States, with case rates in HPV-infected men 45-170 times that of age- and risk-matched populations [67]. The men-who-have-sex-with-men population is particularly at risk for this infection, but high rates of rectal dysplasia have also been identified in women [68, 69]. Finally, HPV can have similar pathogenesis of the oropharynx, with men in the United States being more likely to develop oral cancer than women due to HPV, according to the [CDC](#).

The local immune response to HPV and the role of innate immunity in persistence or regression of HPV infection are not well understood. Although an initial inflammatory infiltrate may contribute to disease regression, sustained inflammation at the HPV-induced lesions, characterized by macrophage and neutrophil infiltration, has been observed in persistence. With regard to adaptive immunity, a key indicator of regression in humans is infiltration of the lesion with both CD4+ and CD8+ T cells. Thus, in individuals with persistent lesions, HPV-specific responses may confound mucosal immune assays [70].

## Considerations

Important information to record related to abnormal cervical, rectal, or oral cytology and dysplasia may include:

- History of HPV vaccination
- Whether participant has received genital and oral examinations, cytological tests in past
- Location (cervix, anus, or oropharynx)
- Presence of high-risk HPV
  - Results from genetic HPV screening, if available
- Degree of abnormality and type of test
- Anal Pap smear vs. high-resolution anoscopy (HRA) with directed biopsies
- Cervical cytology vs. colposcopy with directed biopsies
  - any data on women who had visual inspection with acetic acid, with or without treatment, in low resource settings
- Anal cytology performs poorly due to poor inter-rater reliability compared to HRA-directed biopsies (although the technical training needs for HRA are higher)
- High-risk HPV serotype
- Histopathology is required for the diagnosis of cancer and the “gold standard” for screening tests; infrastructure and training requirements are high. DAIDS has developed a robust external quality assurance program for interested users [71]

---

## 3. Medical History



### 3.1. Medical Conditions

#### Rationale

Inclusion of past and current medical conditions and any ongoing conditions such as mental illness, alcoholism, drug abuse, and chronic conditions (controlled or not controlled by medication) will assist in inclusion/exclusion criteria, as well as in identifying potential confounders in analysis of mucosal samples. Procedures that result in hormonal dysregulation have potential effects on mucosal surfaces in the GI and GU tracts. Therefore, it is important to note these as potential confounders. Additionally, a history of non-GI/GU surgeries may be relevant to the safety of performing some procedures; for example, interventions in the upper airway and esophagus may affect the ability to perform upper endoscopy in studies where upper GI sampling would be involved, whereas a history of partial colon resection (left side) or peri-anal fissure repair may help explain baseline anatomic



differences observed during lower GI procedures. In addition, it is important to document any oral conditions that may affect oral sampling or the interpretation of data from samples obtained from the oral cavity. Finally, documenting pre-existing respiratory conditions may also inform inclusion/exclusion criteria, as well as the interpretation of data from bronchoalveolar lavage (BAL) samples.

## Considerations

Important information related to medical conditions to record may include:

- Chronic conditions (autoimmune diseases, diabetes, common variable immunodeficiency, chronic liver or kidney disease, endocrine disorders, etc.)
- Key women's health conditions
  - Conditions (endometriosis, Polycystic Ovary Syndrome (PCOS), etc.)
  - History of any pelvic exams and cervical cytology, if known
- Psychiatric conditions and/or use of psychiatric drugs
- Gastrointestinal diseases (irritable bowel syndrome, inflammatory bowel diseases, peptic ulcer disease, diverticulosis/diverticulitis, *Helicobacter pylori*) and their medical and/or surgical treatments
- For oral sampling: history of oral disease (gingival bleeding, ulcers, periodontitis, thrush, or other treatments)
- For respiratory (BAL) sampling: history of asthma, chronic obstructive pulmonary disease (COPD), inhaled steroids, other inhaled medications, and current symptoms
- For urinary sampling: history of urinary tract infections (UTIs), any history of non-specific urethritis, prostate disease, etc.
- Malignancies, including past cancer history
- Vaccinations (see Section 3.3)
- Medications (see Section 3.4)
- Allergies (see Section 3.5)
- Systemic or local infections (hepatitis, tuberculosis, parasites, etc.), including past infection history
- Circumcision status (see Section 2.6)
- Surgeries of female/male genital tract

- 
- For Nasopharyngeal sampling: nasal abnormalities, epistaxis, nasal surgery, or allergic rhinitis
  - Other surgeries:
    - Thyroidectomy
    - Adenoma resection
    - Cardiac surgery
    - Upper-airway surgeries

## 3.2. HIV Status

### Rationale

Mucosal tissues, in particular those in the gastrointestinal tract, are major reservoirs of CD4<sup>+</sup> T cells and antigen-presenting cells, and serve as primary sites of HIV infection and key reservoirs in chronic infection. Acute infection results in massive loss of GI tract CD4<sup>+</sup> T cells, altered profiles of lymphocytic cytokine production, changes in the landscape of gut antigen-presenting cells, and damage to the structural barrier of the GI tract, which contribute to GI tract dysfunction associated with HIV infection. Infection appears to be associated with gut damage and systemic translocation of bowel microbiota, which some suggest drives systemic disease progression [72, 73]. Although mucosal microbial translocation may be an important enhancer of mucosal immune activation, its full clinical implications remain unclear. It is apparent, however, that a different pattern of microbiota populations in the gut is a reproducible, stable observation among HIV-infected individuals on therapy, HIV-infected individuals not on therapy, and healthy individuals [74].



Initiation of cART improves, but does not reverse completely the immunological abnormalities within the GI tract [74]. ART drug levels may vary significantly by medication and between plasma and different tissue compartments [75], which can affect results of mucosal assays such as viral replication in tissue explants. Although numerous studies have demonstrated that concentrations of HIV in plasma compared to the genital tract are highly correlated, HIV can be detected in

cervicovaginal lavage and semen samples collected from HIV-infected women and men in whom cART has suppressed plasma viral loads to undetectable levels [76-80].

## Considerations

Important information to record related to HIV status includes:

- HIV serostatus
  - Duration of HIV infection (last seronegative and first seropositive test dates, if known)
  - CD4 T cell counts (nadir and current)
  - Plasma HIV RNA levels (current “viral loads” and if suppressed, duration of suppression)
- Antiretroviral medications, including detailed information on current use:
  - Duration of current use
  - Self-reported adherence
  - Last time off cART and duration off cART
  - Past treatment history and timing
  - History of resistance
- Mode of acquisition of HIV infection
- History of opportunistic infections
- HCV co-infection
- HIV/STI status of sexual partner(s) (see Section 4.5)

---

## 3.3. Vaccinations

### Rationale

Vaccinations typically invoke systemic immune responses; accordingly, it is important to collect vaccination history from participants who provide mucosal samples for immunologic evaluation. This is particularly critical in the context of HIV vaccine trials. While it is not clear how receipt of a non-study vaccine can affect the immune response to the study product, it is important to collect the information about the proximity of any non-study vaccination(s) relative to the study vaccinations and mucosal sample collection time points. In addition, allowable intervals between non-study and study vaccinations may depend on the type of non-study vaccination received (e.g., live, attenuated or not) and are primarily based on data and/or [Vaccination Guidelines from the Centers for Disease Control](#) (CDC).



### Considerations

Data regarding vaccinations received while on-study (e.g., influenza vaccination, HPV vaccine), is typically collected on the “concomitant medications” section of CRFs.

HIV-vaccine trial participants planning to receive licensed vaccines, allergy treatments, etc., during the course of the trial, should be counseled to schedule these vaccinations outside of study vaccination intervals whenever possible to avoid study vaccination delays or missed vaccinations. Similarly, because the effects of these non-study substances on safety and immunogenicity assessments and their interactions with study vaccines are unknown, if circumstances allow, these non-study substances should also be avoided in the interval between a study vaccination and completion of the post-vaccination follow-up visits.

## 3.4. Medications

### Rationale

All concomitant medications should be logged as they may have intended or un-intended interactions with mucosal immunity.



### Considerations

Relative to medications, it is important to record:

- Use of health supplements, such as multivitamins, herbal/natural remedies, homeopathic therapies, and over-the-counter medications such as decongestants, aspirin, or NSAIDs (non-steroidal anti-inflammatory drugs)
- Use of probiotics; some oral probiotics in current broad over-the-counter use are anti-inflammatory (e.g., ALIGN™), whereas others that could potentially be used in the future may be immune-activating (e.g., used as adjuvants). In either case, changes in gut microbiome may affect mucosal immune responses [81]
- Vaginal probiotics in topical or suppository/capsule presentations
- Hormonal treatments, other than contraception (see Section 2.3)
- Antibiotic treatments
- Record both the trade name and generic name of the medication (if possible) as well as the country of origin (as generic names may vary in different countries)
- Antiretroviral drugs taken either for pre- or post- exposure prophylaxis (PEP/PrEP) or for recreational use; reports for recreational use of the drug efavirenz (trade names: Sustiva®, Stocrin®) are documented and the psychoactive effects of the drug are being studied [82]
- Recreational drugs (See Section 5.1)
- Non-study vaginal or rectal products (see Section 4.2)
- Systemic steroids, or topical with mucosal administration (oral, respiratory, GI, vaginal, anal)
- Recent chemotherapy for systemic autoimmune conditions

---

## 3.5. Allergies

### Rationale

On an operational level, allergies are relevant to safety in interventional studies themselves (e.g., potential allergies to vaccine components) as well as to safety in performing mucosal sampling procedures (e.g., latex, anesthesia, or metal allergies). On an immunological level, although the potential effect of allergies on mucosal immunity is not entirely understood, it is reasonable to expect that such an effect may be present. Thus, it is important to record any allergies. Varying degrees of immune hypersensitivities to agents could directly affect levels of mucosal immune responses. Ultimately the trial sponsors will determine the degree and/or types of allergies reported by a participant that are compatible with the purpose of the study.



