IDENTIFICATION OF HERPES SIMPLEX VIRUS (HSV) SHEDDING IN THE FEMALE GENITAL TRACT OF PREGNANT AND NONPREGNANT WOMEN BY THE XPERT HSV 1/2 ASSAY, ROUTINE PCR, AND CULTURE

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Draft or Version Number: **4.0**

3 July 2018

STATEMENT OF COMPLIANCE

Each investigator must adhere to the protocol as detailed in this document. Each investigator will be responsible for enrolling only those study participants who have met protocol eligibility criteria. This trial will be conducted in compliance with the protocol, International Conference on Harmonization Good Clinical Practice E6 (ICH-GCP), and the applicable regulatory requirements, including:

- U.S. Code of Federal Regulations applicable to clinical studies (45 CFR 46 and 21 CFR including parts 50 and 56 concerning informed consent and IRB regulations, and part 812 subparts C, G and E).
- Completion of Human Subjects Protection Training. Refer to http://grants.nih.gov/grants/guide/notice-files/NOT-OD-01-061.html; http://cme.cancer.gov/clinicaltrials/learning/humanparticipant-protections.asp

PROTOCOL SIGNATURE PAGE

The signature below constitutes the approval of this protocol "**Identification of Herpes Simplex Virus (HSV) Shedding in the Female Genital Tract of Pregnant and Nonpregnant Women by the Xpert HSV 1/2 Assay, Routine PCR, and Culture**" and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines. It is understood that no deviations from the protocol may be made without permission of the Sponsor.

Site Investigator:

Signed: _____ Date: _____
Name

_____
Title

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
CASG	Collaborative Antiviral Study Group
CFR	Code of Federal Regulations
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
DNA	Deoxyribonucleic Acid
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIV	Human Immunodeficiency Virus
HSV	Herpes Simplex Virus
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IDE	Investigational Device Exemption
IEC	Independent or Institutional Ethics Committee
IND	Investigational New Drug Application
IRB	Institutional Review Board
MOP	Manual of Procedures
N	Number (typically refers to subjects)
N/A	Not Applicable
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
OHSR	Office for Human Subjects Research
PCC	Probe Check Control
PCR	Polymerase Chain Reaction
PI	Principal Investigator
SAC	Sample Adequacy Control
SPC	Sample Processing Control
STI	Sexually Transmitted Infections
UAB	University of Alabama at Birmingham
US	United States

PROTOCOL SUMMARY

Title:	Identification of Herpes Simplex Virus (HSV) Shedding in the Female Genital Tract of Pregnant and Nonpregnant Women by the Xpert HSV 1/2 Assay, Routine PCR, and Culture
Phase:	N/A (Device Trial)
Population:	Group 1: nonpregnant women in Sexually Transmitted Infections (STI) Clinics with clinically-apparent HSV lesions; and Group 2: pregnant women admitted with the intent of delivery with no visible evidence of HSV infection
Number of Sites:	15 (listed in Section 1)
Study Duration:	5 years
Subject Participation Duration:	1 day for Group 1 (nonpregnant women); 30-90 days for Group 2 (asymptomatic pregnant women)
Objectives:	Primary: • To estimate the sensitivity of the Xpert HSV 1/2 Assay relative to culture for detecting herpes simplex virus (HSV) DNA in the genital tract of nonpregnant women in sexually transmitted infections (STI) clinics • To estimate the positive percent agreement and negative percent agreement of the Xpert HSV 1/2 Assay relative to routine PCR for detecting HSV DNA in the genital tract of pregnant women admitted with the intent of delivery Secondary: • To estimate the positive predictive value of the Xpert HSV 1/2 Assay relative to culture for detecting HSV DNA in genital tract of nonpregnant women in STI clinics • To estimate the positive percent agreement of the Xpert HSV 1/2 Assay relative to routine PCR for detecting HSV DNA in genital tract of nonpregnant women in STI clinics • To compare the performance of Xpert with routine PCR in the combined group (pregnant and non-pregnant women) in detecting HSV-1 and HSV-2 virus, separately • To quantify HSV viral load, as determined by routine PCR, in the genital tract of women shedding the virus • To assess the type of infection (first-episode primary, first-episode non-primary, recurrent) among women shedding

HSV

Exploratory:

- To determine rates of neonatal HSV disease among neonates delivered to pregnant women without active genital HSV lesions
- To assess likelihood of viral transmission to neonates exposed to HSV during delivery as a manifestation of mode of delivery

Description of Study Design:

The natural history of genital HSV shedding from the genital tract of pregnant and non-pregnant women will be evaluated, and the utility of a new cartridge-based PCR platform (the GeneXpert system with the Xpert HSV 1/2 Assay cartridge) for detection of HSV-1 or HSV-2 will be assessed. Two groups of women will be studied: 1) Group 1 consists of non-pregnant women who present at an STI clinic (likely at one or two study centers) with clinically evident HSV lesions; and 2) Group 2 consists of pregnant women presenting with the intent to deliver with no evidence of HSV lesions (at all study centers).

For Group 1 (n=300), viral shedding will be assessed by viral culture in real time, followed by detection of HSV-1 and HSV-2 DNA by two PCR methods (routine PCR and the Xpert HSV 1/2 PCR using the GeneXpert platform). PCR detection will be completed in batches and not in real time. Results from the GeneXpert system (Xpert HSV 1/2 PCR) will be compared against results from HSV viral cultures and routine PCR.

For Group 2 (n=12,500), viral shedding will be assessed by PCR methodology only (routine PCR and Xpert HSV 1/2 PCR). Approximately half of the women in Group 2 will be tested by routine HSV PCR and Xpert HSV 1/2 PCR; specimens from the rest of the women in Group 2 will be stored for possible testing in the future by routine HSV PCR and Xpert HSV 1/2 PCR. In this manner, we will maximize the data from which to compare Xpert HSV 1/2 PCR results with routine PCR, while maintaining flexibility to ensure an adequate number of specimens are positive for HSV DNA by routine PCR.

All Xpert and routine PCR testing will be performed at the UAB Central Laboratory.

A blood specimen for determination of HSV type-specific

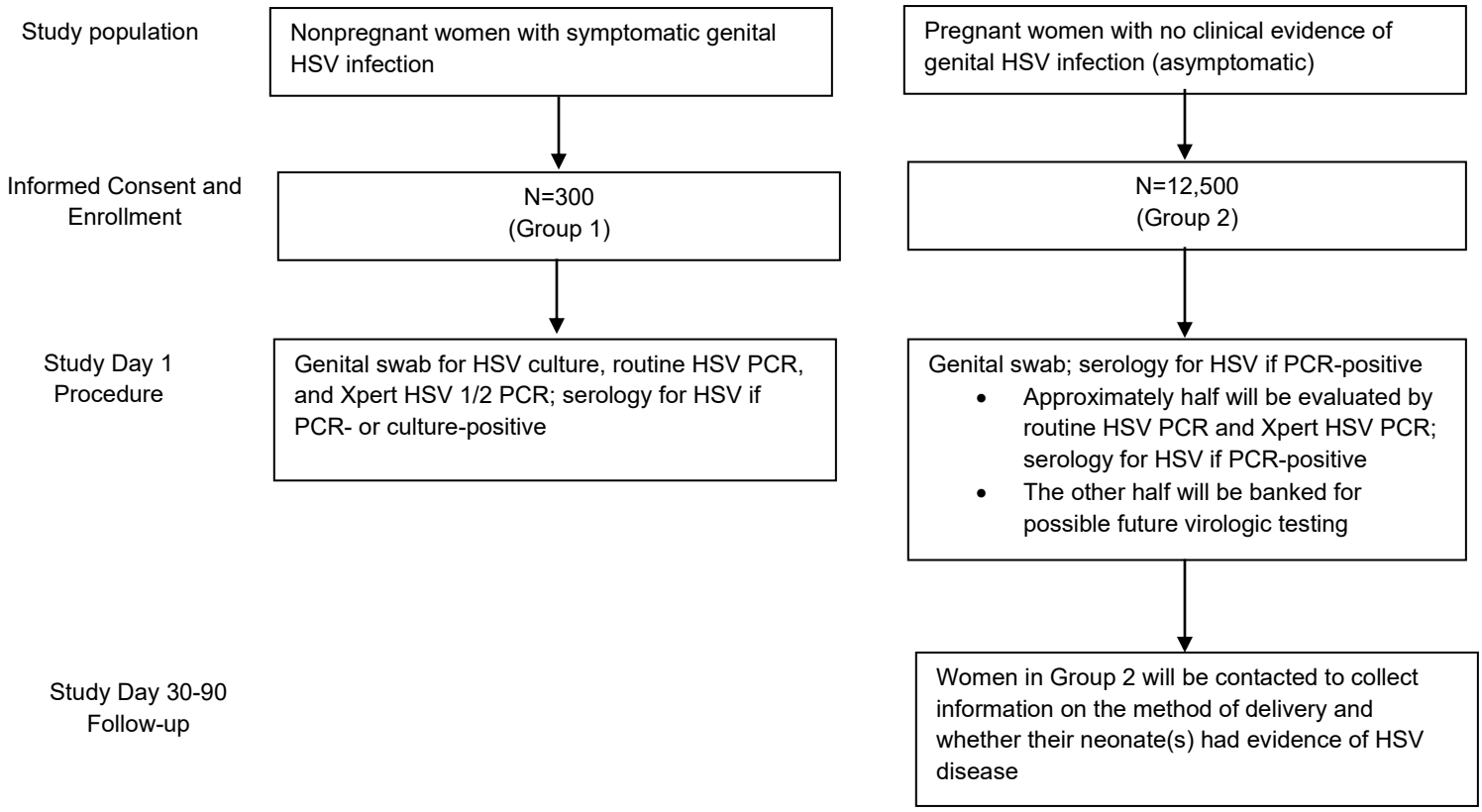
antibodies will be obtained on Study Day 1 from all women in both groups (Group 1 and Group 2). If she is determined to be shedding HSV in the genital tract (using any of the three viral methods), then type-specific serologic testing will be performed. Correlation of viral typing from the virologic sampling with HSV-1 and HSV-2 serostatus will allow for categorization of infection (first-episode primary, first-episode nonprimary, or recurrent infection).

