Abbreviated Title: Study on the safety and immunogenicity of Boostrix vaccine in pregnant Malian women and their infants

A Phase II Double-blind Trial to Evaluate the Safety, Immunogenicity and Effect on Infant Immune Responses of a Single Dose of Tdap in Pregnant Women in Mali

DMID Protocol Number: 16-0024

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IND Sponsor: Division of Microbiology and Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health

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Draft or Version Number: 3.0
24 July 2019

STATEMENT OF COMPLIANCE

This trial will be carried out in accordance with Good Clinical Practice (GCP) as required by the following:

- United States Code of Federal Regulations (CFR) 45 CFR Part 46: Protection of Human Subjects
- Food and Drug Administration (FDA) Regulations, as applicable: 21 CFR Part 50 (Protection of Human Subjects), 21 CFR Part 54 (Financial Disclosure by Clinical Investigators), 21 CFR Part 56 (Institutional Review Boards), 21 CFR Part 11, and 21 CFR Part 312 (Investigational New Drug Application), 21 CFR 812 (Investigational Device Exemptions)
- International Conference on Harmonisation: Good Clinical Practice (ICH E6); 62 Federal Register 25691 (1997); and future revisions
- Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, Report of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research
- National Institutes of Health (NIH) Office of Extramural Research (OER), Research Involving Human Subjects, as applicable
- National Institute of Allergy and Infectious Diseases (NIAID) Clinical Terms of Award, as applicable
- Applicable Federal, State, and Local Regulations and Guidance

Compliance with these standards provides public assurance that the rights, safety, and well-being of trial subjects are protected, consistent with the principles that have their origin in the Declaration of Helsinki.

All key personnel (all individuals responsible for the design and conduct of this trial) have completed Human Subjects Protections Training.

SIGNATURE PAGE

The signature below provides the necessary assurance 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 US federal regulations and ICH E6 Good Clinical Practice (GCP) guidelines.
I agree to conduct the study in compliance with GCP and applicable regulatory requirements.
I agree to conduct the study in accordance with the current protocol and will not make changes to the protocol without obtaining the sponsor's approval and Institutional Review Board (IRB)/Institutional Ethics Committee (IEC) approval, except when necessary to protect the safety, rights, or welfare of subjects.

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Title

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LIST OF ABBREVIATIONS

ACIP	Advisory Committee on Immunization Practices, CDC
ACOG	American College of Obstetrics and Gynecology
AE	Adverse Event/Adverse Experience
AGA	Appropriate for Gestational Age
ASQ-3	Ages and Stages Questionnaire-3
BMI	Body Mass Index
BP	Blood Pressure
bpm	Beats per Minute
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CI	Confidence Interval
cm	Centimeter
CMS	Clinical Materials Services
CNAM	Centre Nationale à la lutte contre la Maladie
CRM_{197}	a form of diphtheria toxoid
CROMS	Clinical Research Operations and Management Support
CSR	Clinical Study Report
CVD	Center for Vaccine Development
CVD-Mali	Center for Vaccine Development- Mali
°C	Degrees Celsius
°F	Degrees Fahrenheit
D	Day(s)
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases
DSMB	Data and Safety Monitoring Board
DTwP	Diptheria, Tetanus, and whole-cell Pertussis Vaccine
eClinical[SM]	Electronic Data Capture System
eCRF	electronic Case Report Form
e.g.	for example
EGA	Estimated Gestational Age
ELISA	Enzyme-Linked Immunosorbent Assay
EPI	Expanded Program on Immunization
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act

FHA	Filamentous Hemagglutinin
FIM 2	Fimbriae 2
FIM 3	Fimbriae 3
FW	Field Worker
FWA	Federalwide Assurance
g/dL	Grams per Deciliter
GA	Gestational Age
GBS	Guillain-Barré Syndrome
GCP	Good Clinical Practices
gDM	Gestational Diabetes Mellitus
GMC	Geometric Mean Concentration
GMR	Geometric Mean Ratio
GMT	Geometric Mean Titer
GSK	GlaxoSmithKline Biologicals
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
IATA	International Air Transport Association
ICF	Informed Consent Form
ICH	International Council for Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
i.e.	that is
IEC	Independent or Institutional Ethics Committee
IFN	Interferon
IgA	Immunoglobulin A
IL	Interleukin
IM	Intramuscular
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ITT	Intent-to-Treat
LGA	Large for Gestational Age
LMP	Last Menstrual Period
MAPT	Multicenter Acellular Pertussis Trial
mcg or µg	Microgram(s)
MD	Doctor of Medicine
MedDRA®	Medical Dictionary for Regulatory Activities
mg	Milligram(s)

mg/dL	Milligram(s) per Deciliter
mL	Milliliter(s)
mm	Millimeter(s)
mm Hg	Millimeters of Mercury
MOP	Manual of Procedures
MPH	Master of Public Health
MS or MSc	Master of Science
MSD	Meso Scale Diagnostic
N	Number of Subjects
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OER	Office of Extramural Research (NIH)
OHRP	Office for Human Research Protections
PBMC	Peripheral Blood Mononuclear Cells
PERCH	Pneumonia Etiology Research for Child Health
PHI	Personal/Protected Health Information
PI	Principal Investigator
PP	Per Protocol
PRN	Pertactin
PRR	Proportional Reporting Ratio
PT	Pertussis Toxin
QA	Quality Assurance
QC	Quality Control
Rh(D)	Rhesus Antigen D (a red blood cell surface protein)
SAE	Serious Adverse Event/Serious Adverse Experience
SBA	Serum Bactericidal Antibody
SGA	Small for Gestational Age
SOC	System Organ Class
SOP	Standard Operating Procedure
Td	Tetanus Diphtheria Toxoid
Tdap	Tetanus Diphtheria Acellular Pertussis Vaccine
Th	T Helper Cell
TNF	Tumor Necrosis Factor
TT	Tetanus Toxoid
UK	United Kingdom
US	United States
USP	United States Pharmacopeial Convention
V	Visit

VAERS	Vaccine Adverse Event Reporting System
vs.	Versus
VTEU	Vaccine and Treatment Evaluation Unit
VVM	Vaccine Vial Monitor
WBC	White Blood Cells
WFI	Water for Injection
WHO	World Health Organization