### Considerations

Participants should be asked for all known allergies (drug and non-drug such as environmental exposures and food), allergy treatments, or plans to receive allergy treatments. Allergy medications will also be captured on the concomitant medications log (see Section 3.4).

A participant's description of their "allergy" is important to record; oftentimes these will be intolerances to side effects and not true immune-mediated allergies. Record:

- ✓ Specific product/agent/food/environmental sensitivity
- ✓ Specific responses to these, including the timing and description of rashes, as this is critical for distinguishing hypersensitivity reactions versus serum sickness versus delayed hypersensitivity reactions, which are distinct immune reactions with different levels of risk

Participants may be unaware of some allergies, for example an allergy to a product used occasionally, such as vaginal douches, perfumed pads, etc. Practices where such products are used should be recorded (see Section 4.2). It will be the role of the investigators to elucidate any potential interactions with mucosal assays.

## 3.6. Body Mass Index

### Rationale

Body Mass Index (BMI), a relative measure of fat and muscle mass in the body based on height and weight, may have a significant influence on vaccine-elicited immunogenicity [83, 84]. Obesity, defined as BMI  $\geq 30$  kg/m<sup>2</sup>, has been associated with impaired adaptive and innate immune system responses to infections or vaccination and increased susceptibility to infectious agents and cancer. Conversely, although less is known about the effect of eating disorders and abnormally low BMIs (<18.5 kg/m<sup>2</sup>) on the immune response of adults, being underweight and/or malnourished affects or impairs the immunogenicity of mucosal vaccines in children [85].



Some human studies have shown that systemic inflammation varies with different levels of adiposity even among individuals with normal weights. It has been proposed that adipose tissue inflammation modulates immune responses to antigens [86-88]. Assessment of adiposity and the pro-inflammatory biomarkers secreted by adipose tissue can thus provide additional insight into immune regulation and may be important to explore in responses to vaccination.

### Considerations

Measurements of BMI and adiposity can be straightforward and relatively inexpensive, or more complex and expensive (e.g., fasting leptin and adiponectin levels). The more complex and expensive methods should be justified as they raise significant operational hurdles. The influence of BMI and gender on vaccine-elicited immunogenicity may be related, which should be considered in analyses.

---

## 3.7. Underexplored Areas and Other Factors

### Rationale

Factors like circadian rhythm [89], duration and intensity of physical exercise [90, 91], exposure to acute or chronic stress [92], and nutrition [93, 94] are all known to affect the immune response.



Although the evidence for the importance of these factors is still an underexplored area, it may be important to consider and document such factors during study design, mucosal sampling, and the interpretation of results.

### Considerations

Some factors to consider depending on the nature of study are:

- Circadian rhythm issues
  - Whether participant's work/wake hours are mainly in the nighttime
  - Exact time of participant's visit to the clinic/study site for blood and/or mucosal sampling
- Current and past history of physical activity or exercise
  - Hours per week
  - Duration and intensity
- History of significant life events causing stress
  - Past or ongoing
  - Acute or chronic
- Nutritional status
  - Anthropometric measurements of body composition
  - Laboratory measurement, if available
    - Serum albumin
    - Vitamin B12
    - Fasting lipid and glucose profiles
  - Dietary intake assessments, if available



---

## 4. Sexual History & Sexual Risk Behaviors



### 4.1. Sexual Practices

#### Rationale

Sexual transmission across mucosal surfaces (whether gastrointestinal or urogenital) is the primary source of new HIV infections and the predominant driver of the ongoing global HIV epidemic [95]. Among sexually-acquired HIV infections, semen is the primary transmission vector [96]. There is increasing research into the potential role that seminal plasma as well as vaginal and rectal fluids may play in HIV transmission, independent of their obvious function as a carrier of infectious virions [97, 98].



Information regarding sexual practices is key not just as a gauge of risk to HIV infection risk, but also due to the possible impact on immune responses (immune activation and immunomodulation) and trauma responses in the vaginal or rectal mucosa. Seminal plasma may also affect innate factors and may alter the antiviral effectiveness of topical microbicides or of innate factors produced by the female genital mucosa [99, 100].

---

Semen has been shown to recruit immune cells such as dendritic cells, macrophages, and memory T cells to the lamina propria soon after coitus [101, 102]. If defining immune parameters in the genital compartment is of interest, it is important to know the time of coitus, and testing for seminal proteins or the Y chromosome may be advised [101, 102]. For women, it is critical to consider dual compartment sexual exposures (vaginal and rectal) and the order in which these sex acts occurs, because of the potential to transmit microorganisms (and later-applied products) from one compartment into the other, with potential impact on the mucosal microbiome.

## Considerations

Important Sexual Practices information to record may include:

- Number of sexual partners in the last day, week, month
- Gender of partner(s)
- Number of partners per session
- Types of sex acts (oral, vaginal, rectal, insertive, and receptive) and potential for tissue trauma (e.g., noted blood)
- Number of acts of sexual intercourse per session
  - with or without competent use of a latex condom (see Section 4.3)
  - with or without pre-exposure prophylaxis
  - with or without contraceptive but no additional barrier methods
- Date of last vaginal sex
- Date of last anal sex (for both men and women)
- Frequency of sexual intercourse
- Frequency and mode (oral, vaginal, rectal, insertive, receptive, combination) of sexual intercourse *without* a latex condom
- Duration and mode (oral, vaginal, rectal, insertive, receptive, combination) of average sexual intercourse
- Duration and mode (oral, vaginal, rectal, insertive, receptive, combination) of last sexual intercourse
- Exposure to ejaculate (genital, non-genital/oral)
- Use of foreign objects (e.g., sex toys, or other natural or man-made products inserted into the vagina or rectum (see Section 4.2)

- 
- Cleaning habits before and after intercourse
  - Use of oils or oil-based lubricants that can affect the integrity of latex condoms during sex

## 4.2. Use of Vaginal or Rectal Products or Devices

### Rationale

Products or devices may be inserted inside the vagina to prepare for sex, to clean inside the vagina before or after sex, or to treat vaginal conditions. For example, in a group of young South African women, 15% reported using intravaginal “drying agents” (e.g. herbs, snuff, or powders) placed in the vagina to reduce wetness because of cultural expectations of men in some regions (Kwon Douglas. Personal Communication, 2013). Such use of products or insertion of objects may alter the vaginal flora and predispose to bacterial vaginosis (BV) and, as a result, enhance risk of other STIs including HIV [37]. A meta-analysis of intravaginal washing or drying practices of women from thirteen prospective cohort studies showed a correlation between some practices such as intravaginal cleaning with soap, and development of BV and HIV risk, but a direct causal link could not be established [103]. Although a common practice globally, vaginal douching has been associated with increased risk of HIV acquisition and may increase the rate of genital viral shedding in HIV-infected women [104, 105]. Finally, rectal insertion of products for sexual or therapeutic purposes may also occur. Rectal douching before receptive anal intercourse may also be common and may be associated with other sexual risk behaviors [106], but the contribution of this practice to the acquisition of HIV in men and women is unknown. Thus, it is important to document the use of vaginal or rectal products/practices which may affect the efficacy of HIV prevention strategies.



---

## Considerations

Important variables to consider in the use of vaginal and rectal products or devices may include:

- Products inserted into the vagina during menstruation (tampons, cotton wool, rags, vaginal softcups, etc.)
- Other products used before/during/after sexual activity
- Douching /cleaning regimen
- Type of products or devices:
  - Vaginal or rectal lubricant (type and name brand of product, if commercial; other characteristics if non-commercial)
  - Vaginal moisturizers different from lubricants (type and name brand of product, if commercial; other characteristics if non-commercial)
  - Other vaginal washing/drying practices
    - Insertion of cloth, paper, and vegetation such as leaves, etc.
    - Use of products that dry or tighten
  - Vaginal yeast medication
  - Vaginal estrogen cream (name brand of product)
  - Other intravaginal treatments (such as probiotics)
  - Use of soap
  - Spermicide
  - Douche (vaginal or rectal, type and name of product, if commercial)
  - Enema
  - Timing of last use (especially within last 48–72 hours)

---

## 4.3. Sexual Risk Behaviors

### Rationale

In general, for men, condomless receptive or insertive anal intercourse with an HIV-infected man or condomless vaginal intercourse with an HIV-infected woman are the leading causes of HIV infection. For women, it is condomless anal or vaginal intercourse with an HIV-infected man. Data suggests that HIV transmission risk is a minimum of twenty-fold greater per receptive rectal vs. vaginal sex act with an HIV-seropositive individual who is not on cART [107, 108]. Co-infections and multiple exposures per episode only increase these reported HIV-transmission rates [109-112].



### Considerations

Important sexual risk behaviors information to record may include the following variables; note that the recall period for these considerations may vary depending on the study:

- Types of sexual partners (primary, concurrent, transactional, or multiple)
- Frequency of condomless sex
- Knowledge of competent use of condoms (condom re-use, transfer to a different partner, exposure to ejaculate collected in condom, and handling a new condom with contaminated hands may be common practices that can negate the protection afforded by condoms).
- Frequency of vaginal or anal sex without a condom with partners who injected drugs
- Frequency of vaginal or anal sex without a condom with partners who used other drugs
- Sex in exchange for money, drugs, goods, services (with or without a condom)
- Concurrent sexual behaviors
- Both insertive and receptive anal sex for men who have sex with men (MSM)
- Single- vs. dual-compartment sex for women (anal or/and vaginal)
- Knowledge of partner(s) HIV seropositivity and their viral load
- Oral sex activity, particularly for studies involving saliva

---

## 4.4. Risk Behaviors of Partner

### Rationale

Some individuals may be monogamous, but at increased risk for HIV infection because of the behaviors of their partner. Thus, trying to ascertain the partner's HIV risks can inform the team of whether a potential trial participant is low or high risk. Because of challenges in disclosure, a negative history may not always clearly define the partner's risk, but record of the partner's high-risk behavior(s) can help the research team more appropriately interpret study findings.