HSV culture results from women in Group 1 will be reported to their treating physician. Virologic results from women in Group 2 will not be reported back to the subject or her physician. The reasons for not reporting results to the Group 2 subject or her physician are two-fold: 1) the PCR analyses are performed in batches, so no results will be available within a timeframe in which clinical decisions on the woman's care could be made; and 2) routine HSV PCR and Xpert HSV 1/2 Assay are not FDA-cleared tests, so at this point the reliability of a positive or a negative test result is not known. Even if PCR results (routine HSV PCR or Xpert HSV 1/2 Assay) could be obtained in a real-time (non-batched) fashion, it would be irresponsible to report results from a non-validated test to the subject and her health care provider, as they may be influenced to make clinical decisions based on this unvalidated information. Furthermore, using the results of this research test for clinical decision making is not the intent of this study protocol. All pregnant women in Group 2 will receive written materials on Study Day 1 educating them on signs and symptoms of neonatal HSV disease. All postpartum women or designated representative will be contacted 30-90 days post-delivery and an inquiry will be made to determine mode of delivery and whether their babies developed neonatal HSV disease.

Data on the incidence of neonatal HSV disease among babies delivered to women in Group 2 will be compared with the incidence data from Brown et al.[1] In their study of almost 60,000 women conducted over a 20 year period, this group of researchers has reported an incidence rate for neonatal HSV disease of 1 in 3,200 live births.

Estimated Time to Complete Enrollment: 4 years from enrollment of the first study subject

***Schematic of Study Design**:



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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Despite important advances over the past thirty years in the treatment of neonatal HSV disease, significant numbers of babies die or are left with lifelong neurologic sequelae. Since the 1970s, the National Institute of Allergy and Infectious Diseases (NIAID) Collaborative Antiviral Study Group (CASG) has completed a series of antiviral treatment studies in neonates with HSV infection. The first two of these trials involved vidarabine.[2,3] These were followed by a comparative study between vidarabine and acyclovir in the 1980s,[4] and then a trial of high dose acyclovir in the 1990s.[5] Each of these investigations defined the standard of care for the management of babies with neonatal herpes, and decreased mortality from disseminated neonatal herpes from over 80% in the pre-antiviral era to 30% with high dose acyclovir therapy.[2,5,6] Throughout the 2000s, the NIAID CASG conducted two placebo-controlled studies of oral acyclovir suppressive therapy following treatment of acute neonatal disease, which again redefined standard of care and simultaneously provided paradigm-shifting insights into the natural history of HSV infections of the central nervous system (CNS).[7] In these suppression studies, babies with neonatal HSV CNS disease had significantly improved neurodevelopmental outcomes when immediately started on active antiviral suppression following acute disease compared with babies in whom oral antiviral suppression was deferred until after skin recurrences occurred or who only received placebo suppressive therapy. These data indicate that subclinical viral replication with concomitant ongoing neurologic damage occurs during and following neonatal HSV CNS disease. Even with immediate antiviral suppression, though, approximately three in 10 survivors of neonatal HSV CNS disease develop significant neurologic sequelae.[7] Each year in the United States, over 100 babies die from neonatal herpes, and 250 survivors are left with significant neurologic damage.[1,5,7-9] Approximately 1,000 more survive without neurologic impairment, yet still require two to three weeks of hospitalization for intravenous therapy for acute disease, which puts significant emotional stress on their families – especially given the fact that this involves a sexually transmitted infection (STI) which the mother passed to her newborn.[1,5,7] While the improvements in outcomes from the antiviral interventions defined by the CASG have been significant, the best mechanism of averting death, neurologic damage, and emotional distress from neonatal HSV is to prevent the disease from occurring in the first place.

Clearly an ideal means of preventing neonatal HSV infection would be to prevent maternal genital infection with an effective vaccine.[10] Since at least 20%, and more recently closer to 50%, of genital herpes is caused by HSV-1,[11] a successful vaccine would need to prevent both types of HSV. No HSV-1 vaccine is in development, and the best HSV-2 vaccine candidate[12] recently failed to provide benefit in a large Phase III study.[13] This trial, which represented a joint

effort between NIAID and GlaxoSmithKline (GSK), enrolled 8,323 women age 18-30 years at 50 study sites in the United States and Canada and was conducted from 2002 to 2010. Overall, the vaccine was not efficacious; vaccine efficacy was 20% (95% confidence interval [CI], −29 to 50) against genital herpes disease.[13] There are no other HSV-2 vaccine candidates that are in Phase II or III development, so vaccine prevention of maternal infection, and subsequent neonatal infection, is not likely for at least 15 years or more, if at all. At a time when approximately 20% of all sexually active women in the United States have HSV-2 genital infection,[14] and an additional 20%-50% of all genital herpes is caused by HSV-1,[11] other means of preventing perinatal HSV transmission are urgently needed.

Most babies developing neonatal herpes are delivered to women who are asymptomatically shedding HSV in their genital tract, have no identifiable signs or symptoms of active infection, and have no history of genital herpes.[6,9,15] While maternal antiviral suppression is routinely provided near the end of pregnancy to women with a history of genital herpes,[16-19] it is ineffective in completely suppressing viral shedding at delivery.[18-21] Importantly, the CASG recently has reported cases of infants who developed neonatal herpes despite compliant maternal antiviral suppression.[22,23] Furthermore, the approach of providing antiviral suppression to women with a history of genital herpes (classified as having “recurrent” infection) is intrinsically flawed, given that 60-80% of women delivering babies that develop neonatal HSV disease have no known history of genital herpes themselves. Further, between 25% and 57% of women acquiring genital herpes during pregnancy (classified as having “first-episode primary” or “first-episode non-primary” infection) who are asymptomatically shedding at delivery will transmit the virus to their babies, compared with only 2% of women with recurrent genital herpes who are asymptomatically shedding at delivery.[1,24-26] Thus, focusing on women with a history of genital herpes not only is ineffective, but also is illogical because these are not the women at highest risk of transmission of virus to their neonates.

As noted above, since 85% of babies developing neonatal HSV disease acquire the virus from their mothers during the birth process,[8] detecting which women are shedding HSV would allow a rational, targeted approach focusing on those neonates at risk. Equally important, knowing that a woman with a history of genital herpes does *not* have HSV present in the genital tract at the time of delivery could have a positive impact on her obstetrical care by preventing unnecessary cesarean deliveries. To accomplish this, however, information on the natural history of asymptomatic HSV shedding in the genital tract of pregnant women at the time of delivery is needed. A simple, standardized, rapid test for detecting HSV in the maternal genital tract also is required. PCR technologies have advanced to the point where this can now be considered. A recent study demonstrated that real-time PCR detection of HSV when women are in labor is feasible.[27] However, the assay used in that study required significant technical preparation of reagents and utilized multiple aliquoting steps on different machines to perform it correctly. Thus, this report served as proof of concept that real-time PCR detection in the labor and delivery suites is possible, but this assay is not a system which can be expanded to large-scale testing and utilization at multiple institutions.