PROTOCOL SUMMARY

Title:	A Phase II Double-Blind Trial to Evaluate the Safety, Immunogenicity, and Effect on Infant Immune Responses of a Single Dose of Tdap in Pregnant Women in Mali
Phase:	2
Population:	200 healthy pregnant women, ages 18 through 39 years, inclusive, who meet all eligibility criteria, and their infants
Number of Sites:	At least one Vaccine and Treatment Evaluation Unit (VTEU)-subcontracted site
Study Site:	Referral Health Center of Commune I (CSREF I) of the district of Bamako, Mali
Study Duration:	21 months: Approximately 2 months in the start-up period, 6 months enrolling subjects, and 13 months (3-7 months while pregnant and 6 months postpartum) from last subject vaccinated until she and her infant complete follow-up
Subject Participation Duration:	Pregnant subjects: Approximately 10-13 months; infants: approximately 6 months
Description of Agent or Intervention:	Test agent: A single dose of Tetanus, diphtheria, acellular pertussis (Tdap) (BOOSTRIX) manufactured by GlaxoSmithKline Biologicals (GSK) and administered intramuscularly at a volume of 0.5 milliliter (mL) or Control agent: a single dose of Tetanus diphtheria toxoid (Td) intramuscularly at a volume of 0.5 mL.
Objectives:	**Primary:** Safety and Tolerability: • To assess the safety and tolerability of a single 0.5 mL intramuscular injection of BOOSTRIX in pregnant women. • To assess the safety of a single maternal BOOSTRIX vaccination on the fetus and infant.

Immunogenicity:

- To assess the level of PT antibody at birth among infants whose mothers received a single dose of BOOSTRIX or Td while pregnant.

Secondary:
Immunogenicity:

- To assess the antibody response to BOOSTRIX vaccine antigens in pregnant women one month after receipt of BOOSTRIX, at the time of delivery, and at 6 months after delivery.
- To compare the antibody levels of BOOSTRIX vaccine antigens at birth (cord blood) and 6 weeks of age (before receiving any infant doses of Diphtheria, Tetanus, and whole-cell Pertussis (DTwP)) in infants whose mothers received BOOSTRIX or Td during pregnancy.
- To assess placental antibody transfer by determining the ratio of maternal and infant BOOSTRIX -specific antibody responses at delivery.
- To assess interference with infant antibody responses to DTwP either prior to the second dose of the primary DTwP series, at approximately 10 weeks of age (in ½ of subjects), or approximately one month after the third dose of the primary DTwP series, at approximately 18 weeks of age (in ½ of subjects), and at 6 months of age (all subjects).

Exploratory:
Immunogenicity:

- To assess the effects of maternal age, parity, gestational age (GA) at which the vaccine is given, GA at delivery, and infant birthweight on antibody responses to BOOSTRIX in pregnant women at time of delivery, and in their newborn infants at birth.

- To assess maternal secretory immunity through measurement of breast milk antibodies to Tdap vaccine antigens in women at the time of delivery, at 6 weeks postpartum, at 10 weeks (in ½ of women) or 18 weeks (in ½ of women) postpartum, and at 6 months after delivery.
- To assess the cytokine profile after stimulation in vitro with diphtheria, tetanus and pertussis antigens of peripheral blood mononuclear cells (PBMC) obtained from women before and one month after receiving BOOSTRIX or Td.
- To assess the cytokine profile after stimulation in vitro with diphtheria, tetanus, and pertussis antigens of PBMC obtained from infants before the first dose of DTwP (approximately 6 weeks of age) in all subjects, one month after the first dose of DTwP (approximately 10 weeks of age) in ½ of infants, one month after the last dose of DTwP (approximately 18 weeks of age) in ½ of infants, and at 6 months of age in all infants.

Outcome Measures:

Primary:

Safety and Tolerability:

- Safety in pregnant women: Frequency and severity of study vaccine-related serious adverse events (SAEs), and all SAEs in pregnant women from study vaccination through 6 months postpartum, description and comparison between those receiving BOOSTRIX and Td.
- Safety in pregnant women and fetuses/infants: Frequency and severity of adverse events specific to pregnancy, in pregnant women and their infants (as delineated in Section 9.2.4), description and comparison between those receiving BOOSTRIX and Td.
- Tolerability in pregnant women: Frequency and severity of solicited injection site and systemic reactogenicity events from study vaccination until 7 days following vaccination (Day 8).

- Tolerability in pregnant women: Frequency and severity of all unsolicited non-serious AEs from day of study vaccination to Day 31, description and comparison between those receiving BOOSTRIX and Td.
- Safety in the infants: Frequency and severity of study vaccine-related serious adverse events, and all SAEs in infants from birth through 6 months of age, description and comparison between infants born to women vaccinated with BOOSTRIX and Td.

Immunogenicity:

- Infant humoral immunity: Geometric Mean Concentration (GMC) of serum IgG antibodies to PT as measured by Enzyme-Linked Immunosorbent Assay (ELISA) at birth between infants born to women vaccinated with BOOSTRIX and Td.

Secondary:

Immunogenicity:

- Maternal humoral immunity: GMC of serum IgG antibodies to Tdap vaccine antigens (PT (Pertussis Toxin), FHA (Filamentous Hemagglutinin), PRN (Pertactin), tetanus, diphtheria) as measured by ELISA in pregnant women one month after receipt of BOOSTRIX or Td, at the time of delivery, and 6 months after delivery.
- Infant humoral immunity: GMC of serum IgG antibodies to Tdap vaccine antigens (PT, FHA, PRN, tetanus, diphtheria) as measured by ELISA at birth and prior to receipt of first DTwP (approximately 6 weeks of age) among infants born to women vaccinated with BOOSTRIX compared to Td.
- Placental antibody transfer: The geometric mean ratio (GMR) of maternal and infant-specific Tdap-specific antibodies (PT, FHA, PRN, tetanus, diphtheria) as

measured by ELISA at delivery after intrapartum receipt of BOOSTRIX versus Td.

