Capturing Participant Information for Mucosal Sampling

AN INVESTIGATOR'S GUIDE
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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 HIV vaccine, as well as other HIV prevention and treatment strategies, research and development. 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 Information for Mucosal Sampling” (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 is now co-funded through the Bill & Melinda Gates...
Foundation’s Collaboration for AIDS Vaccine Discovery (CAVD). 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), and Amapola Manrique 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). The Guide will be reviewed and revised on an as-needed basis and at least annually.

**HOW SHOULD THIS GUIDE BE USED?**

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; 5) 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)
- Selected references (relevant reviews, as well as specific literature cited in the above sections) are listed in the Endnote section
- Clinical Report Forms (CRFs) examples (specific examples of questions in this category from trial Network CRFs)

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

This document should be used in conjunction with Network procedures and policies, protocols, and 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.

**ARE THERE ANY GENERAL CONSIDERATIONS OR CAVEATS TO REMEMBER WHEN USING THIS GUIDE?**

The Guide was purposely written to be fairly 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 or not.

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.

**WHERE CAN I GET MORE INFORMATION OR HOW CAN I PROVIDE FEEDBACK?**

If you have any questions or suggestions, please email:

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. Menopausal Status
   2.2. Menstrual History
   2.3. Pregnancy Status and History
   2.4. Contraception
   2.5. Use of Vaginal and Rectal Products or Devices
   2.6. Female Reproductive Tract Surgeries
   2.7. Male Reproductive Tract Surgeries
   2.8. Sexually-transmitted and Reproductive Tract Infections (STIs/RTIs)
   2.9. Abnormal Cervical or Rectal 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

4. Sexual History
   4.1. Sexual Practices
   4.2. HIV Status of Partner(s)

5. Risk Behaviors
   5.1. Drug Use
   5.2. Smoking
   5.3. Alcohol Consumption
   5.4. Sexual Risk Behaviors
   5.5. Risk Behaviors of Partner

6. Symptoms
   6.1. Constitutional Symptoms
   6.2. Vaginal Discharge
   6.3. Rectal Discharge
   6.4. Pelvic Pain
1. Demographics

1.1. Age

RATIONALE

Age, among other factors\(^1,2\) is associated with degree of ectopy displayed by the cervical epithelium, which affects baseline levels of inflammatory and regulatory cytokines/chemokines present in cervico-vaginal secretions\(^3\) and may be a risk factor for heterosexual transmission of HIV\(^4\). A recent study reported substantive differences in several biomarkers of mucosal innate immunity measured in cervico-vaginal lavage samples from sexually active adolescents compared to adult females\(^5\). There are also marked differences in vaginal flora in girls compared to older women as estrogens promote the growth of lactobacilli in the genital tract. These are generally not present in prepubescent girls\(^6\). Changes in the reproductive tract
brought about by menopause and peri-menopause are also important considerations for studies of mucosal immunity (see section 2.1). Equivalent studies in the GI tract are underway, but have not yet been reported.

**CONSIDERATIONS**

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

**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.

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 Rwanda, ethnic distinctions are outlawed so any questions about ethnicity/tribe are omitted.

### 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. 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. In addition, there is evidence that gender bias may be affected and/or reinforced by the host’s microbiome.

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 hormone
    - Use of high doses oral contraception

**I.4. Relationship Status**

**RATIONALE**

Characterization of a 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. 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 ethnicity. 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)

### 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 central Africa; miners in South Africa) because migration can play a large role in HIV risk. Terms reflecting level of education may require adjustment for the educational system in the study locale.

**CONSIDERATIONS**

Important Education/Employment/Income variables to consider may include:
- Highest level of education:
  - Some high school or less
- 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 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 of travel
  - Length of time in current area
2. Reproductive History

2.1. 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\(^ {18}\). A recent report that estradiol directly reduces the susceptibility of target CD4+ T cells and macrophages to HIV infection in an \textit{in vitro/ex vivo} viral challenge model using cervical or cervico-vaginal biopsies raises further questions regarding the relative risk of HIV acquisition in menopausal women\(^ {18,19}\).
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 menopausal phase:
  - Pre-menopausal
  - Peri-menopausal
  - Post-menopausal

- Any peri/post-menopausal hormone replacement therapy

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.