An alternative approach is to use the GeneXpert PCR platform (Figure 1) to rapidly and simply detect HSV in the genital tracts of women presenting to Labor and Delivery suites with the intent of delivery. The GeneXpert system completely automates sample preparation, performing all the complex steps of DNA extraction in a single microfluidic cartridge (Figure 2). Once the sample nucleic acid is extracted, it is moved in a completely automated fashion from the sample processing chamber in the cartridge into the reaction tube where amplification and detection of viral DNA take place (Figure 3). All that is required to perform the test is to collect a genital swab and place it in viral transport media, and then to add approximately 1 mL of viral ansport media to the Xpert HSV 1/2 Assay cartridge, close the artridge cap, place the cartridge in the GeneXpert instrument, and ress "start." The results then come off of the computer in about an our.

Figure 1

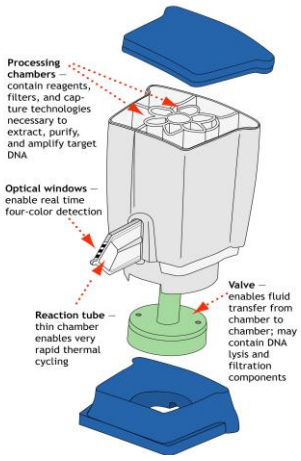
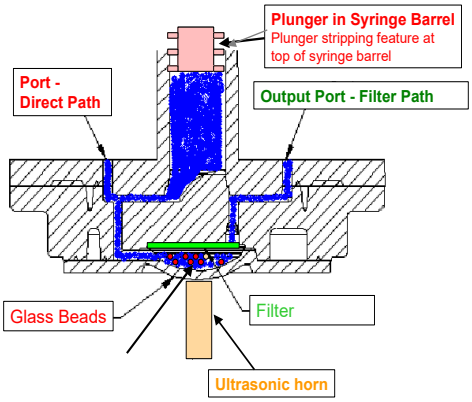


Figure 2

Figure 3



ignificant parallels exist etween the approach we ill study in this staged trial nd the prevention of eonatal disease caused by roup B Streptococcus GBS). Approximately 25% o 30% of pregnant women have genital herpes,[11,14] compared with 10%-30% who are colonized with GBS in the vagina or rectum.[28-30] The incidence of neonatal herpes (0.33 cases per 1,000 live births)[1] is essentially identical to that of neonatal group B Streptococcal disease (0.34-0.37 cases per 1,000 live births).[31] Both infections occur in neonates who are exposed to the pathogen in the mother's vagina. Colonization with GBS is intermittent,[32,33] as is HSV shedding.[34] An important difference, though, is that detection of GBS in women within 5 weeks of delivery serves as a marker of who will be colonized at delivery,[29,35] whereas with HSV cultures obtained in the weeks prior to delivery do not predict which women will be shedding virus at delivery.[36] Furthermore, recent data suggest that HSV may be intermittently shed in the genital tract for only a few hours at a time,[37,38] further necessitating that testing for the virus occurs at the time of delivery.

2.2 Scientific Rationale

With no HSV vaccine in the near- or intermediate-term future, reduction in cases of neonatal HSV disease requires improved identification of those babies at risk of neonatal infection. To do

so on a large scale requires that the natural history of maternal genital shedding of HSV at delivery be fully understood and that methodologies exist to detect virus simply and rapidly. The GeneXpert system provides a powerful opportunity to accomplish this with a test that is simple to perform and scalable to administer from a programmatic standpoint. In this study, The Xpert HSV 1/2 Assay will be compared to routine PCR in both populations, and to viral culture in the symptomatic population. The Xpert's sensitivity and specificity will be determined in symptomatic nonpregnant women, and its positive and negative agreement will be determined in asymptomatic pregnant women. All Xpert and routine PCR testing will be performed at the UAB Central Laboratory, with the Xpert PCR and routine PCR being performed in batches).

The outcomes of neonates delivered to the 12,500 pregnant women enrolled on the current study (approximately half of whom will have virologic testing and neonatal outcomes recorded, and half of whom will have neonatal outcomes recorded and will have specimens banked for possible future virologic testing) will be queried and rates of neonatal HSV disease without preemptive therapy will be determined. These incidence data will be compared with the incidence data from Brown et al.[1] In their study of almost 60,000 women conducted over a 20 year period, this group of researchers has reported an incidence rate for neonatal HSV disease of 1 in 3,200 live births.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

As a natural history and laboratory comparative study that does not include a therapeutic intervention, the potential risks relate to obtaining the vaginal sample or lesion swab, and having blood drawn. For the vaginal (non-lesion) sampling, there is the possibility of minor discomfort of a swab in the vaginal vault. For the lesion swab, there is a possibility also of minor discomfort in the area of the lesion. For the blood sampling, there is the possibility of associated discomfort, bruising at the phlebotomy site, and rarely an infection.

2.3.2 Known Potential Benefits

For those women in Group 1, culture-confirmation of HSV infection will be provided to their clinicians. For those women in Group 2, educational materials on neonatal HSV infection will be provided to the mothers.

3 OBJECTIVES

3.1 Study Objectives

3.1.1 Primary Objectives

- To estimate the sensitivity of the Xpert HSV 1/2 Assay relative to culture for detecting herpes simplex virus (HSV) DNA in the genital tract of nonpregnant women in sexually transmitted infections (STI) clinics
- To estimate the positive percent agreement and negative percent agreement of the Xpert HSV 1/2 Assay relative to routine PCR for detecting HSV DNA in the genital tract of pregnant women admitted with the intent of delivery

3.1.2 Secondary Objectives

- To estimate the positive predictive value between the Xpert HSV 1/2 Assay and culture for detecting HSV DNA in the genital tract of nonpregnant women in STI clinics
- To estimate the positive percent agreement of the Xpert HSV 1/2 Assay relative to routine PCR for detecting HSV DNA in the genital tract of nonpregnant women in STI clinics
- To compare the performance of Xpert with routine PCR in the combined group (pregnant and non-pregnant women) in detecting HSV-1 and HSV-2 virus, separately
- To quantify HSV viral load, as determined by routine PCR, in the genital tract of women shedding the virus
- To assess the type of infection (first-episode primary, first-episode non-primary, recurrent) among women shedding HSV

3.1.3 Exploratory Objectives

- To determine rates of neonatal HSV disease among neonates delivered to pregnant women without active genital HSV lesions
- To assess likelihood of viral transmission to neonates exposed to HSV during delivery as a manifestation of mode of delivery

3.2 Study Outcome Measures

3.2.1 Primary Outcome Measures

- Sensitivity of detection of HSV DNA by Xpert HSV 1/2 Assay from specimens obtained from the genital tract of nonpregnant women in STI clinics as compared with HSV culture

- Positive percent agreement and negative percent agreement of detection of HSV DNA by Xpert HSV 1/2 Assay from specimens obtained from the genital tract of pregnant women admitted with the intent of delivery, as compared with routine PCR detection of viral DNA

3.2.2 Secondary Outcome Measures

- Positive percent agreement of the Xpert HSV 1/2 Assay relative to routine PCR for detecting HSV DNA in genital tract of nonpregnant women in STI clinics
- Positive predictive value of detection of HSV DNA by Xpert HSV 1/2 Assay from specimens obtained from the genital tract of nonpregnant women in STI clinics, as compared with culture
- Comparison of Xpert and routine PCR results for detection of HSV-1 and HSV-2, separately
- HSV viral load, as determined by routine PCR, in the genital tract of women shedding the virus
- Correlation of HSV viral load, as determined by routine PCR, in genital tract of women with type of infection (first-episode primary, first-episode non-primary, recurrent)
-

3.2.3 Exploratory Outcome Measures

- HSV disease among neonates delivered to women without active genital HSV lesions
- Correlation of HSV disease among neonates delivered to women without active genital HSV lesions and mode of delivery

4 STUDY DESIGN

The natural history of genital HSV shedding from the genital tract of pregnant and nonpregnant women will be evaluated, and the utility of a new cartridge-based PCR platform (the GeneXpert system with the Xpert HSV 1/2 Assay cartridge) for the detection of HSV-1 or HSV-2 will be assessed. The study will be conducted at 15 academic medical centers throughout the United States. Virologic assessments will be made in two populations: 1) nonpregnant women in STI clinics (likely at one or two study centers) with clinically-apparent genital HSV lesions (**Group 1, n=300**), and 2) pregnant women admitted with the intent of delivery (at all study centers) with no visible evidence of genital HSV infection (**Group 2, n=12,500**). Women in each group will have specimens obtained from genital lesions (Group 1) or vaginal swabs (Group 2). Specimens from all women in Group 1 will be evaluated by HSV culture, routine HSV PCR, and Xpert HSV 1/2 PCR. For both groups, **Study Day 1** is the day that the genital or vaginal swab is obtained. Approximately half of the women (determined randomly) in Group 2 will be tested by routine HSV PCR and Xpert HSV 1/2 PCR; specimens from the rest of the women in Group 2 will be stored for possible testing in the future by routine HSV PCR and Xpert HSV 1/2 PCR. In this manner, we will maximize the data from which to compare Xpert HSV 1/2 PCR results with routine PCR for women in Group 2, while maintaining flexibility to ensure an adequate number of specimens are positive for HSV DNA by routine PCR.