- Interference with infant responses among infants whose mothers received intrapartum BOOSTRIX compared to Td: GMC of antibodies to DTwP vaccine antigens (PT, FHA, PRN, Fimbriae 2 (FIM2), Fimbriae 3 (FIM3), tetanus, diphtheria) as measured by ELISA one month after the first dose of DTwP (~10 weeks of age, ½ of infants), one month after the third dose of DTwP vaccine (~18 weeks of age, ½ of infants), and at 6 months of age.

Exploratory:
Immunogenicity:

- Maternal immunogenicity cofactors for maternal and neonatal anti-PT antibody responses following intrapartum BOOSTRIX: Maternal age (18-29, 30-39 years old), parity (primiparous vs. multiparous), GA at time of vaccination (14-17, 18-21, 22-26 weeks), GA at time of delivery (28-32, 33-36, 37 or more weeks), and infant birthweight as potential independent associations with PT GMC.
- Maternal secretory immunity: GMC of breast milk IgG and IgA antibodies to Tdap vaccine antigens (PT, FHA, PRN, tetanus, diphtheria) as measured by ELISA in women at the time of delivery, at 6 weeks after deliver, at 10 weeks (in ½ of women) or 18 weeks (in ½ of women) after delivery, and 6 months after delivery after intrapartum receipt of BOOSTRIX versus Td.
- Maternal cytokine responses: Cytokines produced by peripheral blood cells stimulated with DTwP vaccine antigens, as measured by multiplex assays, in women before and one month after receiving Tdap. Cytokines measured will include: Interferon (IFN)-γ, Inerleukin (IL)-10, IL-12p70, IL-12/IL-23p40, IL-13, IL-15, IL-16, IL-17A, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, Tumor Necrosis Factor (TNF)-α, and TNF-β.

- Infant cytokine responses: Cytokines produced by infant peripheral blood cells stimulated with DTwP antigens from infants whose mothers received intrapartum BOOSTRIX versus Td, measured by multiplex assays before the first dose (or umbilical cord blood), one month after the first dose of DTwP (~10 weeks of age, ½ of subjects), one month after the last dose of study vaccine (approximately 18 weeks, ½ of subjects), and at 6 months of age. Cytokines measured will include: IFN-γ, IL-10, IL-12p70, IL-12/IL-23p40, IL-13, IL-15, IL-16, IL-17A, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, TNF-α, and TNF-β.

Description of Study Design: This is a phase II, single center, randomized, double-blind, active-controlled study in which 200 healthy pregnant women, ages 18 through 39 years, inclusive, will be randomly allocated in a 2:1 ratio to receive either Tdap (BOOSTRIX*)* or Td at 14 0/7 weeks through 26 6/7 weeks estimated GA. After signing an informed consent form (ICF), all subjects will undergo a review of current and past medical history, current and prior medication use, and recent and relevant vaccination history. A physical examination performed as part of routine antenatal care or a study-specific focused exam may be used to determine eligibility. For the fetuses of pregnant subjects, GA will be established by ultrasound, whenever possible, in combination with date of last menstrual period (LMP), when available, and fundal height.

Eligibility will be confirmed, and the subject will be enrolled in the study. Each subject will receive prenatal care consistent with national guidelines and with local standards as practiced by clinicians caring for pregnant women and delivering neonates in the study area. In Mali, this includes hemoglobin, sickle cell test, Rh group, HIV, syphilis, toxoplasmosis, and rubella serology and urine glucose and protein at the first presentation for antenatal care, urine albumin and glucose at each subsequent visit and ultrasound at 22 and 32 weeks gestation.

At the time of randomization to vaccine allocation, women will also be randomized to a schedule of postpartum visits for specimen collection. All women-infant pairs will have specimens collected at birth, at 6 weeks after delivery, and at 6 months after delivery. In addition, women-infant pairs will be allocated in a 1:1 ratio to have specimen collections at one month following the first dose of DTwP (approximately 10 weeks of age), or at one month following the third dose of DTwP (approximately 18 weeks of age). Thus, pregnant subjects will typically have 7 clinic visits (only 6 if delivery occurs before the first visit after vaccination, on Day 31): 3 antenatal visits (Screening Visit, Study Day 1, and Study Day 31, unless delivery occurs first), 1 visit at the time of delivery, and 3 postpartum visits – at 6 weeks, at 10 or 18 weeks postpartum, and a final visit 6 months postpartum. The maternal subject will also undergo 2 home visits after vaccination to assess her health status (Days 4 and 8). Infants born to the pregnant subjects will have 4 study visits: at birth, at approximately 6 weeks of age (day of first DTwP), at approximately 10 or 18 weeks of age (one month after first or third dose of DTwP, respectively), and at 6 months of age. Blood and colostrum/breast milk samples for immunologic assessments will be collected as described below:

- Pregnant subjects: approximately 30 mL of whole blood will be collected by venipuncture on Study Day 1 (prior to vaccination), Study Day 31 (one month after vaccination), at delivery, and at 6 months postpartum in all women.
- Infants: up to 30 mL of umbilical cord blood will be collected at delivery and 5 mL peripheral blood will be collected by venipuncture at 6 weeks (prior to first dose of DTwP), either the 10-week or 18-week (per randomized study schedule), and 6 months of age in all infants. If cord blood cannot be obtained, 2-5 mL of peripheral blood will be collected by venipuncture within 72 hours of birth when possible. Cord blood is preferred over venipuncture.

- At delivery, and at the 6-week, either the 10-week or 18-week (per randomized study schedule), and the 6-month postpartum visit, a 10-20mL sample of colostrum or breast milk will be collected. When possible, the first breast milk or colostrum collection will occur while the mother and baby are admitted for delivery and postpartum care. When not possible, it may be collected any time within the first 4 days of life.