### Considerations

Important information to record related to risk behaviors of partner may include:

- If the respondent has asked her/his partner(s) about their sexual behaviors
- Direct or indirect knowledge of sexual partners having sex with another person(s) in the last six months
- If sexual partner(s) are not monogamous or exclusive, record whether any of the following factors apply:
  - Intravenous drug use
  - Alcohol
  - Other drugs
  - Exchange sex
  - Sex with men who have sex with men
  - Sex with a commercial sex worker
  - Sex with partner who was drunk or high on drugs
  - Sex with a known HIV-infected partner

---

## 4.5. HIV and Other STI Status of Partner(s)

### Rationale

Knowledge of the HIV/other STI infection status of recent sexual partners is important to determine [113]. Recent sex with a person who is known to be HIV-infected increases the probability that the participant has acute HIV infection, and may increase the risk for transmission of other HIV-associated co-infections such as HSV-2. In addition, individuals exposed to HIV without infection may demonstrate important differences in genital immunology. Frequently exposed yet uninfected men-who-have-sex-with-men have been demonstrated to have elevated systemic immune activation and even differences in T cell receptor repertoires [114].



### Considerations

The HIV status of partner may be unknown; when asking questions it is important not to assume knowledge of partners' serostatus. For example, consider asking:

- Number of partners with unknown HIV status
- Number of partners known to be HIV positive
- Number of partners known be HIV negative
- Knowledge of HIV or AIDS status of main sex partner
  - Detectable or suppressed viral load
- Other sexually transmitted infections in partners



---

## 5. Other Risk Behaviors



### 5.1. Drug Use

#### Rationale

Drug users may be at high risk of contracting HIV from sharing equipment (e.g., syringes) to inject drugs into the blood stream, but also from [unsafe sexual practices when under the influence of drugs](#), or through sexual activity to get drugs. Some drugs such as methamphetamine, crack cocaine, and amyl nitrite (poppers), are associated with sexual practices that may increase the likelihood of HIV and other STI transmission (e.g., long duration of sex act leading to inflammation or ulcerations, multiple partners, lack of inhibition, decreased condom use) [115]. Methamphetamine may also dry the mucosae, which may lead to more irritation and abrasions that could facilitate HIV entry during sexual activity.



Drug use is also linked to poor adherence to ART and medication regimens in general, which may lead to higher risk of HIV and STI transmission from infected individuals who are also drug users, though an empirical study has shown that many drug users can be adherent to HIV medications [116]. Drug use may also directly affect immune function, although data are conflicting [117, 118].

### Considerations

Some users may not wish to disclose drug use due to stigma, and/or perceiving that their provider would view them negatively (e.g., be afraid that their provider would judge them as engaging in an illegal behavior versus having a problem that requires appropriate treatment). It is important to emphasize to participants confidentiality and Health Insurance Portability and Accountability Act (HIPAA) protections of this information and stress that drug use information is used only for inclusion/exclusion criteria for study enrollment and to ensure scientific accuracy of mucosal data obtained from participant samples. Drug use of sexual partners is also important to record in order to determine if they may be high-risk individuals.

Important drug use information to record may include:

- Whether the subject has injected/snorted/inhaled/swallowed drugs
- Type of drugs used. Make sure to include routes of administration, including:
  - Inhalants, such as powder cocaine, amyl nitrate (“poppers”)
  - Injectables (intravenous, subcutaneous “skin popping”), such as heroin
  - Pills, such as oxycodone
  - Topically applied to mucosa prior to/during sex
    - e.g., “booty bump”, which is rectal insertion of drugs such as crystal meth, ecstasy, and others
    - vaginal lubricants containing cannabis
  - Marijuana (see Section 5.2)
  - Long-term methadone use
  - Local products such as whoonga
  - Other
- Frequency of drug use

- 
- Most recent substance use
  - Usage in the past month, three months, six months
  - Usage patterns (e.g. daily, weekends, social events, etc.)
  - Unprotected vaginal or anal sex while using drugs, and type(s) of drugs used with sex
  - Limited memory or uncertainty about having unprotected anal or vaginal sex while using drugs
  - Unprotected sex in exchange for drugs or money
  - Drug use of sexual partners

## 5.2. Smoking

### Rationale

Cigarette smoking has both behavioral and immunological consequences. Smoking and nicotine affect both systemic and mucosal immunity, altering a wide range of both innate and adaptive immune functions [119-123]. In the context of mucosal sampling, and depending on the invasiveness of the sampling procedure, it is also important to note the impacts of smoking on wound healing; slower healing has been observed clinically in smokers with wounds resulting from trauma, disease, or surgical procedures [124]. The understanding of the impact of nicotine on post-operative wound healing has been confirmed more recently in a systematic review and meta-analysis [125]. Smoking has also been linked to cervical and anal cancer [126, 127]. Documentation of participants' smoking history may inform the assessment of post-procedure recovery and the interpretation of any observed events related to a wound resulting from a procedure, and possibly the existence of cervical and anal dysplasia.



Finally, smoking and/or dependence to nicotine may be used as an indicator of individuals who have a propensity for risk-taking activities [128-130].

## Considerations

Important smoking information to record may include:

- Date when started
- If having quit, duration of smoking period
- Frequency and volume of smoking (pack per day, week, etc.)
- Variability of smoking patterns (increases under pressure, social smoker, time of day)
- Type of cigarettes (filtered, hand-rolled, other)
- Choice of smoking material (tobacco, chicory, marijuana, other)
- Other tobacco use (cigars, chewing tobacco, other)
- Nicotine use (patches, E-cigarettes, gum)

## 5.3. Alcohol Consumption

### Rationale

For some people who engage in high-risk sex, alcohol use can be an important risk factor for HIV infection because it can be linked to less frequent use of condoms and/or multiple sexual partners. In addition, a single episode of binge alcohol intoxication can cause acute immunomodulatory effects [131]. Furthermore, excessive alcohol consumption can suppress mucosal immunity in the GI tract, and result in more severe cases in progressive liver disease that can contribute to impaired immune function systemically [132, 133]. On the other hand, evidence also shows that moderate alcohol consumption may enhance vaccine immune reactivity [134].



---

## Considerations

Important alcohol consumption information to record may include:

- Frequency and quantity of drinking (number of drinks per day, weekly pattern of drinking)
- Frequency of six or more drinks containing alcohol on one occasion (i.e. binge drinking)
- Triggers for periods of excessive drinking
- Measures of excessive drinking (e.g., the CAGE or AUDIT screening questions, which may be found in the National Institute on Alcohol Abuse and Alcoholism (NIAAA) [Guide for Clinicians and Researchers](#).)
- Sex while intoxicated (number of oral, vaginal, and/or anal sex acts and condom use while intoxicated)
- Limited memory or uncertainty about having had sex while intoxicated



---

## 6. Symptoms



This section complements the Medical History section as new infections and conditions occurring during the course of the study should be recorded here. These events may greatly affect both the protocol and the interpretation of assays and may also reveal conditions that are not obvious to the participant or were not disclosed earlier.

### 6.1. Constitutional Symptoms

For HIV-prevention trials (in contrast to therapeutic trials), potential participants with generalized lymphadenopathy, icterus, cirrhosis, etc. should probably be excluded. For therapeutic trials, as long as a stable and detailed recording of symptoms is in place for both the pre-enrollment and the pre-product exposure phases, enrollment may be approved. Investigators may consider enrolling participants with mild or unrelated symptoms and/or lab results that are unlikely to be affected by responses to study agents.



## Rationale

For any HIV-intervention trial, it is critical to assess whether participants exhibit symptoms consistent with acute HIV infection. These include: fever, malaise, myalgia, maculopapular rash, headache, night sweats, sore throat/oral ulcers, lymphadenopathy, arthralgia, and nasal congestion [135]. The symptom observations may be noted before or after study enrollment.

It is important to understand that these symptoms can be very non-specific; for example, acute Epstein–Barr virus (EBV) infection can have clinical symptoms similar to primary HIV infection (fevers, chills, diffuse lymphadenopathy).

## Considerations

Important information related to constitutional symptoms to record may include:

- Other concomitant signs/symptoms of infection
- RNA collection, deferment of enrollment, product hold in the case of reasonable suspicion of acute HIV infection
- Duration, onset, trigger, treatment, timing with respect to product exposure, and relief of symptoms (related/unrelated) for the following symptoms:
  - Fevers/sweats/chills
  - Headaches
  - Joint aches/arthralgias
  - Skin rashes or changes in skin color
  - Enlarged or painful lymph nodes (notable in a number of genital STIs such as lymphogranuloma venereum, chancroid, syphilis, HSV, etc.)
  - Oral ulcerations or other oral symptoms (discomfort, ulcers, tooth pain, gum pain, loose teeth, bleeding gums, tooth loss)
  - Nausea/vomiting
  - Abdominal pain/discomfort
    - Location of discomfort and radiation of pain
  - Dysuria
  - Urethral discharge
  - Diarrhea with mucus or blood, or other GI symptoms (constipation, pain, dietary intolerance, or flatulence)

- 
- Proctalgia (patient's report of urgency)
  - Urogenital ulcer history (penile, vaginal, or perianal)
  - Rectal, vaginal, or urethral discharge

## 6.2. Vaginal Discharge

### Rationale

Both participant complaints and clinical findings of abnormal vaginal discharge are common among women of reproductive age. While the evaluation of abnormal vaginal discharge may not differ between the two, whether treatment is offered and how the abnormality is reported may. Abnormal vaginal discharge may be associated with cervicitis, yeast, trichomoniasis, and/or BV), among other conditions. Site clinicians are encouraged to thoroughly evaluate complaints and/or findings of abnormal vaginal discharge as well as describe any findings in the genital examination: characteristics of vaginal discharge, vaginal pH, and presence of any ulcerations or odor, presence and description of cervix.