2.2. 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. 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, 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\textsuperscript{24,25}. There is also growing evidence of the relationship between reproductive hormones and adaptive and innate mucosal immunity\textsuperscript{26,27}.

**CONSIDERATIONS**

By itself, date of last menstrual period 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 contraception (see section 2.4)
- Date of first day of last menstrual period
- 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
- Breast feeding
- Time since last childbirth

2.3. **Pregnancy Status and History**

**RATIONALE**

There is evidence that pregnancy is a high-risk period for HIV transmission, both to women and from HIV-infected women to their male partners\textsuperscript{28}. This could reflect a higher frequency of unprotected sexual activity among women who become pregnant versus those who do not become pregnant. 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\textsuperscript{+} T
lymphocytes in the genital mucosa, suggesting a possible mechanism by which pregnancy may increase HIV levels in female genital secretions and HIV infectivity\textsuperscript{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

### 2.4. Contraception

**RATIONALE**

Biological and epidemiological evidence suggests that hormonal contraceptives, including daily oral pills and long-acting injectables, may influence HIV transmission and acquisition. A recent review\textsuperscript{31} observed that most studies assessing the use of oral contraceptive or injectable norethisterone enanthate (NET-EN) showed no association with HIV acquisition, whereas contradictory results were found in studies assessing injectable depot medroxyprogesterone acetate (DMPA), suggesting differential impacts of these hormones. Thus, documenting the use of oral, depot, or injectable hormonal contraceptive methods is important.

Progesterone induces changes in cervical mucus and uterine epithelium that likely affect susceptibility to HIV infection. DMPA has been shown to inhibit cytokine production by peripheral blood mononuclear cells (PBMCs), activated T cells, and plasmacytoid dendritic cells, and women on DMPA have lower interferon-\textalpha levels in genital secretions and plasma. In contrast to \textit{in vitro} studies that suggest DMPA
also prevents CXCR4 and CCR5 down-regulation on the surfaces of T cells after activation and increases HIV replication in PBMC cultures\textsuperscript{32,33}, no vaginal changes in HIV target cell densities or CCR5 expression were observed in a recent study of women on DMPA for one year\textsuperscript{34}. 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\textsuperscript{35}.

The serum concentration of 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 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\textsuperscript{36–38}. Furthermore, when tested as a potential microbicide to prevent HIV acquisition, spermicide nonoxynol-9 caused mucosal inflammation that increased the incidence of HIV. Intra-Uterine Devices (IUDs) and implants, some of which are hormonally impregnated, are an attractive method of contraception, but their effect on the local mucosal milieu and HIV risk women is largely unknown.

**CONSIDERATIONS**

Important contraception practice information to consider may include:

- Type and usage (brand and name) of any contraceptive (oral, device, hormonal) or topical agents (rings, condoms, lubricants)
- Any method(s) of contraception/family planning the participant reports; practices can vary greatly within countries and communities
- Frequency of use of oral hormonal contraception and/or condoms
- 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., depot contraceptive device such as vaginal ring)

- Timing of last administration of long-acting injectable contraceptive or hormone-impregnated device
  - Distinction between IUDs that do or do not contain hormones
  - Name of IUDs and devices to be able to check the nature and concentration of hormones delivered
  - Time since placement of device

- Use of a combination of methods

2.5. 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, powders) placed in the vagina to reduce wetness because of cultural expectations of men in some regions. 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. A meta-analysis of intravaginal washing or drying practices of women from 13 prospective cohort studies showed a correlation between some practices, such as intravaginal cleaning with soap, development of BV, and HIV risk but, a direct causal link could not be established. 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. Finally, rectal insertion of products for sexual or therapeutic purposes may also occur.

CONSIDERATIONS
Important variables to consider in the Use of Vaginal and Rectal Products of Devices may include:

- Used during menstruation
- Used before/during/after sexual activity (see section 4.2)
- Douching /cleaning regimen
- Type of products or devices:
  - Vaginal or rectal lubricant (type and name brand of product)
  - Spermicide
  - Douche (vaginal or rectal)
  - Enema
  - Vaginal yeast medication
  - Other vaginal washing/drying practices:
    - Insertion of cloth, paper, and vegetation such as leaves, etc.
    - Use of products that dry or tighten
    - Use of soap
  - Timing of last use (especially within last 48–72 hours)

2.6. Female Reproductive Tract Procedures/Surgeries

RATIONALE

Gynecological procedures such total hysterectomy 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.
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 including endocervical curettage
- Biopsies
- Genital reassignment surgery for transgender individuals
- Colposcopy/biopsy
- 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: cytoceles, rectoceles, enteroceles
- Female genital modifications

2.7. 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%\(^44-46\). As well, circumcision reduces the risk of Herpes Simplex Virus (HSV)\(^-2\), Human Papilloma Virus (HPV), and genital ulcerative diseases which are also associated with HIV infection risk and/or recruitment of HIV target cells\(^45,47,48\). Lastly, circumcision procedures cause alterations to the penile microbiome, which may affect inflammatory mediators at the foreskin\(^49\).