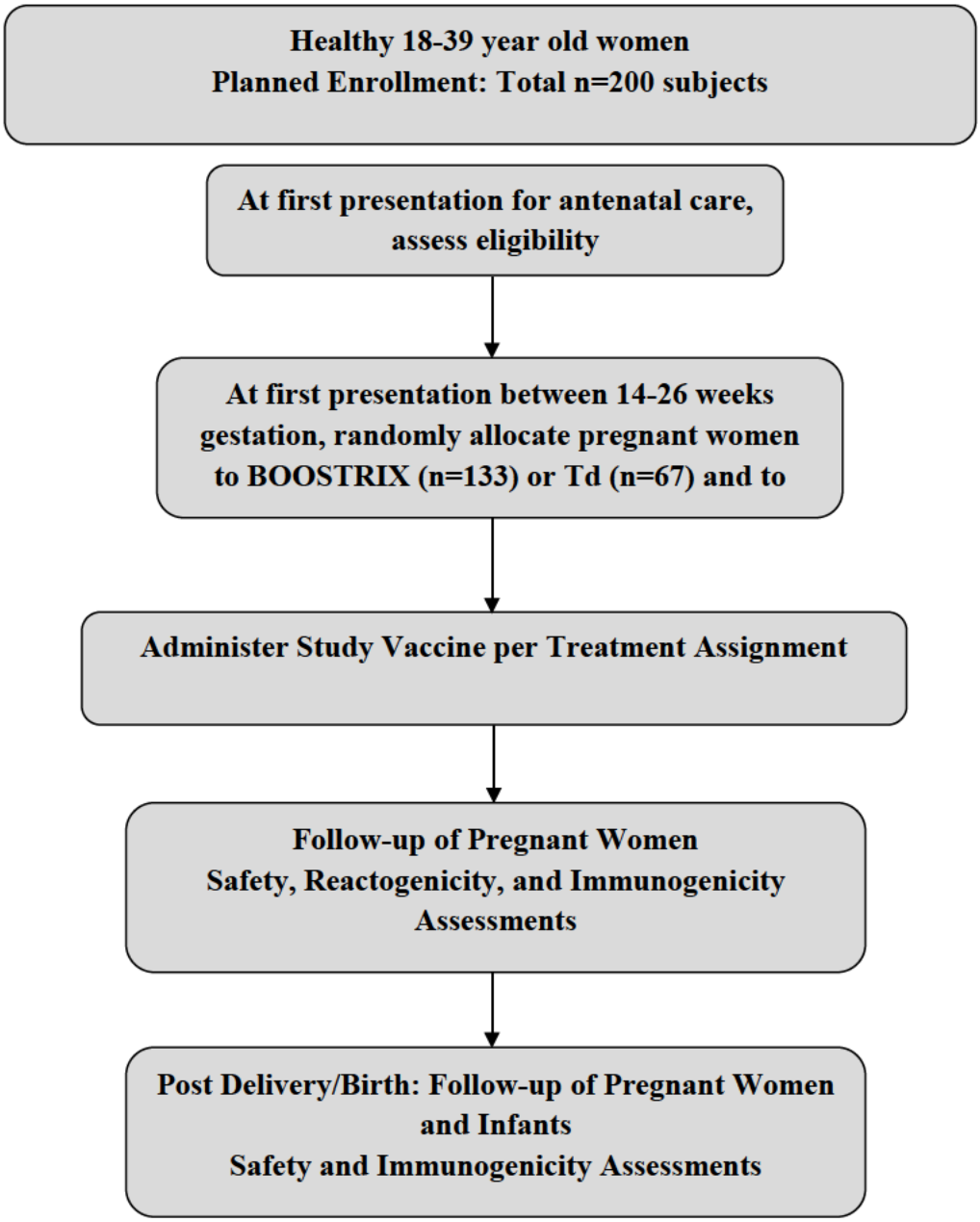
Safety will be monitored from the time of signing of the ICF until trial termination.

Estimated Time to Complete Enrollment: Approximately 6 months from the first vaccinated subject

Table 1: Treatment Arms and Study Vaccine to be Administered

	Treatment Arm		
Subject Group	**BOOSTRIX**	**Td**	**Total**
Pregnant women	133	67	200

Figure 1: Schematic of Study Design



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2. BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1. Background Information

2.1.1. Disease Caused by Bordetella Pertussis

Pertussis, or whooping cough, is a disease of the upper and lower respiratory tract caused by Bordetella pertussis (B. pertussis), a Gram-negative bacterium that lives in the upper respiratory tract. Pertussis is highly contagious. The bacteria attach to the cilia that line part of the upper respiratory system, and release toxins which damage the cilia and inflame the airways. While disease usually starts with cold-like symptoms and maybe a mild cough or fever, it may progress to paroxysms of coughing, post-tussive vomiting, and exhaustion. Pertussis is particularly dangerous in young infants, in whom it may lead to pneumonia, pulmonary hypertension, convulsions, apnea, encephalopathy, and death.

2.1.2. Burden of Illness

The introduction of pertussis vaccination in the past century has led to dramatic decreases in pertussis disease throughout the world. Nevertheless, pertussis burden remains high globally, as an estimated 16 million cases of pertussis occurred worldwide as recently as 2008.[1] Moreover, there has been a resurgence in pertussis disease in industrialized countries in recent years, with over 48,000 cases reported in the US alone for 2012. [2] This rise is likely due to a combination of factors, including improved diagnostics, heightened awareness of the disease, a decrease in natural immunity due to low circulation, and decreased duration of protection from acellular pertussis vaccines.[3]

Even in settings where vaccination coverage is high, the highest incidence of serious disease and mortality occurs in infants under two months of age, as they are too young to receive protection through current pertussis vaccines. In the US, this age group accounts for 57% of all infant pertussis hospitalizations. [4, 5]

Less is known about pertussis in low resource settings. A modeling study estimated there were 16 million cases of pertussis and over 160,000 deaths in children younger than 5 years due to pertussis worldwide in 2014, with most deaths occurring in developing countries. [1, 2, 6] Recent data from the Pneumonia Etiology Research for Child Health (PERCH) study, a case-control study of pneumonia etiology conducted in 7 low and middle resource countries, identified pertussis in 2.2% of severe pneumonia hospitalizations and 2.9% of pneumonia deaths. Younger, unimmunized, and HIV-exposed infants, and those in Africa, were most at risk. The case-fatality rate among infants 1-5 months of age at the 5 African sites where cases were detected was 12.5%. As PERCH excluded children younger than 1 month of age, these are undoubtedly underestimates.[7]