### Considerations

Whether to treat the underlying cause of the abnormal Vaginal Discharge will depend on the underlying diagnosis and whether the participant is symptomatic. If the evaluation reveals an underlying sexually transmitted infection such as trichomoniasis, the participant and her partner(s) should be offered treatment regardless of symptoms. If the evaluation reveals BV or yeast, the participant should be offered treatment only if she is symptomatic.

Identification and treatment of symptomatic BV is important given the high risk of HIV acquisition and transmission [110, 111]. Participant complaint of vaginal discharge must be distinguished from clinician-observed discharge. If testing for typical STI is negative but meets criteria for BV (or vulvovaginal candidiasis), offer treatment ONLY if participant is symptomatic. A standardized scoring system for the interpretation of Gram-stained vaginal smears is recommended. The scoring system provides a 0- to 10-point scale for the evaluation of vaginal flora; the scale is based on a weighted sum of the following bacterial morphotypes with good to excellent intercenter reliability: *Lactobacilli spp.*, *G. vaginalis*, and *Mobiluncus spp.* When the most reliable of

the bacterial morphotypes are used to produce a summary score, that score can be used to assess the degree of alteration in vaginal flora as a continuum rather than as a forced dichotomy [136].

## 6.3. Rectal Discharge

### Rationale

The differential diagnosis of rectal discharge is extensive and includes inflammatory and infectious etiologies. The presence of this symptom requires clinical evaluation and is likely to exclude participants from enrolling in a study requiring mucosal sampling [109, 137] .



### Considerations

Rectal discharge could signify an ulcerative STI or other process that may influence the risk of HIV transmission. Causes of rectal discharge include:

- Anorectal STIs:
  - Chlamydial infection including lymphogranuloma venereum
  - Gonorrhea
  - Anal condyloma
- Non-STI causes:
  - Inflammatory bowel disease (ulcerative colitis and Crohn’s disease)
  - Anal fissure
  - Hemorrhoids
  - Irritable bowel syndrome
  - Anal and colorectal cancer

The majority of patients with anorectal STIs (e.g., syphilis) are asymptomatic, which is why screening is mandatory for studies that involve sampling of the rectal compartment. Rectal discharge can signify non-STI conditions as well that would also warrant work-up (inflammatory bowel disease, etc.).

---

## 6.4. Pelvic Pain

### Rationale

Evaluation is key for assessment of pelvic infection (chlamydia, gonorrhea, and *Trichomonas vaginalis*, especially) and pelvic inflammatory disease. Pelvic pain may warrant a full gynecologic assessment to rule out other conditions, which may need attention and could impact study-related outcomes. Pelvic pain has obvious impact on the ability and willingness to participate in clinical practices (e.g., taking an oral study drug, or inserting either a vaginal or rectal study product). Any number of these conditions could affect eligibility in a trial and impact study participation for those already enrolled.



### Considerations

- It is important to distinguish pelvic pain from abdominal/gastrointestinal pain.
- Testing ought to include evaluation for complicated pregnancy

## 6.5. Oral, Respiratory or other GI Symptoms

### Rationale

Symptoms related to medical situations or conditions described in Section 3.1 may arise after enrollment and during the course of the study. Many are listed here again for clarity.



### Considerations

Important information related to symptoms and/or medical conditions to record may include:

- Previously undetected chronic conditions (autoimmune diseases, diabetes, common variable immunodeficiency, chronic liver or kidney disease, endocrine disorders, etc.)
- Appearance of gastrointestinal diseases (irritable bowel syndrome, inflammatory bowel diseases, peptic ulcer disease, diverticulosis/diverticulitis, *Helicobacter pylori*) and their medical and/or surgical treatments
- Appearance of oral disease (gingival bleeding, ulcers, or periodontitis)
- Appearance of STI's such as gonorrhea and chlamydia, which can have carriage in multiple mucosal compartments, including the oropharynx [138] (see Section 2.7)
- Appearance of respiratory diseases and treatments: asthma, chronic obstructive pulmonary disease (COPD), inhaled steroids, other inhaled medications, current symptoms
- Appearance of urinary tract infections (UTIs), or non-specific urethritis, prostate disease, etc.
- Allergies (see Section 3.5)
- Appearance of cancer/malignancies
- Vaccinations (see Section 3.3)
- Appearance of systemic or local infections (hepatitis, tuberculosis, parasites, etc.)
- Any surgeries or medical emergency procedures

---

## Endnote

### References

1. Hickey, R.J., et al., *Vaginal microbiota of adolescent girls prior to the onset of menarche resemble those of reproductive-age women*. MBio, 2015. 6(2).
2. Petrova, M.I., et al., *Vaginal microbiota and its role in HIV transmission and infection*. FEMS Microbiol Rev, 2013. 37(5): p. 762-92.
3. Madan, R.P., et al., *Altered biomarkers of mucosal immunity and reduced vaginal Lactobacillus concentrations in sexually active female adolescents*. PLoS One, 2012. 7(7): p. e40415.
4. Wira, C.R., M. Rodriguez-Garcia, and M.V. Patel, *The role of sex hormones in immune protection of the female reproductive tract*. Nat Rev Immunol, 2015. 15(4): p. 217-30.
5. Yamamoto, T., et al., *Bacterial populations in the vaginas of healthy adolescent women*. J Pediatr Adolesc Gynecol, 2009. 22(1): p. 11-8.
6. Rollenhagen, C. and S.N. Asin, *Enhanced HIV-1 replication in ex vivo ectocervical tissues from post-menopausal women correlates with increased inflammatory responses*. Mucosal Immunol, 2011. 4(6): p. 671-81.
7. Mabbott, N.A., et al., *Aging and the mucosal immune system in the intestine*. Biogerontology, 2015. 16(2): p. 133-45.
8. Sato, S., H. Kiyono, and K. Fujihashi, *Mucosal Immunosenescence in the Gastrointestinal Tract: A Mini-Review*. Gerontology, 2015. 61(4): p. 336-42.
9. Wang, H. and D.P. Kotler, *HIV enteropathy and aging: gastrointestinal immunity, mucosal epithelial barrier, and microbial translocation*. Curr Opin HIV AIDS, 2014. 9(4): p. 309-16.
10. Kaul, R., et al., *Biological factors that may contribute to regional and racial disparities in HIV prevalence*. Am J Reprod Immunol, 2011. 65(3): p. 317-24.
11. Peipert, J.F., et al., *Bacterial vaginosis, race, and sexually transmitted infections: does race modify the association?* Sex Transm Dis, 2008. 35(4): p. 363-7.
12. Markle, J.G. and E.N. Fish, *SeXX matters in immunity*. Trends Immunol, 2014. 35(3): p. 97-104.

13. Oertelt-Prigione, S., *The influence of sex and gender on the immune response*. *Autoimmun Rev*, 2012. 11(6-7): p. A479-85.
14. Sankaran-Walters, S., et al., *Sex differences matter in the gut: effect on mucosal immune activation and inflammation*. *Biol Sex Differ*, 2013. 4(1): p. 10.
15. Markle, J.G., et al., *Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity*. *Science*, 2013. 339(6123): p. 1084-8.
16. Marrazzo, J.M., et al., *Tenofovir-based preexposure prophylaxis for HIV infection among African women*. *N Engl J Med*, 2015. 372(6): p. 509-18.
17. Kposowa, A.J., *Marital status and HIV/AIDS mortality: evidence from the US National Longitudinal Mortality Study*. *Int J Infect Dis*, 2013. 17(10): p. e868-74.
18. Lurie, M.N., et al., *The impact of migration on HIV-1 transmission in South Africa: a study of migrant and nonmigrant men and their partners*. *Sex Transm Dis*, 2003. 30(2): p. 149-56.
19. Mmbaga, E.J., et al., *The role of in-migrants in the increasing rural HIV-1 epidemic: results from a village population survey in the Kilimanjaro region of Tanzania*. *Int J Infect Dis*, 2008. 12(5): p. 519-25.
20. Wira, C.R. and J.V. Fahey, *A new strategy to understand how HIV infects women: identification of a window of vulnerability during the menstrual cycle*. *Aids*, 2008. 22(15): p. 1909-17.
21. Hel, Z., E. Stringer, and J. Mestecky, *Sex steroid hormones, hormonal contraception, and the immunobiology of human immunodeficiency virus-1 infection*. *Endocr Rev*, 2010. 31(1): p. 79-97.
22. Jespers, V., et al., *Assessment of mucosal immunity to HIV-1*. *Expert Rev Vaccines*, 2010. 9(4): p. 381-94.
23. Wira, C.R., et al., *Innate immunity in the human female reproductive tract: endocrine regulation of endogenous antimicrobial protection against HIV and other sexually transmitted infections*. *Am J Reprod Immunol*, 2011. 65(3): p. 196-211.
24. Keller, M.J., et al., *PRO 2000 elicits a decline in genital tract immune mediators without compromising intrinsic antimicrobial activity*. *Aids*, 2007. 21(4): p. 467-76.
25. Oertelt-Prigione, S., *Immunology and the menstrual cycle*. *Autoimmun Rev*, 2012. 11(6-7): p. A486-92.