All testing of samples on the GeneXpert platform and routine PCR in Group 1 and Group 2 will be performed in batches, while viral cultures performed in Group 1 will be performed in real time analysis. All Xpert and routine PCR testing will be done at the UAB Central Laboratory. In the conduct of this study, clinical staff may perform research activities (e.g., swabs for virologic testing, blood for serologic testing), under the direction of the site Principal Investigator or designee.

Lesion swabs from women in Group 1 (non-pregnant women from STI clinics) will be placed in viral transport media, refrigerated at 4°C, and sent to the laboratory for processing. The laboratory will perform real time analysis by HSV culture on a portion of the transport media, and freeze the remainder of the transport media in aliquots for subsequent batch testing by routine HSV PCR and Xpert HSV 1/2 PCR at the UAB Central Laboratory.

Vaginal swabs from women in Group 2 (pregnant women admitted with the intent of delivery) will be placed in viral transport media, frozen at -20°C or colder, and batch-shipped to the UAB Central Laboratory for future PCR analysis by routine HSV PCR and Xpert HSV 1/2 PCR. Specimens from approximately half of the Group 2 women will be analyzed by routine PCR. All specimens evaluated by routine PCR will also be evaluated by Xpert HSV 1/2 PCR. The laboratory personnel performing each type of procedure (routine PCR for Groups 1 and 2; culture for Group 1; and Xpert HSV 1/2 PCR for Groups 1 and 2) will be masked to the results of the other tests for a given subject. A blood specimen will be obtained from each nonpregnant

(Group 1) and pregnant (Group 2) woman on Study Day 1, and if she is determined to be shedding HSV by routine PCR, Xpert HSV 1/2 PCR, or culture then type-specific serologic testing will be performed. All virologic and serologic testing in this protocol is for research and not for standard of care. Correlation of viral typing from the virologic sampling with HSV-1 and HSV-2 serostatus will allow for categorization of infection (first-episode primary, first-episode nonprimary, or recurrent infection).

HSV culture results from women in Group 1 will be reported to their treating physician. Virologic results from women in Group 2 will not be reported back to the subject or her physician. The reasons for not reporting results to the Group 2 subject or her physician are two-fold: 1) the PCR analyses are performed in batches, so no results will be available within a timeframe in which clinical decisions on the woman's care could be made: and 2) neither routine HSV PCR nor Xpert HSV 1/2 Assay are FDA-cleared for evaluation of HSV testing on women without active lesions, and thus the reliability of a positive or a negative test result in this population is not known. Even if PCR results (routine HSV PCR or Xpert HSV 1/2 Assay) could be obtained in a real-time (non-batched) fashion, it would be irresponsible to report results from a non-validated test to the subject and her health care provider, as they may be influenced to make clinical decisions based on this unvalidated information. Furthermore, using the results of this research test for clinical decision making is not the intent of this study protocol. All pregnant women in Group 2 will receive written materials on Study Day 1 educating them on signs and symptoms of neonatal HSV disease. All postpartum women or designated representative will be contacted 30-90 days post-delivery and an inquiry will be made to determine mode of delivery and whether their babies developed neonatal HSV disease.

Data on the incidence of neonatal HSV disease among babies delivered to women in Group 2 will be compared with the incidence data from Brown et al.[1] In their study of almost 60,000 women conducted over a 20 year period, this group of researchers has reported an incidence rate for neonatal HSV disease of 1 in 3,200 live births.

Study involvement for women in Group 1 will be limited to only 1 day on Study Day 1 (day of virologic sampling). Women in Group 2 will be involved in the study for up to 90 days, with virologic sampling obtained and educational materials provided on Study Day 1, and with follow-up contact performed between Study Day 30 and Study Day 90. Full study accrual will occur within 4 years of enrollment of the first study subject.

4.1 Substudies (if applicable)

Not Applicable

5 STUDY POPULATION

Enrollment will be confined to women 18 years of age and older who are nonpregnant and have active genital HSV lesions (Group 1, n=300) or women 19 years of age and older who are pregnant and do not have visible genital HSV lesions or prodromal symptoms (Group 2, n=12,500). Women of all races will be included.

5.1 Recruitment Strategies

Identification of study subjects will be done based on the local standards of practice and IRB approved means. Clinicians who are care providers for these patients will identify patients who would generally qualify for inclusion in Group 1 or Group 2 of the study and will confirm that it is acceptable by the patient for the research team to approach them to discuss inclusion in the study. Group 1 subjects will be recruited from the STI clinic at the Health Department and/or similar outpatient facility. Group 2 subjects will be recruited from labor and delivery facilities and/or identified through obstetric practices. Subjects can sign informed consent in the labor and delivery facilities, or can sign informed consent prior to that time if the woman is 34 weeks estimated gestational age or older. However, no study related samples/procedures can be initiated until subject is admitted to the hospital labor and delivery area with the intent of delivering her baby. Each participating facility will determine the appropriate setting from which to recruit subjects. IRB approval will be obtained for each site's recruitment plan. Site investigators (who may also be the treating clinician) or study coordinators will provide information about the intended study population to the clinicians to aid in identifying appropriate patients to be approached for inclusion and consent. Consenting will be conducted by the research team (e.g., investigator and/or study coordinator/trained designee) for Group 1 and Group 2. Informed consent will be obtained prior to any study required procedures. The State of Alabama has recently (2015) changed the age of consent from 19 years to 18 years. As a result, Group 1 (which is still enrolling at the time of amendment of the protocol to version 4.0) will now enroll women who are 18 years of age or older. Recruitment and enrollment into Group 2 is not affected by this change because the cohort is already fully enrolled.

5.2 Subject Inclusion Criteria

- Signed Informed Consent from woman
- ≥ 18 years of age (Group 1); ≥ 19 years of age (Group 2)
- Female gender

- Group 1: women not known to be pregnant and being evaluated in STI clinics for herpetic genital lesions; OR Group 2: pregnant women ≥ 34 weeks estimated gestational age

5.3 Subject Exclusion Criteria

- For women in Group 2 (pregnant women admitted with the intent of delivery), active herpetic lesions or prodromal symptoms in the genital region

- Receipt of acyclovir, valacyclovir, or famciclovir within the previous 14 days

- Known HIV infection

6 STUDY PROCEDURES/EVALUATIONS

6.1 Test Device

The Xpert HSV1/2 Assay, performed on the GeneXpert Instrument Systems, is a new qualitative *in vitro* PCR test for the automated and rapid detection and qualitative differentiation of HSV 1 and HSV 2 in clinician-collected genital swabs. The Xpert HSV1/2 Assay system performs multiplex polymerase chain reaction (PCR) for detection of DNA targets after an initial sample processing step. The system consists of a GeneXpert® instrument, personal computer, and disposable fluidic cartridges that are designed to complete sample preparation and real-time PCR for detection of HSV1 and/or HSV2 in about 1 hour.

The single-use cartridges contain: 1) eleven chambers for holding sample, reagents, or other materials; 2) a valve body composed of a plunger and syringe barrel; 3) a rotary valve system for controlling the movement of fluids between chambers; 4) an area for capturing, concentrating, washing, and lysing of virus; 5) dry, real-time PCR reagents; 6) liquid reagents for PCR; and 7) an integrated PCR reaction tube that can be automatically filled by the instrument. To eliminate test-to-test contamination, all fluids including amplicons are contained within the disposable cartridge. The instrument never comes into contact with any fluids within the cartridge. Each cartridge is intended to test one sample.

The primers and probes in the Xpert HSV1/2 Assay detect and differentiate HSV-1 and HSV-2 DNA sequences. Each test cartridge includes a Sample Processing Control (SPC), a Sample Adequacy Control (SAC), and a Probe Check Control (PCC). The SPC is present to control for adequate processing of the target bacteria and to monitor the presence of inhibitors in the PCR reaction. The SAC reagents detect the presence of a single copy human gene and monitor whether the sample contains human DNA. The PCC verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

Cepheid (Sunnyvale, CA) will provide the GeneXpert system (instrument and cartridges). Xpert HSV 1/2 Assay cartridges will be appropriately labeled in accordance with regulations, and will be stored as indicated in the package insert. The UAB Central Laboratory personnel will be responsible for maintaining logs of receipt, accountability, storage conditions of the Xpert HSV 1/2 Assay investigational device and associated records and results. Documents and the investigational device will be stored in a secure and accessible location.