Vasectomies and vasectomy reversal surgeries seem to alter the antibody composition of the seminal fluid of men who underwent surgery\(^50\). 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. Vasectomy, however, does not seem to affect total HIV measurements in semen.\textsuperscript{51}.

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\textsuperscript{52,53}. Most HIV in semen comes from distal genitourinary sources rather than the prostate\textsuperscript{51,54}, but surgeries and trauma might facilitate communication between genital and systemic sources of HIV. Lastly, procedures for treatment of benign 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\textsuperscript{55,56}.

**CONSIDERATIONS**

Important Male Reproductive Tract Procedures/Surgeries information to record may include:

- Vasectomy and vasectomy reversal
- Circumcision
- Prostatic or testicular surgeries
- Prostate radiation
- Hormonal therapy or orchiectomy for prostate cancer
- Genital reassignment surgery and hormone therapy for transgender individuals
- Rectal prolapse repair surgeries: rectoceles, enteroceles

### 2.8. 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\textsuperscript{57}. 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\textsuperscript{57,58}.

Accordingly, screening for STIs (e.g., gonorrhea, chlamydia, syphilis, trichomonas, lymphogranuloma venereum) 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. 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.

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 (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.

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 treatment for STIs
- Genital or rectal sores, ulcers, fistulas, fissures
- Genital or rectal discharge
- Genital or rectal pain
- Skin rashes in the genital and rectal areas, and in general

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 an important STI (e.g., chlamydia) 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, this factor may substantially increase the study size. 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.

2.9. Abnormal Cervical or Rectal 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 2008. More than 85% of the global burden occurs in developing countries, where it accounts for 13% of all female cancers. 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. Screening with regular gynecologic examinations and cytologic testing (Pap smear) 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 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. The men-who-have-sex-with-men population is particularly at risk for this infection. Finally, HPV can have similar pathogenesis of the oropharynx.

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.

CONSIDERATIONS

Important information to record related to Abnormal Cervical or Rectal Cytology and Dysplasia may include:

- Location (cervix, anus)
- Presence of high-risk HPV
- Degree of abnormality and type of test
- Anal Pap smear vs. high-resolution anoscopy (HRA) with directed biopsies
- 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
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 or a history of partial
Colon resection (left side) or peri-anal fissure repair may help explain baseline anatomic differences observed during the procedure.

**CONSIDERATIONS**

Important information related to Medical Conditions to record may include:

- Chronic conditions (autoimmune diseases, diabetes, common variable immunodeficiency, chronic liver or kidney disease, etc.)
- Gastrointestinal diseases (irritable bowel syndrome, inflammatory bowel diseases, peptic ulcer disease, diverticulosis/diverticulitis, Helicobacter pylori) and their medical and/or surgical treatments
- Allergies (see 3.5)
- Medications (see 3.4)
- Malignancies, including past cancer history
- Vaccinations (see 3.3)
- Systemic or local infections (hepatitis, tuberculosis, parasites, etc.), including past infection history
- Circumcision status (see section 2.7)
- Surgeries of female/male genital tract
- 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+ 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+ 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\textsuperscript{65,66}. 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\textsuperscript{67}. Initiation of cART improves, but does not reverse completely the immunological abnormalities within the GI tract\textsuperscript{67}. ART drug levels may vary significantly by medication and between plasma and different tissue compartments\textsuperscript{68}, 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\textsuperscript{69–72}.

**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 (“viral loads”)
- 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
- Mode of HIV infection
• History of opportunistic infections

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 guidelines from the Centers for Disease Control (CDC).