- 
26. Shacklett, B.L. and R.M. Greenblatt, *Immune responses to HIV in the female reproductive tract, immunologic parallels with the gastrointestinal tract, and research implications*. Am J Reprod Immunol, 2011. 65(3): p. 230-41.
  27. Drake, A.L., et al., *Incident HIV during pregnancy and postpartum and risk of mother-to-child HIV transmission: a systematic review and meta-analysis*. PLoS Med, 2014. 11(2): p. e1001608.
  28. Mugo, N.R., et al., *Increased risk of HIV-1 transmission in pregnancy: a prospective study among African HIV-1-serodiscordant couples*. Aids, 2011. 25(15): p. 1887-95.
  29. Kutteh, W.H. and R.D. Franklin, *Quantification of immunoglobulins and cytokines in human cervical mucus during each trimester of pregnancy*. Am J Obstet Gynecol, 2001. 184(5): p. 865-72; discussion 872-4.
  30. Donders, G.G., et al., *Vaginal cytokines in normal pregnancy*. Am J Obstet Gynecol, 2003. 189(5): p. 1433-8.
  31. Polis, C.B., et al., *Hormonal contraceptive methods and risk of HIV acquisition in women: a systematic review of epidemiological evidence*. Contraception, 2014. 90(4): p. 360-90.
  32. Polis, C.B. and K.M. Curtis, *Use of hormonal contraceptives and HIV acquisition in women: a systematic review of the epidemiological evidence*. Lancet Infect Dis, 2013. 13(9): p. 797-808.
  33. Michel, K.G., et al., *Effect of hormonal contraception on the function of plasmacytoid dendritic cells and distribution of immune cell populations in the female reproductive tract*. J Acquir Immune Defic Syndr, 2015. 68(5): p. 511-8.
  34. Huijbregts, R.P., et al., *Hormonal contraception and HIV-1 infection: medroxyprogesterone acetate suppresses innate and adaptive immune mechanisms*. Endocrinology, 2013. 154(3): p. 1282-95.
  35. Chandra, N., et al., *Depot medroxyprogesterone acetate increases immune cell numbers and activation markers in human vaginal mucosal tissues*. AIDS Res Hum Retroviruses, 2013. 29(3): p. 592-601.
  36. Mitchell, C.M., et al., *Long-term effect of depot medroxyprogesterone acetate on vaginal microbiota, epithelial thickness and HIV target cells*. J Infect Dis, 2014. 210(4): p. 651-5.

37. Bright, P.L., et al., *Hormonal contraception and area of cervical ectopy: a longitudinal assessment*. *Contraception*, 2011. 84(5): p. 512-9.
38. Gupta, K., et al., *Effects of contraceptive method on the vaginal microbial flora: a prospective evaluation*. *J Infect Dis*, 2000. 181(2): p. 595-601.
39. Dezzutti, C.S., et al., *Is wetter better? An evaluation of over-the-counter personal lubricants for safety and anti-HIV-1 activity*. *PLoS One*, 2012. 7(11): p. e48328.
40. Fuchs, E.J., et al., *Hyperosmolar sexual lubricant causes epithelial damage in the distal colon: potential implication for HIV transmission*. *J Infect Dis*, 2007. 195(5): p. 703-10.
41. Van Damme, L., et al., *Effectiveness of COL-1492, a nonoxynol-9 vaginal gel, on HIV-1 transmission in female sex workers: a randomised controlled trial*. *Lancet*, 2002. 360(9338): p. 971-7.
42. Anderson, B.L. and S. Cu-Uvin, *Clinical parameters essential to methodology and interpretation of mucosal responses*. *Am J Reprod Immunol*, 2011. 65(3): p. 352-60.
43. Rodríguez-García, M., et al., *Estradiol reduces susceptibility of CD4+ T cells and macrophages to HIV-infection*. *PLoS One*, 2013. 8(4): p. e62069.
44. Rahn, D.D., et al., *Vaginal estrogen for genitourinary syndrome of menopause: a systematic review*. *Obstet Gynecol*, 2014. 124(6): p. 1147-56.
45. Fashemi, B., et al., *Effects of feminine hygiene products on the vaginal mucosal biome*. *Microb Ecol Health Dis*, 2013. 24.
46. Harlow, S.D., et al., *Executive summary of the Stages of Reproductive Aging Workshop +10: addressing the unfinished agenda of staging reproductive aging*. *Climacteric*, 2012. 15(2): p. 105-14.
47. Auvert, B., et al., *Effect of male circumcision on the prevalence of high-risk human papillomavirus in young men: results of a randomized controlled trial conducted in Orange Farm, South Africa*. *J Infect Dis*, 2009. 199(1): p. 14-9.
48. Gray, R.H., et al., *Male circumcision for HIV prevention in men in Rakai, Uganda: a randomised trial*. *Lancet*, 2007. 369(9562): p. 657-66.
49. Bailey, R.C., et al., *Male circumcision for HIV prevention in young men in Kisumu, Kenya: a randomised controlled trial*. *Lancet*, 2007. 369(9562): p. 643-56.
50. Tobian, A.A., et al., *Male circumcision for the prevention of HSV-2 and HPV infections and syphilis*. *N Engl J Med*, 2009. 360(13): p. 1298-309.

- 
51. Sobngwi-Tambekou, J., et al., *Male circumcision and Neisseria gonorrhoeae, Chlamydia trachomatis and Trichomonas vaginalis: observations after a randomised controlled trial for HIV prevention*. Sex Transm Infect, 2009. 85(2): p. 116-20.
  52. Price, L.B., et al., *The effects of circumcision on the penis microbiome*. PLoS One, 2010. 5(1): p. e8422.
  53. Fowler, J.E., Jr. and M. Mariano, *Immunoglobulin in seminal fluid of fertile, infertile, vasectomy and vasectomy reversal patients*. J Urol, 1983. 129(4): p. 869-72.
  54. Krieger, J.N., et al., *Vasectomy and human immunodeficiency virus type 1 in semen*. J Urol, 1998. 159(3): p. 820-5; discussion 825-6.
  55. Rumke, P., *The origin of immunoglobulins in semen*. Clin Exp Immunol, 1974. 17(2): p. 287-97.
  56. Pudney, J. and D.J. Anderson, *Immunobiology of the human penile urethra*. Am J Pathol, 1995. 147(1): p. 155-65.
  57. Coombs, R.W., et al., *Lower genitourinary tract sources of seminal HIV*. J Acquir Immune Defic Syndr, 2006. 41(4): p. 430-8.
  58. Chung, E. and G. Brock, *Sexual rehabilitation and cancer survivorship: a state of art review of current literature and management strategies in male sexual dysfunction among prostate cancer survivors*. J Sex Med, 2013. 10 Suppl 1: p. 102-11.
  59. Friebe, R.W., et al., *The impact of minimally invasive surgeries for the treatment of symptomatic benign prostatic hyperplasia on male sexual function: a systematic review*. Asian J Androl, 2010. 12(4): p. 500-8.
  60. Mlisana, K., et al., *Symptomatic vaginal discharge is a poor predictor of sexually transmitted infections and genital tract inflammation in high-risk women in South Africa*. J Infect Dis, 2012. 206(1): p. 6-14.
  61. Masson, L., et al., *Defining genital tract cytokine signatures of sexually transmitted infections and bacterial vaginosis in women at high risk of HIV infection: a cross-sectional study*. Sex Transm Infect, 2014. 90(8): p. 580-7.
  62. Rebe, K., et al., *A Cross Sectional Analysis of Gonococcal and Chlamydial Infections among Men-Who-Have-Sex-with-Men in Cape Town, South Africa*. PLoS One, 2015. 10(9): p. e0138315.
  63. Shannon, B., et al., *Impact of asymptomatic herpes simplex virus type 2 infection on mucosal homing and immune cell subsets in the blood and female genital tract*. J Immunol, 2014. 192(11): p. 5074-82.

64. Silverberg, M.J., et al., *Risk of anal cancer in HIV-infected and HIV-uninfected individuals in North America*. Clin Infect Dis, 2012. 54(7): p. 1026-34.
65. Einstein, M.H., et al., *Clinician's guide to human papillomavirus immunology: knowns and unknowns*. Lancet Infect Dis, 2009. 9(6): p. 347-56.
66. Scott, M., D.P. Stites, and A.B. Moscicki, *Tb1 cytokine patterns in cervical human papillomavirus infection*. Clin Diagn Lab Immunol, 1999. 6(5): p. 751-5.
67. Palefsky, J.M. and M. Rubin, *The epidemiology of anal human papillomavirus and related neoplasia*. Obstet Gynecol Clin North Am, 2009. 36(1): p. 187-200.
68. Kojic, E.M., et al., *Human papillomavirus infection and cytologic abnormalities of the anus and cervix among HIV-infected women in the study to understand the natural history of HIV/AIDS in the era of effective therapy (the SUN study)*. Sex Transm Dis, 2011. 38(4): p. 253-9.
69. Stier, E.A., et al., *Prevalence of anal human papillomavirus infection and anal HPV-related disorders in women: a systematic review*. Am J Obstet Gynecol, 2015. 213(3): p. 278-309.
70. Mhatre, M., et al., *Cervical intraepithelial neoplasia is associated with genital tract mucosal inflammation*. Sex Transm Dis, 2012. 39(8): p. 591-7.
71. Godfrey, C.C., et al., *Improving diagnostic capability for HPV disease internationally within the NIH-NIAID Division of AIDS Clinical Trial Networks*. Am J Clin Pathol, 2013. 140(6): p. 881-9.
72. Brenchley, J.M., *Mucosal immunity in human and simian immunodeficiency lentivirus infections*. Mucosal Immunol, 2013. 6(4): p. 657-65.
73. Nazli, A., et al., *Exposure to HIV-1 directly impairs mucosal epithelial barrier integrity allowing microbial translocation*. PLoS Pathog, 2010. 6(4): p. e1000852.
74. Shacklett, B.L., *Immune responses to HIV and SIV in mucosal tissues: 'location, location, location'*. Curr Opin HIV AIDS, 2010. 5(2): p. 128-34.
75. Kwara, A., et al., *Antiretroviral drug concentrations and HIV RNA in the genital tract of HIV-infected women receiving long-term highly active antiretroviral therapy*. Clin Infect Dis, 2008. 46(5): p. 719-25.
76. Cu-Uvin, S., et al., *Genital tract HIV-1 RNA shedding among women with below detectable plasma viral load*. Aids, 2010. 24(16): p. 2489-97.