6.2 Study Procedures

If a patient is eligible for the study based on information regarding inclusion/exclusion criteria that is collected as part of the standard medical care (and not obtained for study purposes), then

the clinical team, following consultation with the patient indicating that she is interested to hear more about the study, will contact the study staff who will approach the patient and initiate the informed consent process (Section 11). For Group 1, signing of the informed consent document will take place on the same day as study procedures. For Group 2, signing of the informed consent document may occur on or before Study Day 1. Patients may be provided with study information prior to confirmation of eligibility for enrollment. After consent is obtained, the study participant will begin participation in the study procedures.

Following informed consent and confirmation of eligibility criteria, virologic sampling will be obtained from women in Group 1 and Group 2. No laboratory tests will be done to confirm eligibility. For the Group 1 women (nonpregnant women with active genital HSV lesions), a swab of the lesion(s) will be obtained and placed in viral transport media. For the Group 2 women (pregnant women without active lesions), following admission to the hospital labor and delivery area with the intent of delivering her baby a swab of the vaginal vault/canal will be obtained and placed in viral transport media; utilization of a speculum examination is not required in this sampling process. The specimen should be obtained before delivery, but may be obtained following delivery as long as it is collected within 24 hours following delivery. Women in both Groups 1 and 2 will have a venipuncture for collection of blood for serologic testing. This test will be performed only if the subject's virologic studies identify HSV shedding.

Women in Group 2 will be provided with printed material describing neonatal HSV signs and symptoms. These women (Group 2) or their designated representative also will be contacted between Study Day 30 and Study Day 90 for self-reporting of the delivery mode (vaginal vs. Cesarean delivery) and the development of neonatal HSV symptoms and signs post-delivery. It is acceptable to initiate contact shortly following the 30 day window and multiple attempts may be needed to reach the mother or her designated representative. The initial maternal Informed Consent document will include obtaining parental permission for the neonate in order to ask these questions. Verbal reporting from the mother on maternal receipt of antiviral therapy at any point following enrollment also will be captured from all women in Group 2.

HSV culture results (not PCR) from women in Group 1 will be reported to their treating physician. Virologic results from women in Group 2 will not be reported back to the subject or her physician. The reasons for not reporting results to the Group 2 subject or her physician are two-fold: 1) the PCR analyses are performed in batches, so no results will be available within a timeframe in which clinical decisions on the woman's care could be made: and 2) neither routine HSV PCR nor Xpert HSV 1/2 Assay are FDA-cleared for evaluation of HSV testing on women without active lesions, and thus the reliability of a positive or a negative test result in this population is not known. Even if PCR results (routine HSV PCR or Xpert HSV 1/2 Assay) could be obtained in a real-time (non-batched) fashion, it would be irresponsible to report results from a non-validated test to the subject and her health care provider, as they may be influenced to make clinical decisions based on this unvalidated information. Furthermore, using the results of this research test for clinical decision making is not the intent of this study protocol.

Subjects are not required to be seen in person after their involvement on Study Day 1. However, for Group 2 study staff may visit or contact the subject before she is discharged from the hospital to confirm the follow-up contact information. No additional contact is made with the subject following Study Day 1 for Group 1, and following the Study Day 30-90 contact for Group 2.

6.3 Laboratory Evaluations

6.3.1 Laboratory Evaluations/Assays

Virologic Testing

Samples obtained from women in Group 1 and Group 2 will be tested in batches for HSV DNA by Xpert HSV 1/2 PCR and routine PCR. Additionally, samples from women in Group 1 will be tested in real time by HSV culture. In this trial, all of these virologic tests will be Research Laboratory Evaluations (i.e., they would not be performed if the subject were not enrolled in the study). Routine HSV PCR from genital mucosa is not an FDA-cleared test. All Xpert and routine PCR testing will be conducted at the UAB CLIA-certified Central Laboratory. Bi-directional sequencing will be performed on specimens with discordant results between the Xpert HSV 1/2 PCR and routine PCR from asymptomatic pregnant women admitted with the intent of delivery, and between Xpert HSV 1/2 PCR and HSV culture in nonpregnant women. The primers used for bi-directional sequencing will be different from those used in the Xpert HSV 1/2 PCR and the routine PCR methods. Note: The results of discrepant analysis are for informational purposes only and will not be used to recalculate the performance of the Xpert HSV 1/2 PCR Assay.

Venipuncture

Women in both Groups 1 and 2 will have a venipuncture on Study Day 1 for collection of at least 0.5 mL of serum for serologic testing for HSV type-specific antibody. This serologic test will be performed for subjects in either group only if the subject's virologic studies identify HSV shedding. In this trial, all serologic tests will be Research Laboratory Evaluations (i.e., they would not be performed if the subject were not enrolled in the study). The window of time for collection of this specimen from women in Group 2 is ± 3 days. Remnant blood samples from clinical laboratory draws may be retrieved for this serologic testing. Informed consent will be obtained prior to any study required procedures.

6.3.2 Specimen Preparation, Handling, and Shipping

6.3.2.1 Instructions for Specimen Preparation, Handling, and Storage

Group 1: Lesion swabs from nonpregnant women in STI clinics will be placed in viral transport media, refrigerated at 2-8°C, and transported by the study coordinator or designee in real time to the laboratory for analysis by HSV culture in real time, and by Xpert HSV 1/2 PCR and routine HSV PCR (batched analysis at the UAB Central Laboratory).

Group 2: Vaginal swabs from pregnant women admitted with the intent of delivery will be placed in viral transport media, frozen at -20°C or colder, and batch-shipped to the UAB Central Laboratory for batched analysis by Xpert HSV 1/2 PCR and by routine HSV PCR. Only one freeze-thaw cycle will be required for each type of PCR test. Specimens will be assigned by the Study Biostatistician to either batched processing by routine PCR or to storage for possible testing by PCR in the future. Those that are batch processed by routine PCR also will be analyzed subsequently by Xpert HSV 1/2 PCR.

Serum from blood samples will be frozen at -20°C or colder and batch-shipped to the UAB Central Laboratory for HSV serologic testing. If remnant blood is retrieved for this purpose, it should have been stored according to local laboratory standard operating procedures until retrieval for research purposes.

Specific instructions on specimen preparation, handling, and shipping will be provided in the Manual of Procedures (MOP) for this study. Residual specimens will be stored at the UAB Central Laboratory if consent is provided by the study subject. Access to these specimens is detailed in Section 10.1.

6.3.2.2 Specimen Shipment

Specific instructions on specimen shipments, including frequency and location, will be provided in the Manual of Procedures (MOP) for this study.

7 STATISTICAL CONSIDERATIONS

7.1 Study Hypotheses

For pregnant women, we hypothesize that the long term expected positive percent agreement of HSV DNA using the Xpert HSV 1/2 Assay is 95% as evidenced by confidence interval containing this value. Therefore, with a sample size of between 135 and 150 we will have over a 95% probability of obtaining a point estimate greater than 91.8% with a lower bound of a 95% CI greater than 85%. This can be summarized in the form of hypothesis testing as:

H_0: Positive Percent Agreement = 95%
H_A: Positive Percent Agreement < 85%
Sample Size 135-150
Critical Value = 11FN, (or point estimate > 91.85% with lower 95% CI > 85%)
Target Alpha Value = 5%, Power > 95%.

For non-pregnant women, we hypothesize that the long term expected sensitivity of HSV DNA using the Xpert HSV 1/2 Assay is 95.34% as evidenced by a 95% confidence interval containing this value. Therefore, with a sample size of between 270 and 300 we will have a 95% probability of obtaining a point estimate greater 93.3% with a lower bound of a 95% CI greater than 90%. This can be summarized in the form of hypothesis testing as:

H_0: Sensitivity = 95.34%
H_A: Sensitivity < 90%
Sample Size 270-300
Critical Value = 18 false negative (FN), (or point estimate > 93.3% with lower 95% CI > 89%)
Target Alpha Value = 5%, Power > 95%.