2.1.3. Prevention of Pertussis in Young Infants through Maternal Vaccination in Developed Countries

Serious disease and death due to pertussis overwhelmingly occur in infants under two months of age, including in settings with excellent access to medical care and high vaccination coverage. [3] The rising incidence of pertussis in industrialized countries among infants too young to complete their primary vaccination series has prompted recommendations for pertussis vaccination during pregnancy in the US, UK, New Zealand, and several Latin American and European countries. [1-4, 8]The goal of these recommendations is to achieve protection of young infants through transplacental transfer of maternal immunoglobulin G antibodies until active immunization of the infant can occur. The US Advisory Committee on Immunization Practices (ACIP) first recommended antenatal pertussis immunization in 2011 for women who had not previously received Tdap vaccine, [9]and expanded this recommendation to all pregnant women during every pregnancy, regardless of prior receipt of Tdap. [10] In support of this policy, both observational [11, 12] and interventional [13] studies have shown that antenatal Tdap immunization results in increased infant antibody levels. The benefits of this intervention were further supported through an evaluation of the national antenatal pertussis immunization program in the UK begun in 2012, in which a case-control study design demonstrated a vaccine effectiveness of 91% against laboratory-confirmed clinical pertussis among children younger than 3 months of age. [14] In the US, a case control study estimated that maternal Tdap vaccine given in the third trimester of pregnancy was 77.7% effective against the outcome of pertussis in infants younger than 2 months of age. This estimate rose to 90.5% against hospitalized cases of pertussis in infants. [15]

2.1.4. Interference of Maternal Antibody with Infant Immune Responses

The beneficial effects of maternal antibody early in life must be balanced with any detrimental effects on primary infant responses to pertussis vaccines. In the maternal pertussis immunization studies from the 1930s and 1940s in the US, infant agglutinin antibody responses to whole-cell pertussis vaccine were lower in infants born to mothers who received whole-cell pertussis vaccine as compared to mothers who received no vaccine. Subsequent studies in the 1990s examining the effect of pre-existing antibodies in unvaccinated pregnant women showed that infant responses to whole-cell vaccines were suppressed by pre-existing maternal antibody, while responses to acellular vaccines were not affected, or at least were suppressed to a lesser degree. [16-18] One of these studies [18]evaluated this issue within the NIAID-funded Multicenter Acellular Pertussis Trial (MAPT), which allowed direct comparison between whole-cell and multiple acellular pertussis vaccines. In this evaluation, pre-existing anti-PT antibodies had a strongly negative effect on PT responses for the two whole-cell DTwP vaccines, but only a minimal reduction for the acellular DTaP vaccines.

Several recent studies in in the US, Europe, and Vietnam have described the immunogenicity of acellular pertussis vaccines in pregnancy, and in all cases the infants also received acellular

pertussis vaccines. [10, 19-22] In all studies, the first dose of infant vaccine was given at or after 2 months of age. An NIAID-sponsored study of Tdap vs. placebo in 48 women in the US found that Tdap was immunogenic in pregnant women and that infants born to such women had statistically higher concentrations of pertussis antibodies at delivery and 2 months of age. Antibody titers for FHA at age 7 months were significantly lower in infants whose mothers received Tdap, although the difference was no longer significant at 13 months of age following a Tdap booster dose. [10] In a nonrandomized study in Belgium, pregnant women who received Tdap and their infants through 2 months of life had higher concentrations of all antigens included in the vaccine. Blunting was reported for infant diphtheria and PT responses one month after the third dose of infant vaccine, and a minor blunting effect persisted for PT responses after the booster dose. [20] In Vietnam, women and infants up to 2 months of age had significantly higher GMCs for PT, PRN, and FHA. One month after completion of the primary infant vaccination schedule, anti-PRN GMC was significantly lower in the vaccinated group. [19] In the US, the blunting of infant responses to FHA was overcome by the booster dose in the second year of life; studies in Vietnam are on-going to determine the effect of the booster dose on infant responses. [10, 19, 20]

The clinical significance of any blunting effect of maternal antibody on infant primary responses is complicated by the lack of an agreed correlate of protection for pertussis. High concentrations of antibodies to PT, FHA, PRN, Fim 2, and Fim 3 are known to be protective against disease. Small amounts of antibody to the A subunit of PT will prevent death in infants although it will not prevent infection. [22] Therefore, while all responses may be clinically important, the postulated critical role of anti-PT antibodies in protection against severe infant pertussis justifies an emphasis on this response in the first months of life.

2.1.5. Cytokines Produced in Response to Vaccine Antigens

The development of robust antibody responses relies on the stimulation of T cells, which supports antibody development through direct interactions with B lymphocytes and the secretion of cytokines. Th1 and Th17 cytokines have been shown to contribute to protection against pertussis in mice, following infection or vaccination. [23] Most recently, IL-17 induced by DTwP has been associated with enhanced pertussis clearance in the baboon model. [24] There is limited information on cytokine T cell responses to Tdap during pregnancy. A recent study reported transient and impaired IFN-γ responses in pregnant women who received Tdap, [25] but these results were derived from a very small number of subjects and using whole blood (as opposed to purified cells) stimulated with polyclonal, not pertussis-specific antigens. It is possible that the passive transfer of maternal antibodies might interfere with the ability of pertussis antigens to elicit protective cellular responses. Cytokine measurements in vaccinated infants would allow assessment of the potential impact of maternal immunization on the infant's T cell responses following vaccination.