- 
77. Lambert-Niclot, S., et al., *Detection of HIV-1 RNA in seminal plasma samples from treated patients with undetectable HIV-1 RNA in blood plasma on a 2002-2011 survey*. *Aids*, 2012. 26(8): p. 971-5.
78. Sheth, P.M., et al., *Mucosal correlates of isolated HIV semen shedding during effective antiretroviral therapy*. *Mucosal Immunol*, 2012. 5(3): p. 248-57.
79. Politch, J.A., et al., *Highly active antiretroviral therapy does not completely suppress HIV in semen of sexually active HIV-infected men who have sex with men*. *Aids*, 2012. 26(12): p. 1535-43.
80. Cu-Uvin, S. and A.M. Caliendo, *Genital tract HIV-1 RNA shedding among women with below detectable plasma viral load*. *Aids*, 2011. 25(6): p. 880-1.
81. Konieczna, P., et al., *Portrait of an immunoregulatory Bifidobacterium*. *Gut Microbes*, 2012. 3(3): p. 261-6.
82. Gatch, M.B., et al., *The HIV antiretroviral drug efavirenz has LSD-like properties*. *Neuropsychopharmacology*, 2013. 38(12): p. 2373-84.
83. de Bruyn, G., *Cofactors that may influence vaccine responses*. *Curr Opin HIV AIDS*, 2010. 5(5): p. 404-8.
84. Montefiori, D.C., et al., *Demographic factors that influence the neutralizing antibody response in recipients of recombinant HIV-1 gp120 vaccines*. *J Infect Dis*, 2004. 190(11): p. 1962-9.
85. Prendergast, A.J., *Malnutrition and vaccination in developing countries*. *Philos Trans R Soc Lond B Biol Sci*, 2015. 370(1671).
86. Jin, X., et al., *Multiple factors affect immunogenicity of DNA plasmid HIV vaccines in human clinical trials*. *Vaccine*, 2015. 33(20): p. 2347-53.
87. Kaminski, D.A. and T.D. Randall, *Adaptive immunity and adipose tissue biology*. *Trends Immunol*, 2010. 31(10): p. 384-90.
88. Schaffler, A. and J. Scholmerich, *Innate immunity and adipose tissue biology*. *Trends Immunol*, 2010. 31(6): p. 228-35.
89. Scheiermann, C., Y. Kunisaki, and P.S. Frenette, *Circadian control of the immune system*. *Nat Rev Immunol*, 2013. 13(3): p. 190-8.
90. Simpson, R.J., et al., *Exercise and the Regulation of Immune Functions*. *Prog Mol Biol Transl Sci*, 2015. 135: p. 355-80.
91. West, N.P., et al., *The effect of exercise on innate mucosal immunity*. *Br J Sports Med*, 2010. 44(4): p. 227-31.

92. Dhabhar, F.S., et al., *Stress-induced redistribution of immune cells--from barracks to boulevards to battlefields: a tale of three hormones--Curt Richter Award winner*. Psychoneuroendocrinology, 2012. 37(9): p. 1345-68.
93. Hood, M.I. and E.P. Skaar, *Nutritional immunity: transition metals at the pathogen-host interface*. Nat Rev Microbiol, 2012. 10(8): p. 525-37.
94. Kau, A.L., et al., *Human nutrition, the gut microbiome and the immune system*. Nature, 2011. 474(7351): p. 327-36.
95. Hladik, F. and M.J. McElrath, *Setting the stage: host invasion by HIV*. Nat Rev Immunol, 2008. 8(6): p. 447-57.
96. Anton, P. and B.C. Herold, *HIV transmission: time for translational studies to bridge the gap*. Sci Transl Med, 2011. 3(77): p. 77ps11.
97. Sabatte, J., et al., *The role of semen in sexual transmission of HIV: beyond a carrier for virus particles*. Microbes Infect, 2011. 13(12-13): p. 977-82.
98. Doncel, G.F., T. Joseph, and A.R. Thurman, *Role of semen in HIV-1 transmission: inhibitor or facilitator?* Am J Reprod Immunol, 2011. 65(3): p. 292-301.
99. Patel, S., et al., *Seminal plasma reduces the effectiveness of topical polyanionic microbicides*. J Infect Dis, 2007. 196(9): p. 1394-402.
100. Herold, B.C., et al., *Female genital tract secretions and semen impact the development of microbicides for the prevention of HIV and other sexually transmitted infections*. Am J Reprod Immunol, 2011. 65(3): p. 325-33.
101. Sharkey, D.J., et al., *Seminal fluid induces leukocyte recruitment and cytokine and chemokine mRNA expression in the human cervix after coitus*. J Immunol, 2012. 188(5): p. 2445-54.
102. Sharkey, D.J., et al., *Seminal plasma differentially regulates inflammatory cytokine gene expression in human cervical and vaginal epithelial cells*. Mol Hum Reprod, 2007. 13(7): p. 491-501.
103. Low, N., et al., *Intravaginal practices, bacterial vaginosis, and HIV infection in women: individual participant data meta-analysis*. PLoS Med, 2011. 8(2): p. e1000416.
104. McClelland, R.S., et al., *Vaginal washing and increased risk of HIV-1 acquisition among African women: a 10-year prospective study*. Aids, 2006. 20(2): p. 269-73.
105. Clark, R.A., et al., *Frequent douching and clinical outcomes among HIV-infected women*. Sex Transm Dis, 2007. 34(12): p. 985-90.

- 
106. Javanbakht, M., et al., *Prevalence and types of rectal douches used for anal intercourse: results from an international survey*. BMC Infect Dis, 2014. 14: p. 95.
  107. Baggaley, R.F., R.G. White, and M.C. Boily, *HIV transmission risk through anal intercourse: systematic review, meta-analysis and implications for HIV prevention*. Int J Epidemiol, 2010. 39(4): p. 1048-63.
  108. Grulich, A.E. and I. Zablotska, *Commentary: probability of HIV transmission through anal intercourse*. Int J Epidemiol, 2010. 39(4): p. 1064-5.
  109. Ward, H., et al., *The prevalence of lymphogranuloma venereum infection in men who have sex with men: results of a multicentre case finding study*. Sex Transm Infect, 2009. 85(3): p. 173-5.
  110. Atashili, J., et al., *Bacterial vaginosis and HIV acquisition: a meta-analysis of published studies*. Aids, 2008. 22(12): p. 1493-501.
  111. Cohen, C.R., et al., *Bacterial vaginosis associated with increased risk of female-to-male HIV-1 transmission: a prospective cohort analysis among African couples*. PLoS Med, 2012. 9(6): p. e1001251.
  112. Ward, H. and M. Ronn, *Contribution of sexually transmitted infections to the sexual transmission of HIV*. Curr Opin HIV AIDS, 2010. 5(4): p. 305-10.
  113. Golden, M.R., et al., *Importance of sex partner HIV status in HIV risk assessment among men who have sex with men*. J Acquir Immune Defic Syndr, 2004. 36(2): p. 734-42.
  114. Killian, M.S., et al., *Persistent alterations in the T-cell repertoires of HIV-1-infected and at-risk uninfected men*. Aids, 2004. 18(2): p. 161-70.
  115. Buchacz, K., et al., *Amphetamine use is associated with increased HIV incidence among men who have sex with men in San Francisco*. Aids, 2005. 19(13): p. 1423-4.
  116. Binford, M.C., S.Y. Kahana, and F.L. Altice, *A systematic review of antiretroviral adherence interventions for HIV-infected people who use drugs*. Curr HIV/AIDS Rep, 2012. 9(4): p. 287-312.
  117. Maccarrone, M., et al., *Endocannabinoid signaling at the periphery: 50 years after THC*. Trends Pharmacol Sci, 2015. 36(5): p. 277-96.
  118. Shoptaw, S., et al., *Cumulative exposure to stimulants and immune function outcomes among HIV-positive and HIV-negative men in the Multicenter AIDS Cohort Study*. Int J STD AIDS, 2012. 23(8): p. 576-80.