7.2 Sample Size Considerations

Approximately 6,000 pregnant women (of the 12,500 enrolled) admitted with the intent of delivery will be tested to detect 144 (2.4%) who are shedding HSV by PCR. A subject who enrolls on the study but does not have a vaginal specimen obtained will not count toward the target sample size. The process of determining the 6,000 to be tested by PCR is as follows: IDs of pregnant women enrolled in a given 3 month period (each quarter) will be randomly assigned by site to either (1) the group to be tested by PCR or (2) the group not to be tested by PCR. This will enable us to have better representation over time and across sites. Specimens will be collected and tested from an additional 300 nonpregnant women presenting to STI clinics with active herpetic lesions who are shedding HSV by both PCR and culture. A 95% confidence interval will be constructed for the sensitivity of Xpert HSV 1/2 Assay relative to culture for the nonpregnant women population. Since we do not anticipate many negative results in the

symptomatic nonpregnant population, we may not be able to estimate the specificity of the GeneXpert in this population. For the pregnant women population, 95% confidence intervals will be constructed for the true positive and negative percent agreement between Xpert and routine PCR. The sample size justification considers the width and lower bound of these 95% confidence intervals for a single proportion using Clopper-Pearson method (also known as the Fisher's Exact CI) for small samples and large-sample confidence intervals for samples of greater than 100 (Ref Nonparametric Statistical Methods, Hollander and Wolf, Wiley, NY, NY 1973). SAS version 9.2 was used for these calculations.

Table 1 displays the minimum number of Xpert HSV 1/2 Assay positives from the total number of routine PCR positives in order to get a lower bound of a 95% confidence interval of at least 86%. With the expected 144 routine PCR positives from the pregnant women admitted with the intent of delivery, if the Xpert HSV 1/2 Assay finds 133 positives, then the corresponding 95% confidence interval for the true positive percent agreement will have a width of 9.4% and the resulting interval estimate is [86.7%, 96.1%]. Similar interpretations may be applied to the other cases considered in this table. With an expected 5856 negatives out of 6000 pregnant women, the 95% confidence interval for the negative percent agreement will be very tight. In particular, if the observed negative percent agreement is 97% (i.e., Xpert HSV 1/2 Assay finds 5680 negatives out of the 5856 routine PCR negatives), then the corresponding 95% confidence interval for the true negative percent agreement of Xpert HSV 1/2 Assay and routine PCR will have a width of 0.9% and the resulting interval estimate is [96.5%, 97.4%]. The reason for using positive and negative percent agreement instead of sensitivity and specificity is that routine PCR is not considered a gold standard.

Table 1: Minimum number of Xpert HSV 1/2 Assay positives, corresponding 95% Confidence Interval (CI) for Positive Percent Agreement, and width of the interval

Number of PCR Positives	**135**	**137**	**139**	**142**	**144**	**150**
Number of Xpert HSV 1/2 Assay Positives	124	126	128	131	133	138
Greatest number of Xpert HSV 1/2 Assay FN Allowed	11	11	11	11	11	12
95% CI for positive % agreement	85.9, 95.9	86.1, 95.9	86.3,96.0	86.6, 96.1	86.7, 96.1	86.4, 95.8
Width of CI	10.0	9.8	9.9	9.5	9.4	9.4

For nonpregnant women presenting to STI clinics with active herpetic lesions, Table 2 displays the minimum number of Xpert HSV 1/2 Assay positives from the total number of culture positives out of 300 subjects in order to get a lower bound of a 95% confidence interval of approximately 90%. Since we conservatively expect 270 of the 300 (90%) to be shedding HSV by culture, this is the smallest number of positive cultures considered in Table 2. To interpret, if

Xpert HSV 1/2 Assay finds 252 positives out of the 270 culture positives, the corresponding 95% confidence interval for the true sensitivity will have a width of 6.3% and the resulting interval estimate is [89.7, 96.0%]. Similar interpretations may be applied to the other cases considered in this table. The specificity of Xpert HSV 1/2 Assay may not be estimated in this population because we do not expect many culture-negative women. The main purpose of including this population is to enable us to get a precise estimate of the sensitivity of Xpert HSV 1/2 Assay relative to viral culture in women with symptomatic HSV lesions.

Table 2: Minimum number of positives and corresponding 95% Confidence Interval (CI) for Sensitivity

Number of Culture Positives	**270**	**275**	**280**	**285**	**290**	**295**	**300**
Number of Xpert HSV 1/2 Assay Positives	252	257	261	266	271	276	280
Greatest number of Xpert HSV 1/2 Assay FN Allowed	18	18	19	19	19	19	20
95% CI for sensitivity	89.7, 96.0	89.9, 96.1	89.6, 95.9	89.8, 95.9	90.0,96.0	90.1, 96.1	89.9, 95.9
Width of CI	6.3	6.5	6.3	6.1	6.0	6.0	6.0

7.3 Participant Enrollment and Follow-Up

Subjects will be enrolled in one of two groups based upon their clinical status. Group 1 (n=300) will consist of women with clinically apparent herpetic genital lesions in STI Clinics. Group 2 (n=12,500) will consist of women without clinically apparent genital HSV lesions who are admitted with the intent of delivery. For women in Group 2, all study procedures will be uniform at each study site; once specimens from a given subject are received in the UAB Central Laboratory, the Study Biostatistician will randomly assign them to either batched processing by routine PCR or to storage for possible testing by PCR in the future. Those that are batch processed by routine PCR also will be analyzed subsequently by Xpert HSV 1/2 PCR. We anticipate that approximately half of women enrolled in Group 2 will have batched PCR analysis of their specimens performed, with the other half having their specimens stored.

Subjects in Group 1 and Group 2 are not seen in person after their involvement on Study Day 1. HSV culture results from women in Group 1 will be reported to their treating physician. Women in Group 2 or their designated representative will be contacted between Study Day 30 and Study Day 90 to assess for neonatal treatment for HSV infection and for maternal mode of delivery.

7.4 Analysis Plan

Analyses of Group 1 and Group 2 may occur at different times, if Group 1 (n=300) fully accrues earlier than Group 2 (n=12,500). Demographics and clinical characteristics will be summarized using means and standard deviations for continuous variables and counts and proportions for categorical variables. For continuous variables and chi-squared tests for categorical variables, ANOVA (or its nonparametric analog Kruskall-Wallis) will be utilized to compare these characteristics among groups of participants.

For pregnant women, the primary goal of this study is to estimate the positive and negative percent agreement of the Xpert HSV 1/2 Assay relative to routine PCR methods. For nonpregnant women, we will be able to estimate the sensitivity of the Xpert HSV 1/2 Assay relative to culture. As a secondary analysis, we will also estimate the positive percent agreement of the Xpert HSV 1/2 Assay relative to PCR for this population. For all these quantities, we will utilize the exact Clopper-Pearson method for small sample and large-sample methods for samples greater than 100 to construct 95% confidence intervals for a single proportion. To compare the performance of Xpert with routine PCR for the combined groups (pregnant and non-pregnant women) in detecting HSV-1 and HSV-2 virus, we present the results of all samples tested in a 2x2 table separately for each type of virus. We will perform McNemar's test and construct 95% confidence interval estimates for the positive and negative percent agreement for each type of virus.

Since it is also of interest to estimate the true proportion of babies developing HSV infection from the pregnant women participants, we also will construct a confidence interval for the true proportion using either exact or large-sample methods.

8 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Documentation of source data is necessary for the reconstruction, evaluation, and validation of clinical findings, observations, and other activities during a clinical trial. Source documentation serves to substantiate the integrity of the trial data, to confirm observations that are recorded, and to confirm the existence of study participants. This standard also serves to ensure data quality by creating audit trails and enabling verification that data are present, complete, and accurate. This study will be monitored using these standards.

The site will maintain appropriate medical and research records for this trial, in compliance with ICH E6, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects. As part of participating in a DMID-sponsored, DMID-affiliated, or manufacturer-sponsored study, the site will permit authorized representatives of the sponsor(s), DMID, the UAB Central Unit, and regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress.

Examples of these original documents and data records may include, but are not limited to, Source Document Worksheets, hospital records, clinical and office charts, recorded data from automated instruments, and subject files and records kept at the laboratories.

Sites that are participating in this trial should consult the MOP and DMID/NIAID Source Document Standards (most current version) for specific instruction and forms.

The study monitor or other authorized representatives of the sponsor, or other governmental regulatory agencies may inspect all documents and records required to be maintained by the investigator for this trial. The clinical study site will permit access to such records.

9 QUALITY CONTROL AND QUALITY ASSURANCE

The study site will implement a quality management plan. The quality management procedures are described herein, as well as in the Manual of Procedures and the study site quality management plan (QMP). Per the QMP, data will be evaluated for compliance with the protocol and accuracy in relation to source documents. The study will be conducted in accordance with procedures identified in the protocol. Items to be reviewed include, but are not limited to: eligibility (including informed consent), any required AE reporting, study/clinical endpoints, follow-up visits, regulatory documents, missed calls, and review of clinical records. Data that will be reviewed, who is responsible for implementation, and the schedule for internal reviews will be specified or referenced in the quality management plan.