2.1.6. Role of Breastfeeding

Breast feeding has been associated with reduced risk of respiratory illnesses early in life. [26, 27] Pertussis-specific IgA and IgG have been detected in breast milk from women who had received DTwP during childhood [28]as well as IgA in colostrum of Tdap vaccinated pregnant women[29, 30], and it has been proposed that breastfeeding may have a protective role against infection. [28, 29] Investigating antibodies in breast milk of Tdap vaccinated women will provide a more comprehensive evaluation of immunogenicity and test the hypothesis that women vaccinated during pregnancy develop mucosal antibodies that are also available for protection of the offspring. Studies that have examined the effect of maternal antibodies on infant responses to acellular pertussis vaccines have not investigated antibodies in maternal milk. [9, 10]

2.1.7. Pertussis Vaccines in Pregnant Women

In Mali, there are no recommendations for vaccinating pregnant women routinely with a pertussis-containing vaccine. In the US, the UK, and elsewhere, both Adacel and BOOSTRIX are licensed. Adacel is a pregnancy category C and BOOSTRIX is a pregnancy category B. The US and UK recommend that either vaccine be given to pregnant women. Only BOOSTRIX is pre-qualified by the WHO, and therefore is the vaccine of choice for developing countries, and the vaccine that will be used for this study. [14] Standard of care in Mali is to administer to pregnant women at the first prenatal visit a dose of Td vaccine manufactured by Biological E. Limited and prequalified by the WHO. The whole-cell vaccines received by infants varies by country, with most low-income countries using a pentavalent vaccine (DTwP-HepB-Hib) manufactured by one of 8 different WHO-prequalified manufacturers. [14]

In the United States, post licensure surveillance data of Tdap during pregnancy, including studies looking at repeated doses, have revealed no association with medically attended acute adverse events during pregnancy or adverse birth outcomes. [1-4] A recent review of 392 reports to the Vaccine Adverse Event Reporting System (VAERS) of AEs in pregnant women who received Tdap vaccine after the routine recommendation (11/01/2011–6/30/2015) was compared to published data before the routine Tdap recommendation (01/01/2005–06/30/2010). An increase in proportion of reports for stillbirths (1.5–2.8%) and injection site reactions/arm pain (4.5–11.9%) and a decrease in reports of spontaneous abortion (16.7–1%) occurred after the recommendation compared to the period before the routine recommendation for Tdap during pregnancy. In most recent reports, vaccination (79%) occurred during the third trimester. Overall, no new or unexpected AEs were noted among pregnant women who received Tdap after routine recommendations for maternal Tdap vaccination. Changes in reporting patterns would be expected, given the broader use of Tdap in pregnant women in the third trimester after the routine recommendation as compared to before.

2.1.8. Timing of Maternal Immunization

In the US, the ACIP states that the optimal timing for Tdap administration is between 27 and 36 weeks gestation although Tdap may be given at any time during pregnancy. [10] Two recent studies support that earlier vaccination may be more beneficial. In Israel, a prospective study that included women in their 20th week of pregnancy or later demonstrated that IgG GMCs to PT were higher in newborn cord sera when women were immunized at 27-30 weeks compared with 31-36 weeks of gestation. [20] Likewise, in a prospective observation cohort study in Geneva, Switzerland, early second-trimester maternal Tdap immunization significantly increased neonatal antibodies compared to later dosing. [21]

In Mali, and elsewhere in Africa, tetanus-containing vaccines are recommended to be given at the first pregnancy encounter. The rationale for this recommendation is that many women in Mali will not present for multiple, regular prenatal visits, as is done in the United States. Thus, the recommendation for first encounter vaccination ensures that a woman receives the vaccine and has adequate time to develop immunity and transfer antibody to her unborn child. Delaying the administration of tetanus-containing vaccines until the third trimester of pregnancy would be a deviation from standard of care, and would likely be unacceptable to the community. Therefore, second trimester vaccination may be the best option in this trial for consistency with local practices. Any pregnant women who presents in the first trimester and wishes to participate in the study will be asked to return during the second trimester for randomization and receipt of a tetanus-containing vaccine.

2.2. Rationale

Unfortunately, the positive impact of maternal pertussis vaccination cannot be assumed to be generalizable to low resource settings, where most pertussis deaths occur. Maternal antibody transfer in low resource settings may be adversely affected by malnutrition, HIV, malaria, and other factors. [12] In addition any diminution of primary infant antibody responses by the presence of maternal antibody may be more pronounced in low resource countries for two main reasons. First, infants in Africa and many low resource countries in Asia receive their primary pertussis-containing vaccines at earlier time points – 6, 10, and, 14 weeks as compared to 2, 4, and 6 months in industrialized settings. It might be expected that any interference would be greater at younger ages, when maternal antibody concentrations are higher. While booster doses may overcome any deleterious effect of maternal antibody on infant primary responses to pertussis vaccines in developed countries, most low resource countries do not include a pertussis booster in the second year of life. [1, 10] The second reason that interference with infant immunization may be more pronounced in developing countries is that they continue to use whole-cell vaccines in the primary infant series. As above, prior studies suggest that higher levels of maternal antibodies were associated with lower infant responses to whole-cell pertussis

vaccines, with less of an effect seen in infants given acellular pertussis vaccines. [13] These two factors highlight the need for context-specific data on maternal pertussis immunization.
Maternal pertussis vaccination would be feasible in low resource countries. Existing antenatal tetanus immunization programs have been successful worldwide and may serve as a platform for pertussis vaccine introduction as Tdap combinations could be substituted for Td or tetanus toxoid (TT). In Mali, standard of care is to administer Td to pregnant women during their first contact with the health care system, which for most women is in the second trimester.
Realizing the importance of pertussis prevention in infants too young to receive vaccine, and the lack of data on the impact of maternal vaccination on infant responses in developing country settings where whole-cell vaccines are used, a study is necessary to examine the safety, immunogenicity, and effect on infant antibody responses of a Tdap vaccine administered during pregnancy among women in Mali.