119. Sopori, M., *Effects of cigarette smoke on the immune system. Nat Rev Immunol*, 2002. 2(5): p. 372-7.
120. Kikuchi, H., J. Itoh, and S. Fukuda, *Chronic nicotine stimulation modulates the immune response of mucosal T cells to Th1-dominant pattern via nAChR by upregulation of Th1-specific transcriptional factor. Neurosci Lett*, 2008. 432(3): p. 217-21.
121. Feldman, C. and R. Anderson, *Cigarette smoking and mechanisms of susceptibility to infections of the respiratory tract and other organ systems. J Infect*, 2013. 67(3): p. 169-84.
122. Nouri-Shirazi, M. and E. Guinet, *Exposure to nicotine adversely affects the dendritic cell system and compromises host response to vaccination. J Immunol*, 2012. 188(5): p. 2359-70.
123. Yanagita, M., et al., *Nicotine modulates the immunological function of dendritic cells through peroxisome proliferator-activated receptor-gamma upregulation. Cell Immunol*, 2012. 274(1-2): p. 26-33.
124. Silverstein, P., *Smoking and wound healing. Am J Med*, 1992. 93(1a): p. 22s-24s.
125. Sorensen, L.T., *Wound healing and infection in surgery. The clinical impact of smoking and smoking cessation: a systematic review and meta-analysis. Arch Surg*, 2012. 147(4): p. 373-83.
126. Sood, A.K., *Cigarette smoking and cervical cancer: meta-analysis and critical review of recent studies. Am J Prev Med*, 1991. 7(4): p. 208-13.
127. Botteri, E., et al., *Smoking and colorectal cancer: a meta-analysis. Jama*, 2008. 300(23): p. 2765-78.
128. Storholm, E.D., et al., *Cigarette smoking as part of a syndemic among young men who have sex with men ages 13-29 in New York City. J Urban Health*, 2011. 88(4): p. 663-76.
129. Hershberger, S.L., et al., *Nicotine dependence and HIV risk behaviors among illicit drug users. Addict Behav*, 2004. 29(3): p. 623-5.
130. Cavazos-Rehg, P.A., et al., *Brief report: Pregnant by age 15 years and substance use initiation among US adolescent girls. J Adolesc*, 2012. 35(5): p. 1393-7.
131. Afshar, M., et al., *Acute immunomodulatory effects of binge alcohol ingestion. Alcohol*, 2015. 49(1): p. 57-64.

- 
132. Molina, P.E., et al., Focus on: *Alcohol and the immune system*. Alcohol Res Health, 2010. 33(1-2): p. 97-108.
133. Friedman, H., S. Pross, and T.W. Klein, *Addictive drugs and their relationship with infectious diseases*. FEMS Immunol Med Microbiol, 2006. 47(3): p. 330-42.
134. Messaoudi, I., et al., *Moderate alcohol consumption enhances vaccine-induced responses in rhesus macaques*. Vaccine, 2013. 32(1): p. 54-61.
135. Daar, E.S., et al., *Diagnosis of primary HIV-1 infection. Los Angeles County Primary HIV Infection Recruitment Network*. Ann Intern Med, 2001. 134(1): p. 25-9.
136. Nugent, R.P., M.A. Krohn, and S.L. Hillier, *Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation*. J Clin Microbiol, 1991. 29(2): p. 297-301.
137. Kent, C.K., et al., *Prevalence of rectal, urethral, and pharyngeal chlamydia and gonorrhea detected in 2 clinical settings among men who have sex with men: San Francisco, California*, 2003. Clin Infect Dis, 2005. 41(1): p. 67-74.
138. Danby, C.S., et al., *Patterns of Extragenital Chlamydia and Gonorrhea in Women and Men Who Have Sex With Men Reporting a History of Receptive Anal Intercourse*. Sex Transm Dis, 2016. 43(2): p. 105-9.

## Index of Cited Case Report Forms

The following are examples of existing CRFs generously provided by organizations and networks conducting HIV clinical trials, including ACTG, HVTN, IAVI, IMPAACT, and MTN. The HVTN and MTN forms were provided by the protocol operation managers from the Statistical Center for HIV/AIDS Research & Prevention (SCHARP). Portions of these materials are referred to throughout the Guide as illustration of how information is being collected from volunteers via questionnaires during the conduct of a study. These sample forms are provided as examples and may not necessarily reflect the current documents.

### AIDS Clinical Trials Group

[ACTG GYNECOLOGIC STATUS GYN0004- REVISED](#)

[ACTG STD TESTS – III DGW0048](#)

Relates to: Reproductive History, Symptoms

### HIV Vaccine Trials Network

[Behavioral Risk Assessment HVTN](#)

Relates to: Medical History, Risk Behaviors, Sexual History

### International AIDS Vaccine Initiative

[IAVI Protocol-C DEM-1](#)

Relates to: Demographics

### International Maternal Pediatric Adolescent AIDS Clinical Trials Group

[IMPAACT GYN EXAM 5850](#)

Relates to: Reproductive History

### Microbicide Trials Network

[MTN m017 Key CRF Male](#)

Relates to: Demographics, Medical History, Symptoms

[MTN m020 Key CRF](#)

Relates to: Demographics, Reproductive History, Medical History, Sexual History, Risk Behavior, Symptoms

---

## Glossary

ACTG: AIDS Clinical Trial Group

AIDS: Acquired Immunodeficiency Syndrome

BV: Bacterial Vaginosis

cART: Combination Antiretroviral Therapy

CDC: Centers for Disease Control

CRF: Case Report Form

EBV: Epstein-Barr Virus

HIV: Human Immunodeficiency Virus

HPTN: HIV Prevention Trials Network

HPV: Human Papilloma Virus

HSV: Herpes Simplex Virus

HVTN: HIV Vaccine Trials Network

IAVI: International AIDS Vaccine Initiative

IUD: Intra-Uterine Device

IMPAACT: International Maternal Pediatric Adolescent AIDS Clinical Trials Group

MSM: Men who have sex with men

MTN: Microbicide Trials Network

PrEP: Pre-exposure prophylaxis

STI: Sexually Transmitted Infection

RTI: Reproductive Tract Infection

VOICE: Vaginal and Oral Interventions to Control the Epidemic

WHO: World Health Organization

## Appendix (checklist)

This checklist aims to provide a quick-reference summary of participant data to record. Details for each category are found in the corresponding sections of the Guide. As discussed throughout the Guide, collecting some of these participant data may be relevant in some studies and not in others; therefore this checklist is not meant to be used as a template to collect participant data in mucosal studies.

### 1. Demographics

Age (if unknown, record best estimate)

Race/Ethnicity/Tribe

Sex at birth

Self-identified gender

- ✓ If transgender, record stage of transition, gender reassignment surgeries, and any use of exogenous hormones.

Relationship status

- ✓ Length of relationship(s)
- ✓ Cohabitation status
- ✓ Number and gender of sexual partners
- ✓ Types of partners, number of primary and non-primary partners

Education/Employment/Income variables:

- ✓ Highest level of education
- ✓ Current employment status or source of income
- ✓ Travel to the area from home for work purposes
- ✓ Leisure travel
- ✓ Indirect SES indicators

### 2. Reproductive History

Menstrual History

- ✓ Current use of hormonal or non-hormonal contraception
- ✓ Date of first day of last menstrual period
- ✓ Date of first day of menstrual period prior to the last one (to establish cycle length)
- ✓ Duration of last menstrual period, date of last day of last menstrual cycle
- ✓ Average interval between cycles, any recent changes
- ✓ Regularity
- ✓ The presence or absence of inter-menstrual bleeding

- 
- ✓ Intravaginal practices (e.g. douching frequency and tampon use)
  - ✓ Breast feeding
  - ✓ Time since last pregnancy
  - ✓ Time since last childbirth
  - ✓ Time since last vaginal coitus

#### Pregnancy status and history

- ✓ Currently pregnant, including gestational age or at a minimum which trimester
- ✓ Prior pregnancy/abortion/miscarriage
- ✓ Currently breastfeeding
- ✓ Undergoing fertility treatment
- ✓ Undergoing hormone therapy or treatments

#### Contraception

- ✓ Type and usage (brand and name) of any contraceptive (oral, patch, intrauterine device, implant hormonal) or topical agents (rings, condoms, other barrier devices, lubricants)
- ✓ Any method(s) of contraception/family planning the participant reports; practices can vary greatly within countries and communities (the rhythm method, withdrawal, use of local techniques like herbs, fruits, etc.)
- ✓ Frequency of use of oral hormonal contraception (and missed pills)
- ✓ Frequency of use of condoms and frequency of condomless sex (for consistency of condom use)
- ✓ Type of topical contraceptive, including product information and frequency of use, especially with respect to timing relative to collection of mucosal samples and/or product administration because of potential for inflammatory effects on exposed mucosa from spermicide or lubricant components, (administered separately or present in coated condoms) or physical abrasion (e.g., from use of depot contraceptive device such as vaginal ring)
- ✓ Timing of last administration of long-acting injectable contraceptive, implant, or hormone-intrauterine device (IUD)
- ✓ Use of a combination of methods

#### Menopausal status

- ✓ Current (or estimated) menopausal phase
- ✓ Any peri/post-menopausal hormone replacement therapy
- ✓ Use of vaginal douches, moisturizers, vaginal estrogen creams

#### Female Reproductive Tract Procedures/Surgeries

- ✓ Hysterectomy (with or without oophorectomy)
- ✓ Dilation and curettage (D&C)

- ✓ Biopsies of the genital tract (excluding cervix)
- ✓ Genital reassignment surgery for transgender individuals
- ✓ Colposcopy/biopsy of the cervix
- ✓ Cervical excisional treatment
- ✓ Drainage removal of tubal/ovarian abscess
- ✓ Tubal ligation
- ✓ Ectopic pregnancy
- ✓ Hysteroscopy/polypectomy/myomectomy/noninvasive treatment for uterine fibroids
- ✓ Vaginal and rectal prolapse repair surgeries: cystoceles, rectoceles, enteroceles
- ✓ Female genital modifications

#### Male Reproductive Tract Procedures/Surgeries

- ✓ Vasectomy and vasectomy reversal
- ✓ Circumcision
- ✓ Prostatic or testicular surgeries
- ✓ Any recent prostate biopsies that could result in inflammation or abscess
- ✓ Prostate radiation
- ✓ Hormonal therapy or orchiectomy for prostate cancer
- ✓ Genital reassignment surgery and hormone therapy for transgender individuals
- ✓ Rectal prolapse repair surgeries: rectoceles, enteroceles