The UAB Statistical and Data Coordinating Center will implement quality control procedures beginning with the data entry system, and will generate data quality control checks that will be run on the database within 24 hours of new data entered into the system. Full documentation of these checks will be provided to the UAB Central Unit so that any resulting queries can easily be understood and transmitted to the respective site for resolution within a short period of time. These processes are validated by a second programmer and also tested with faulty test data (refer to Section 13 for additional details).

Site monitoring will also be implemented by a DMID directed independent monitoring group. See Section 12 (*Clinical Monitoring Structure)* for additional details.

10 SUBJECT CONFIDENTIALITY

The information obtained during the conduct of this clinical study is confidential, and disclosure to third parties other than those noted below is prohibited. The results of the research study may be published, but study participant's names or identities will not be revealed. Records will remain confidential. To maintain confidentiality, the principal investigators at each site will keep records in locked cabinets and the results of tests will be coded to prevent association with volunteers' names. Data entered into computerized files will be accessible only by authorized personnel directly involved with the study and will be encoded. Data received by DMID and forwarded to Cepheid will not include subject specific data but only encoded data. However, subject specific information will be available to the clinical monitors, and to health authorities where provided by law.

The study investigator is obliged to provide the NIAID/DMID and the UAB Central Unit with complete test results and all data developed in this study. The NIAID/DMID or the UAB Central Unit may disclose this information to appropriate regulatory authorities or clinical practice management groups (such as Pediatric Infectious Disease Society) as deemed necessary by the NIAID/DMID or the UAB Central Unit.

Subject-specific information may be provided to other appropriate medical personnel only with the study participant's permission. To ensure compliance with current ICH guidelines, data generated by this study must be available for inspection upon request by representatives of national and local health authorities, NIAID/DMID, the UAB Central Unit, and the Central Facilitated IRB/IEC overseeing the study (or for each study site, if the site does not utilize the Central Facilitated IRB).

10.1 Future Use of Stored Specimens

Approximately half of the 12,500 women enrolled in Group 2 will have their specimens stored for possible future HSV testing by PCR and serology. This will be fully explained in the Informed Consent document and will not be subject to "Future Use of Specimens" documentation since this testing is the primary purpose of the study. Likewise, any additional comparator PCR assays for HSV DNA that might be required by the FDA for this study will not be subject to "Future Use of Specimens" documentation since this comparison is the primary purpose of the study.

Subjects will be asked at the time of enrollment for permission to keep any remaining specimens for possible future use. Some of the specimens obtained from study participants during this study will be stored indefinitely in the UAB Central Laboratory at the University of Alabama at Birmingham and may be used in future viral or other sexually transmitted infection

research. These specimens will be labeled with a code number and not with the study participant's name. At the time of consent for study participation, study participants will have the opportunity to either agree to have their specimens used in future research or decline to have their specimens used in future research. The study participant will indicate her preference by initialing the appropriate line or checking the appropriate box of the Consent Form in the section entitled, "Future Use of Specimens". Non-protocol designated, future testing of samples will be performed only on samples from study participants who have consented for future testing of samples.

Samples may be shared with other investigators at other institutions or with the manufacturer of the GeneXpert cartridge. The samples will not be sold or used directly for production of any commercial product. No human genetic tests will be performed on samples. Long term storage of residual samples will be established according to OHRP guidelines ensuring that codes or other personally identifying links will not be distributed to future researchers.

The specimens will be stored indefinitely in the UAB Central Virology Laboratory at the University of Alabama at Birmingham. Specimens from study participants will be labeled and coded without study participant's identifiers. If the study participant has indicated in the signed consent form that she does not agree to allow the future use of specimens for future research, then her specimens will be destroyed at the completion of the study.

11 INFORMED CONSENT PROCESS

The process of obtaining informed consent must be documented in the medical records, clinic chart, and/or research chart. The consent form must be signed and dated by the study participant before participation in the study. A copy of the signed consent form must be provided to the study participant. Signed consent forms must remain in each study participant's study file and must be available for verification by study monitors at any time.

The investigational nature and research objectives of this trial, the procedure, and its attendant risks and discomforts will be carefully explained to the study participant. A signed informed consent document will be obtained from each study participant prior to entry into this study. This informed consent document will describe the study-related procedures for the woman, and for women in Group 2 it also will include permission to contact them at 30 to 90 days to inquire about the status of their baby and, if necessary, to inspect the infant's medical chart. Waiver of Assent (or obtaining parental permission) will be provided at the time the signature is obtained on the Informed Consent document (see Section 11.1). At any time during participation in the protocol, if new information becomes available relating to risks, this information will be provided orally or in writing to all enrolled or prospective study participants. Documentation will be provided to the Central Facilitated IRB/IEC overseeing the study (or to each study site, if the site does not utilize the Central Facilitated IRB) and, if necessary, the informed consent will be amended to reflect any relevant information.

There will be two consent forms, one for Group 1 and one for Group 2. The informed consent will reflect that the HSV culture results from women in Group 1 will be reported to their treating physician, and that virologic results from women in Group 2 will not be reported back to the subject or her physician. The reasons for not reporting results to the Group 2 subject or her physician are two-fold: 1) the PCR analyses are performed in batches, so no results will be available within a timeframe in which clinical decisions on the woman's care could be made: and 2) routine HSV PCR and Xpert HSV 1/2 Assay are not FDA-cleared tests, so at this point the reliability of a positive or a negative test result is not known. Even if PCR results (routine HSV PCR or Xpert HSV 1/2 Assay) could be obtained in a real-time (non-batched) fashion, it would be irresponsible to report results from a non-validated test to the subject and her health care provider, as they may be influenced to make clinical decisions based on this unvalidated information. Furthermore, using the results of this research test for clinical decision making is not the intent of this study protocol.

An investigator shall seek such consent only under circumstances that provide the prospective subject sufficient opportunity to consider whether or not to participate and that minimize the possibility of coercion or undue influence. The information that is given to the subject shall be in language understandable to the subject or the representative.

Subjects will sign the informed consent document prior to any procedures being done specifically for the study. Subjects should have the opportunity to discuss the study with their family, friends or personal physician, or think about it prior to agreeing to participate. Subjects may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

11.1 Informed Consent/Assent Process (in Case of a Minor or Others Unable to Consent for Themselves)

The site principal investigator will ensure that this study is conducted in full conformity with principles of the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR 46, 21 CFR 50 and 56, and ICH E6; 62 Federal Regulations 25691 (1997), if applicable. The site principal investigator's Institution will hold a current Federal Wide Assurance (FWA) issued by the Office of Human Research Protection (OHRP) for federally funded research.

For women in Group 2, parental/legal guardian permission will be obtained for the minor's (the subject's baby) information to be gathered on or between Study Days 30-90. Assent age will be determined by the Central Facilitated IRB/IEC overseeing the study (or by each study site, if the site does not utilize the Central Facilitated IRB). Appropriate documentation will be required for subjects who are the age of assent, whether mature enough to read and capable of providing signed assent, or whether too young to read but capable of providing verbal assent, as determined by the IRB in compliance with 45CFR46.408. The Central Facilitated IRB/IEC overseeing the study (or each study site, if the site does not utilize the Central Facilitated IRB) will review and assign the risk level.

11.2 Institutional Review Board

Reviewing IRBs will be registered with the OHRP and have Federal Wide Assurance (FWA) to conduct US federally-funded studies.

Prior to enrollment of subjects into this trial, the sponsor's approved protocol, informed consent documents, relevant supporting information, and all types of volunteer recruitment, advertisements, and information provided to subjects (summary, pamphlets, etc.) will be reviewed and approved by the appropriate IRB listed on the institution's FWA.

In addition, the IRB/REC will review and approve this protocol in compliance with applicable US federal regulations for the protection of pregnant women, human fetuses, and neonates in this study.

The responsible official for the IRB will sign the IRB letter of approval of the protocol prior to the start of this trial and a copy will be provided to the UAB Central Unit, who will then provide a copy to DMID.

Should amendments to the protocol be required, the amendments will be approved by the sponsor and provided by the UAB Central Unit to the site principal investigator for submission to the IRB. Any amendments to the protocol or consent materials must be approved by the IRB/REC before they are placed into use, unless for immediate safety to the subject.

The investigator is responsible for keeping the IRB apprised of the progress of the study and of any changes made to the protocol as deemed appropriate, but in any case at least once per year. The investigator must also keep the IRB informed of any significant AEs. All IRB approved documents as well as relevant study correspondence should be copied and sent to the UAB Central Unit.

For this study, clinical sites will have the opportunity to participate in a Centralized IRB process supported by the UAB Institutional Review Board and the UAB Central Unit. Participating sites will be provided with detailed instructions on required responsibilities for participating in an OHRP compliant Centralized IRB.