2.3. Potential Risks and Benefits

2.3.1. Potential Risks and Discomforts

The potential risks of participating in this study are those associated with having blood drawn, intramuscular (IM) injection of and possible reactions to the study vaccine, accompanying risks to the fetus and infant of the vaccinated mother, risks of breast milk collection, and breach of confidentiality.
Ultrasound: All women will have an ultrasound to date the pregnancy. This procedure is not harmful to the mother or fetus, but it may feel uncomfortable. Mothers will be informed if the ultrasound reveals any implications for the pregnancy or the health of the child. It is possible this news may cause emotional distress; there may also be additional costs to women who choose to perform further testing based on this information.
Phlebotomy: Drawing blood is typically well tolerated and uneventful. However, it often causes transient mild discomfort. Some subjects may have near syncope (2%) or true syncope (fainting). Fainting is a transient autonomic (vasovagal) response to phlebotomy that occurs in approximately 1% of blood donors and less than 1% of those having diagnostic phlebotomy. [24] It is managed by having the subject lie down, loosening his or her clothes, monitoring vital signs, reassuring the subject, and giving fluids. Minor bruising (up to 12%) or a hematoma (2-3%) may occur at the blood draw site. It can be prevented or decreased in frequency by employing experienced phlebotomists and can be ameliorated, when it occurs, by applying pressure to the draw site for several minutes. Rarely, following phlebotomy, persons may have nerve injury (typically lateral antebrachial nerve) causing pain, paresthesias, and motor or sensory loss. This phenomenon is uncommon and typically resolves quickly with watchful waiting only. [25] Infection at the site of the blood draw is rare, and can be avoided by using antiseptics and other appropriate techniques. In a study of over 4000 persons undergoing routine venipuncture for insurance application, there were no serious local reactions such as cellulitis or phlebitis. [24]

Intramuscular Injection: The injection of a vaccine into the deltoid muscle, as will be done in this study, is typically well tolerated. However, it often causes transient discomfort. As with phlebotomy, although less commonly, it can cause near syncope or syncope. It can also cause an abscess, a hematoma, injury to blood vessels and peripheral nerves, or tingling or numbness. Proper technique reduces the risks of infection, bleeding, and injury.

The Vaccine: BOOSTRIX manufactured by GSK, is indicated for active booster immunization against tetanus, diphtheria, and pertussis. BOOSTRIX is approved for use as a single intramuscular injection for persons aged 10 years of age and older in the US. [31]

The safety of BOOSTRIX has been evaluated in multiple studies in adolescents and adults. The most relevant to this age group was a study in US adults 19 to 64 years of age that compared BOOSTRIX (n=1,522) to Adacel (Sanofi Pasteur) (n=762). Solicited local and systemic reactions were monitored for 14 days after vaccination, and medically significant AEs and SAEs for 6 months. Solicited adverse reactions (any/severe), reported by adult BOOSTRIX recipients were pain at the injection site (61%/1.6%), erythema (21%/1.6%), induration (17.6%/1.4%), headache (30%/2.2%), fatigue (28.1%/2.5%), any gastrointestinal symptoms (15.9%/1.2%), and fever (5.5% >99.5°F/0.1% >102.2°F). The reactogenicity profile closely mirrored that of Adacel, the other US licensed Tdap. [31]

The incidence of unsolicited adverse events reported in the 31 days after vaccination was comparable in the 2 groups (17.8% and 22.2% for BOOSTRIX and Adacel). Serious adverse events were reported to occur during the entire study period (0-6 months) by 1.4% and 1.7% of subjects who received BOOSTRIX and Adacel. During the 6-month extended safety period, no serious adverse events of a neuroinflammatory nature or with information suggesting an autoimmune etiology were reported in subjects who received BOOSTRIX. [31]

Since licensure, additional events have been voluntarily reported, but none have prompted concern for common or severe events caused by the vaccine.

Rare or uncommon reactions include anaphylaxis or other acute allergic reactions and syncope. Acute and potentially life-threatening allergic reactions (i.e., anaphylaxis) are also possible. These reactions occur in about 1 in 4 million people given a vaccination. These reactions can manifest as skin rash (urticaria), swelling around the mouth, throat or eyes (angioedema), difficulty breathing (bronchospasm), a fast pulse (tachycardia), or decrease in blood pressure (hypotension). If these reactions occur, they can usually be stopped by the administration of emergency medications by the study personnel. As with any vaccine or medication, there is a very small chance of a fatal reaction (death), although researchers do not expect this to occur. [31]

If Guillain-Barré syndrome (GBS) occurred within 6 weeks of receipt of a prior vaccine containing tetanus toxoid, the risk of Guillain-Barré syndrome may be increased following a subsequent dose of tetanus toxoid-containing vaccine, including BOOSTRIX. A review by the

Institute of Medicine (IOM) found evidence for a causal relationship between receipt of tetanus toxoid and both brachial neuritis and Guillain-Barré syndrome. [31]

Progressive or unstable neurologic conditions (e.g., cerebrovascular events and acute encephalopathic conditions) are reasons to defer vaccination with a pertussis-containing vaccine, including BOOSTRIX. It is not known whether administration of BOOSTRIX to persons with an unstable or progressive neurologic disorder might hasten manifestations of the disorder or affect the prognosis. Administration of BOOSTRIX to persons with an unstable or progressive neurologic disorder may result in diagnostic confusion between manifestations of the underlying illness and possible adverse effects of vaccination. [31]

The vaccine manufacturer maintains a surveillance registry to collect data on pregnancy outcomes and newborn health status outcomes following vaccination with BOOSTRIX during pregnancy. In the event of an observed safety signal from that registry, the manufacturer would share that information with the sponsor and appropriate actions for trial subjects would be taken.

It is unknown if the vaccine to be used in this study poses any risks to an unborn child or to children born to mothers who are vaccinated during pregnancy. The vaccine is currently deemed as pregnancy category B. A developmental toxicity study has been performed in female rats at a dose approximately 40 times the human dose (on a mL/kg basis) and revealed no evidence of harm to the fetus due to BOOSTRIX. Animal fertility studies have not been conducted with BOOSTRIX.

The effects of the vaccine on lactation and nursing, as well as the effects on the breastfed infant of a woman vaccinated with BOOSTRIX during pregnancy, are unknown.

Breast Milk Collection: Women will provide breast milk within the 4 days following delivery and at 3 time points postpartum (at 6 weeks, either 10 or 18 weeks, and 6 months postpartum). There is no significant risk of this collection. Some women may experience mild tenderness from milk expression.

Potential Risks to Infants: As with the risks to pregnant women, there is a possibility that obtaining a blood sample from your infant may cause some pain, swelling, bruising, or infection at the site of the blood draw. To avoid infection, a qualified and trained staff member will collect the blood from the infant.