CONSIDERATIONS
Data regarding vaccinations received while on-study (e.g., influenza vaccination), 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 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.
- Hormonal treatments, other than contraception (see section 2.4)
- 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 prophylactic or 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.
- Recreational drugs
- Non-study vaginal or rectal products (see section 2.5)
- Systemic steroids
- 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 (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 as part of section 2.5. 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 impact on vaccine-elicited immunogenicity.\(^{76,77}\) Obesity, defined as BMI \(\geq 30\ \text{kg/m}^2\), has been associated with impaired adaptive and innate immune system responses to infections or vaccination and increased susceptibility to infectious agents and cancer.

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.\(^{78-80}\) 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.
4. Sexual History

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. Among sexually-acquired HIV infections, semen is the primary transmission vector. 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 virion. 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. Semen has been shown to recruit immune cells such as dendritic cells, macrophages, and memory T cells to the lamina propria soon after coitus (two refs below). 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. For women, it is critical to consider dual compartment sexual exposures (vaginal and rectal) and the order in which these sex acts occur 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, receptive) and potential for tissue trauma (e.g., noted blood)
- Number of acts of sexual intercourse per session:
  - with or without a condom
  - 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 of sexual intercourse without a condom
- Duration of average sexual intercourse
- Duration of last sexual intercourse
- Exposure to ejaculate
- Use of foreign objects (e.g., sex toys, or drying agents such as leaves, which may be used in parts of Africa)
4.2. HIV Status of Partner(s)

RATIONALE

Knowledge of the HIV infection status of recent sexual partners is important to determine. 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.

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. 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). Methamphetamine may also dry the mucosa, 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\textsuperscript{93}. Drug use may also directly affect immune function, although data are conflicting\textsuperscript{94}.

**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
    - Marijuana (see section 5.2)
    - 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
  - 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\(^95\)–\(^99\). 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\(^100\). The understanding of the impact of nicotine on post-operative wound healing has been confirmed more recently in a systematic review and meta-analysis\(^101\). Smoking has also been linked to cervical and anal cancer\(^102,103\). 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 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\(^104\)–\(^106\).

CONSIDERATIONS

Important Smoking information to record may include:

- Date when started
- If have 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. 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 \(^{107,108}\). On the other hand, recent evidence shows that moderate alcohol consumption may enhance vaccine immune reactivity\(^{109}\).

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
• Triggers for periods of excessive drinking
• Measures of excessive drinking (e.g., the CAGE or AUDIT screening questions\(^{110}\))
• Sex while intoxicated (number of oral, vaginal, and/or anal sex acts and condom use while intoxicated)
5.4. 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 20-fold greater per receptive rectal vs. vaginal sex act with an HIV-seropositive individual who is not on cART. Co-infections and multiple exposures per episode only increase these reported HIV-transmission rates.

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
- 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
5.5. 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:

- Knowledge of sexual partners having sex with another person in the last six months
- If sexual partner is not monogamous, record whether any of the following factors apply:
  - Intravenous drug use
  - Alcohol
  - Other drugs
  - Exchange sex
  - Sex with a commercial sex worker
  - Sex with partner who was drunk or high on drugs
  - Sex with a known HIV-infected partner
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. 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 also have similar clinical symptoms (fevers, chills, diffuse lymphadenopathy) than primary HIV infection.

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:
  - Dysuria
  - Urethral discharge
  - Diarrhea with mucus or blood
  - Nausea/vomiting
  - Urticaria
  - Icterus
  - Headaches
  - Fevers/sweats/chills
  - Joint aches/arthralgias
  - Abdominal pain/discomfort
    - Location of discomfort and radiation of pain
- Proctalgia (patient’s report of urgency)
- Urogenital ulcer history (penile, vaginal, perianal)
- Rectal, vaginal, urethral discharge
- Enlarged or painful lymph nodes (notable in a number of genital STIs such as lymphogranuloma venereum, chancroid, syphilis, HSV, etc.)

### 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.

**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\textsuperscript{115,116}. 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 vulvo-vaginal 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, 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.  

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.

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 are asymptomatic (e.g., syphilis), which is why screening is mandatory for studies that involve sampling of the rectal 

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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, 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, 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
Endnote

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Index of Cited Clinical Report Forms

The following are examples of existing CRFs generously provided by organizations and networks conducting HIV clinical trials, including: ACTG, HVTN, IAVI, IMPAACT, 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

Relate 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: Clinical Report Form
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
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

About Timely Topics in HIV Vaccines

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