12 CLINICAL MONITORING STRUCTURE

12.1 Site Monitoring Plan

Site Monitoring will be conducted to ensure adequate human subjects protection and correct handling/shipping of laboratory specimens. NIAID/DMID, which has contracted for the conduct of this study, or its designee will conduct the site monitoring visits. A Clinical Monitoring Plan will be developed to include specifics about monitoring, including a description of the number and type of subjects monitored, the frequency of visits, the tasks to be completed during the visits, and the different types of visits that will be used during the monitoring. The monitoring plan will be risk based.

Site visits typically may be made at standard defined intervals. More frequent visits may be made if needed. Remote site monitoring may also occur. Monitoring visits may include, but are not limited to; review of the regulatory file, informed consent forms, case report forms, and protocol compliance. Study monitors will meet with investigators to discuss any problems and actions to be taken and will document visit finding and discussions.

Every effort will be made to maintain the anonymity and confidentiality of subjects during this study. However, because of the experimental nature of this sampling study, the investigator agrees to allow representatives of the NIAID/DMID, or the UAB Central Unit to inspect the facilities used in this study and to inspect, for purposes of verification, the hospital, clinic or study records of all subjects enrolled.

As outlined in the clinical monitoring plan for this study, the study monitors will verify that the subjects are consented and the clinical trial is conducted in compliance with the protocol, ICH E6(R1), and the applicable regulatory requirements. Reports will be submitted to DMID and UAB on monitoring activities. The study site research team will provide direct access to all trial-related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor and their designees.

13 DATA HANDLING AND RECORD KEEPING

The investigator is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported.

Electronic case report forms (eCRFs) will be developed by the UAB Statistical and Data Coordinating Center. The eCRFs will be provided electronically by the UAB Central Unit. Original data will be recorded on source documents (e.g., medical records, research progress notes, Source Document Worksheets documenting research related procedures). The DMID 11-0070 Data Management Plan provides a description of how the system will function. Source Document Worksheets that mirror each data field on the eCRF will be available for use by sites as a tool to record and maintain data for each study participant enrolled in the study when other source documents are not used to collect original data. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. When making a change or correction, cross out the original entry with a single line and initial and date the change. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL. Specific guidance to investigators and study staff on making corrections to source documents and eCRFs will be provided in the MOP for this study.

Data recorded on the eCRF that differ from source documents must be explained on the Comments eCRF and in the subject's source documents.

13.1 Data Management Responsibilities

All eCRFs must be reviewed by the investigator's research team, under the supervision of the investigator, who will ensure that they are accurate and complete. All data must be supported by source documents, which will remain available for review by regulatory personnel and monitors. Adverse events must be graded, assessed for intensity and causality, and reviewed by the site investigator or designee.

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site PI. During the study, the investigator must maintain complete and accurate documentation for the study.

The UAB Central Unit and Statistical and Data Coordinating will be responsible for data management, quality review, analysis of the study data, and writing of the clinical study report. These tasks are detailed in the DMID 11-0070 DMP.

13.2 Data Capture Methods

Clinical and laboratory data will be entered into a 21 CRF Part 11 compliant electronic Data Entry System (eDES) provided by the UAB Statistical and Data Coordinating Center. The data

system includes password protection and internal quality checks, such as automated range checks, to identify data that appear to be inconsistent, incomplete, or inaccurate.

13.3 Types of Data

Data for this study will include clinical laboratory and virologic results, and clinical and outcome measures (e.g., virology, demographics).

13.4 Timing/Reports

There are no planned interim analyses or safety reviews for this trial. There also are no stopping rules.

13.5 Study Records Retention

Records and documents pertaining to the conduct of this study, including source documents and consent forms, must be retained by the investigator for at least 2 years following completion of the study. No study records shall be destroyed without prior authorization from the UAB Central Unit and NIAID/DMID. These documents should be retained for a longer period, however, if required by local regulations. It is the responsibility of the sponsor to notify the UAB Central Unit, which will notify the investigators, when these documents no longer need to be retained.

13.6 Protocol Deviations

Each investigator must adhere to the protocol as detailed in this document and agree that any changes to the protocol must be approved by the UAB Central Unit and NIAID/DMID prior to seeking approval from the IRB/IEC. Each investigator will be responsible for enrolling only those study participants who have met protocol eligibility criteria.

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or Manual of Procedures requirements. The noncompliance may be either on the part of the study participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6 GCP sections:

4.5 Compliance with Protocol, Sections 4.5.1, 4.5.2, and 4.5.3

5.1 Quality Assurance and Quality Control, Section 5.1.1

5.20 Noncompliance, Sections 5.20.1 and 5.20.2.

It is the responsibility of the site to use continuous vigilance to identify and report deviations within 1 business day of identification of the protocol deviation that increases subject risk.

Deviations that do not increase subject risk can be reported within 5 business days of knowledge of the event. All deviations must be promptly reported to the UAB Central Unit. UAB will report all deviations to DMID in accordance with DMID's instructions.

All deviations from the protocol must be addressed in the source documents. A completed copy of the DMID protocol deviation form must be maintained in the regulatory file (Project Notebook or designated location) as well as in the subject's source documents. A log of protocol deviation will be maintained in the Project Notebook. Protocol deviations must be sent to the local IRB per the IRB's guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB/IEC requirements.

14 PUBLICATION POLICY

Following completion of this study, the investigators are expected to publish the results in a scientific journal. All research reports and other publications resulting from the work completed in this protocol shall:

- Acknowledge the support of the National Institutes of Health whenever publicizing the results from this clinical trial in any media.
- Be submitted to the Project Director in the form of advance copies for review and comment prior to the publication to ensure appropriate coordination of the research results.
- Be furnished in a list of publications resulting from the research as part of the annual progress report submitted to the principal investigator.

The International Committee of Medical Journal Editors (ICMJE) member journals has adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies.

Unless exempted, this trial will be registered prior to enrollment of study subjects. It is the responsibility of the study's PI (e.g., Dr. Kimberlin) to register the non-exempted trials and post results in compliance with Public Law 110-85, the Food and Drug Administration Amendments Act of 2007 (FDAAA).

15 LITERATURE REFERENCES

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SUPPLEMENTS/APPENDICES

APPENDIX A: SCHEDULE OF EVENTS

Table 1. Schematic of Study Design								
	Study Day							
	≤ 1	1	5	10	20	30	60	90
Nonpregnant Women in STI Clinics (Group 1)								
Verification that inclusion/exclusion criteria are met, and informed consent is obtained	X							
Lesion swab from women		X[a,b]						
Collect whole blood for type-specific serology from women (at least 0.5 mL of serum required)		X[c]						
Pregnant Women Admitted with the Intent of Delivery (Group 2)								
Verification that inclusion/exclusion criteria are met, and informed consent is obtained	X							
Vaginal swab from women		X[d]						
Collect whole blood for type-specific serology from women (at least 0.5 mL of serum required)		X[e]						
Provide educational fact sheet about neonatal herpes		X[f]						
Follow-up contact re: treatment for neonatal HSV and maternal mode of delivery						X[g]		

a. Lesion swabs from nonpregnant women will be placed in viral transport media, refrigerated at 2-8°C, and sent to the laboratory for real time analysis by HSV culture on a portion of the transport media, and the remainder of the transport media will be frozen in aliquots for subsequent batch testing by routine HSV PCR and Xpert HSV 1/2 PCR at the UAB Central Laboratory
b. HSV culture results from women in Group 1 will be reported to their treating physician
c. Blood for serologic testing will be collected from all nonpregnant women enrolled, but testing will be performed only on samples from those women who are determined to be shedding HSV genitally by culture, routine PCR, or Xpert HSV 1/2 PCR
d. Vaginal swabs from pregnant women will be placed in viral transport media, frozen at -20°C or colder, and batch-shipped to the UAB Central Laboratory for batched analysis as follows: specimens from approximately half of the women in Group 2 will be tested by routine HSV PCR and Xpert HSV 1/2 PCR; specimens from the rest of the women in Group 2 will be stored for possible testing in the future by routine HSV PCR and Xpert HSV 1/2 PCR.
e. Blood for serologic testing will be collected (or remnant sample may be retrieved) from all pregnant women enrolled (window: ± 3 days), but testing will be performed only on samples from pregnant women who are determined to be shedding HSV genitally by routine HSV PCR or Xpert HSV 1/2 PCR
f. Printed material about neonatal HSV signs and symptoms will be provided to all pregnant women enrolling in the study
g. Follow-up contact will involve all Group 2 women enrolled on the study, or their designated representative. Inquiry will be made regarding information suggesting development of neonatal HSV disease in the first 4-6 weeks of life. Maternal receipt of antiviral therapy at any point following enrollment will be captured.