#### Sexually-transmitted and Reproductive Tract Infections (STIs/RTIs)

- ✓ History of a diagnosis and/or treatment for STIs in participant or sexual partners/contacts
  - Chlamydia, gonorrhea, syphilis, trichomoniasis, HSV
- ✓ Asymptomatic HPV/HSV genital and/or rectal shedding of HSV
- ✓ Genital or rectal sores, ulcers, fistulas, fissures
- ✓ Ulcers or abscesses in the inguinal area
- ✓ Lumps, bumps or warts
- ✓ Genital or rectal discharge
- ✓ Genital or rectal pain
- ✓ Skin rashes in the genital and rectal areas, and in general

#### Abnormal Cervical, Rectal, or Oral Cytology/Dysplasia

- ✓ History of HPV vaccination
- ✓ Whether participant has received genital and oral examinations, cytological tests in past
- ✓ Location (cervix, anus, oropharynx)
- ✓ Presence of high-risk HPV and location
  - Results from genetic HPV screening, if available
- ✓ Degree of abnormality and type of test

- 
- ✓ Anal Pap smear vs. high-resolution anoscopy (HRA) with directed biopsies
  - ✓ Cervical cytology vs. colposcopy with directed biopsies

### 3. Medical History

#### Medical Conditions

- ✓ Chronic conditions
- ✓ GYN health conditions
- ✓ Psychiatric conditions
- ✓ Gastrointestinal diseases
- ✓ For oral sampling: history of oral disease (gingival bleeding, ulcers, periodontitis, thrush, other treatments)
- ✓ For respiratory (BAL) sampling: history of asthma, chronic obstructive pulmonary disease (COPD), inhaled steroids, other inhaled medications, current symptoms
- ✓ For urinary sampling: history of urinary tract infections (UTIs), any history of non-specific urethritis, prostate disease, etc.
- ✓ Malignancies, including past cancer history
- ✓ Systemic or local infections (hepatitis, tuberculosis, parasites, etc.), including past infection history
- ✓ Circumcision status (see Section 2.6)
- ✓ For Nasopharyngeal sampling: nasal abnormalities, epistaxis, nasal surgery, allergic rhinitis
- ✓ Other surgeries

#### HIV Status

- ✓ HIV serostatus: duration of HIV infection, CD4+ nadir and current and dates, plasma HIV levels
- ✓ Antiretroviral medications, including detailed information on current use
- ✓ Mode of acquisition of HIV infection
- ✓ History of opportunistic infections
- ✓ HCV co-infection
- ✓ HIV/STI status of sexual partner(s)

#### Medications

- ✓ Use of health supplements
- ✓ Use of probiotics
- ✓ Vaginal probiotics in topical or suppository/capsule presentations
- ✓ Hormonal treatments, other than contraception
- ✓ Antibiotic treatments
- ✓ Antiretroviral drugs taken either for pre- or post- exposure prophylaxis

- ✓ Recreational drugs
- ✓ Non-study vaginal or rectal products
- ✓ Systemic steroids, or topical with mucosal administration (oral, respiratory, GI, vaginal, or anal)
- ✓ Recent chemotherapy for systemic autoimmune conditions

Allergies

- ✓ Specific product/agent/food/environmental sensitivity

Body Mass Index

## 4. Sexual History & Sexual Risk Behaviors

### Sexual Practices

- ✓ Number of sexual partners in the last day, week, month
- ✓ Gender of partner(s)
- ✓ Number of partners per session
- ✓ Types of sex acts (oral, vaginal, rectal, insertive, and receptive) and potential for tissue trauma (e.g., noted blood)
- ✓ Number of acts of sexual intercourse per session
- ✓ Date of last vaginal sex
- ✓ Date of last anal sex (for both men and women)
- ✓ Frequency of sexual intercourse
- ✓ Frequency and mode (oral, vaginal, rectal, insertive, receptive, combination) of sexual intercourse *without* a condom
- ✓ Duration and mode (oral, vaginal, rectal, insertive, receptive, combination) of average sexual intercourse
- ✓ Duration and mode (oral, vaginal, rectal, insertive, receptive, combination) of last sexual intercourse
- ✓ Exposure to ejaculate (genital, non-genital/oral)
- ✓ Use of foreign objects (e.g., sex toys, or other natural or man-made products inserted into the vagina or rectum)
- ✓ Cleaning habits before and after intercourse

---

### Use of Vaginal or Rectal Products or Devices

- ✓ Products inserted into the vagina during menstruation (tampons, cotton wool, rags, vaginal softcups, etc.)
- ✓ Other products used before/during/after sexual activity
- ✓ Douching /cleaning regimen
- ✓ Type of products or devices

### Sexual Risk Behaviors

- ✓ Types of sexual partners (primary, concurrent, transactional, or multiple)
- ✓ Frequency of condomless sex
- ✓ Frequency of vaginal or anal sex without a condom with partners who injected drugs
- ✓ Frequency of vaginal/anal sex without a condom with partners who used other drugs
- ✓ Sex in exchange for money, drugs, goods, services (with or without a condom)
- ✓ Concurrent sexual behaviors
- ✓ Both insertive and receptive anal sex for men who have sex with men (MSM)
- ✓ Single- vs. dual-compartment sex for women (anal or/and vaginal)
- ✓ Knowledge of partner(s) HIV seropositivity and their viral load
- ✓ Oral sex activity, particularly for studies involving saliva

### Risk Behaviors of Partner

- ✓ If the respondent has asked her/his partner(s) about their sexual behaviors
- ✓ Direct or indirect knowledge of sexual partners having sex with another person(s) in the last six months
- ✓ If sexual partner(s) are not monogamous or exclusive, record additional factors shown in Section 4.4

### HIV and Other STI Status of Partner(s)

- ✓ Number of partners with unknown HIV status
- ✓ Number of partners known to be HIV positive
- ✓ Number of partners known be HIV negative
- ✓ Knowledge of HIV or AIDS status of main sex partner
- ✓ Other sexually transmitted infections in partners

## 5. Other Risk Behaviors

### Drug Use

- ✓ Whether the subject has injected/snorted/inhaled/swallowed drugs
- ✓ Type of drugs used. Make sure to include routes of administration, including any of those listed in Section 5.1
- ✓ Unprotected vaginal or anal sex while using drugs, and type(s) of drugs used with sex
- ✓ Limited memory or uncertainty about having unprotected anal or vaginal sex while using drugs
- ✓ Unprotected sex in exchange for drugs or money
- ✓ Drug use of sexual partners

### Smoking

- ✓ Date when started
- ✓ If quit, duration of smoking period
- ✓ Frequency and volume of smoking (pack per day, week, etc.)
- ✓ Variability of smoking patterns (increases under pressure, social smoker, time of day)
- ✓ Type of cigarettes (filtered, hand-rolled, other)
- ✓ Choice of smoking material (tobacco, chicory, marijuana, other)
- ✓ Other tobacco use (cigars, chewing tobacco, other)
- ✓ Nicotine use (patches, E-cigarettes, gum)

### Alcohol Consumption

- ✓ Frequency and quantity of drinking (number of drinks per day, weekly pattern of drinking)
- ✓ Frequency of six or more drinks containing alcohol on one occasion (i.e. binge drinking)
- ✓ Triggers for periods of excessive drinking
- ✓ Measures of excessive drinking
- ✓ Sex while intoxicated (number of oral, vaginal, and/or anal sex acts and condom use while intoxicated)
- ✓ Limited memory or uncertainty about having had sex while intoxicated

---

## 6. Symptoms

Constitutional Symptoms

Vaginal Discharge: characteristics

Rectal Discharge (Anorectal STI's or non-STI causes)

Pelvic Pain

Oral, Respiratory, and Other GI Symptoms

- ✓ Medical conditions to report found during examination or upon results
- ✓ Oral disease (gingival bleeding, ulcers, or periodontitis, other treatments)
- ✓ STI's such as gonorrhea and chlamydia Trichomoniasis, HSV, syphilis
- ✓ Urinary tract infections (UTIs), or non-specific urethritis, prostate disease, etc.
- ✓ Other systemic or local infections (hepatitis, tuberculosis, parasites, etc.)
- ✓ Any surgeries or medical emergency procedure

---

## About the Global HIV Vaccine Enterprise

The Global HIV Vaccine Enterprise (the Enterprise) is a unique collaboration of the world's leading HIV vaccine research funding, policymaking, advocacy and stakeholder organizations dedicated to working together to advance HIV vaccine research and development.

Recognizing that no single institution, country or individual can develop an HIV vaccine in isolation, the Enterprise promotes and facilitates coordination, collaboration, knowledge sharing and resource optimization.

A small Secretariat supports the Enterprise, helping to catalyze the activities of this collaboration and implement programming to move its mission forward. For more information on the Global HIV Vaccine Enterprise, please visit:

[www.vaccineenterprise.org](http://www.vaccineenterprise.org)

## About Timely Topics in HIV Vaccines

The first edition of this Guide was developed as part of the Timely Topics in HIV Vaccines a strategy series launched in 2012, convening experts as rapidly as possible to analyze, address, and respond to unresolved and emerging priority issues in the field to help accelerate HIV vaccine research and development. Through an open call for proposals, the Enterprise is working to identify the most important strategic needs of the field and sponsoring think tanks, meetings, forums and other events to tackle these issues.

For more on Timely Topics, visit:

[www.vaccineenterprise.org/content/timely-topics-hiv-vaccines](http://www.vaccineenterprise.org/content/timely-topics-hiv-vaccines)





Global HIV Vaccine  
**Enterprise**

64 BEAVER STREET, # 352

NEW YORK, NY 10004

+1 212-461-3692 OR TOLL FREE AT

+1-866-966-4483

[WWW.VACCINEENTERPRISE.ORG](http://WWW.VACCINEENTERPRISE.ORG)