The standard of care in Mali is to vaccinate infants with the pentavalent Diphtheria and Tetanus and Pertussis and Haemophilus influenzae and Hepatitis B vaccine at 6, 10, and 14 weeks of age; this vaccination should provide protection. There is a possibility that the infant's response to the pertussis vaccine could be lower after the usual infant vaccinations because of the mother's study vaccine.

Privacy and Confidentiality: Subjects will be asked to provide and allow research team members access to personal/protected health information (PHI) of the woman and her infant. All attempts will be made to keep this PHI confidential within the limits of the law. However, there is a chance that unauthorized persons will see subject PHI. All paper records will be kept in a

locked file cabinet or maintained in a locked room at each participating VTEU site. Electronic files will be password protected. Only people who are involved in the conduct, oversight, monitoring, or auditing of this study will be allowed access to the PHI that is collected. Any publications from this study will not use information that will identify subjects by name.
Organizations that may inspect and/or copy research records maintained at the participating site for quality assurance and data analysis include groups such as the National Institute of Allergy and Infectious Diseases (NIAID) and/or its representatives and the US Food and Drug Administration (FDA). They also include the Malian Research Ethics Committee of the School of Medicine, Pharmacy, and Dentistry and the Malian National Drug Authority.
A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by US law. This web site will not include information that can identify subjects. At most, this web site will include a summary of the results.
There may be other unknown risks, discomforts, or side effects.

2.3.2. Potential Benefits

BOOSTRIX is licensed in the US for use in persons 10 years old and older and per WHO pre-qualification guidelines for persons 4 years and older, and therefore includes the age range of young adult women who are eligible for this trial. Although not routinely recommended for pregnant women in Mali, BOOSTRIX is not contra-indicated in pregnancy. Therefore, a potential benefit of participation is protection against pertussis in the women and the infants. Td recipients will not accrue any treatment-related benefits while enrolled.
For pregnant subjects, their infants may receive the benefit of transplacental pertussis antibody transfer and/or breast milk antibodies. These antibodies could potentially provide complete or partial protection against pertussis. Contacts of subjects, including infants, could benefit by herd immunity whereby the subjects are protected from pertussis disease and as a result cannot spread pertussis to their contacts. In summary, the prospect of direct benefit due to participation in this study exists for pregnant subjects and their fetuses/infants.
There may be no direct personal benefit of participation. There is potential that BOOSTRIX administered to pregnant women may cause harm in the infants if primary responses to pertussis-containing vaccines are adversely affected. The potential societal benefit is that BOOSTRIX may be found to be safe, well tolerated, and immunogenic in pregnant women and lead to levels of antibodies in infants thought to protect them, thus informing scientists and policymakers on the benefits of Tdap for pregnant women and their babies in Mali and other African countries.
Close follow-up of pregnant subjects, and infants will potentially afford them additional benefit due to frequent access to experts in obstetrics, pediatrics, and immunizations. Pregnant women enrolled in this study will receive an ultrasound, which is standard of care in Mali, but unaffordable to the majority. Likewise, pregnant women will receive the recommended set of pregnancy blood tests (glucose, syphilis, toxoplasmosis, HIV serologies) in Mali at no additional

charge. While these are recommended in Mali, they are unaffordable to many women who must forego these tests.

3. OBJECTIVES AND OUTCOME MEASURES

3.1. Study Objectives

3.1.1. Primary

Safety and Tolerability:

- To assess the safety and tolerability of a single 0.5 mL intramuscular injection of BOOSTRIX in pregnant women.
- To assess the safety of a single maternal BOOSTRIX vaccination on the fetus and infant.

Immunogenicity:

- To assess the level of PT antibody at birth among infants whose mothers received a single dose of BOOSTRIX or Td while pregnant.

3.1.2. Secondary

Immunogenicity:

- To assess the antibody response to BOOSTRIX vaccine antigens in pregnant women one month after receipt of BOOSTRIX, at the time of delivery, and at 6 months after delivery.
- To compare the antibody levels of BOOSTRIX vaccine antigens at birth (cord blood) and 6 weeks of age (before receiving any infant doses of Diphtheria, Tetanus, and whole-cell Pertussis (DTwP)) in infants whose mothers received BOOSTRIX or Td during pregnancy.
- To assess placental antibody transfer by determining the ratio of maternal and infant BOOSTRIX -specific antibody responses at delivery.
- To assess interference with infant antibody responses to DTwP either prior to the second dose of the primary DTwP series, at approximately 10 weeks of age (in ½ of subjects), or approximately one month after the third dose of the primary DTwP series, at approximately 18 weeks of age (in ½ of subjects), and at 6 months of age (all subjects).

3.1.3. Exploratory

Immunogenicity:

- To assess the effects of maternal age, parity, gestational age (GA) at which the vaccine is given, GA at delivery, and infant birthweight on antibody responses to BOOSTRIX in pregnant women at time of delivery, and in their newborn infants at birth.
- To assess maternal secretory immunity through measurement of breast milk antibodies to Tdap vaccine antigens in women at the time of delivery, at 6 weeks postpartum, at 10 weeks (in ½ of women) or 18 weeks (in ½ of women) postpartum, and at 6 months after delivery.
- To assess the cytokine profile after stimulation in vitro with diphtheria, tetanus and pertussis antigens of peripheral blood mononuclear cells (PBMC) obtained from women before and one month after receiving Tdap or Td.
- To assess the cytokine profile after stimulation in vitro with diphtheria, tetanus, and pertussis antigens of PBMC obtained from infants before the first dose of DTwP (approximately 6 weeks of age) in all subjects, one month after the first dose of DTwP (approximately 10 weeks of age) in ½ of infants, one month after the last dose of DTwP (approximately 18 weeks of age) in ½ of infants, and at 6 months of age in all infants.

3.2. Study Outcome Measures

3.2.1. Primary

Safety and Tolerability:

- Safety in pregnant women: Frequency and severity of study vaccine-related serious adverse events (SAEs), and all SAEs in pregnant women from study vaccination through 6 months postpartum, description and comparison between those receiving BOOSTRIX and Td.
- Safety in pregnant women and fetuses/infants: Frequency and severity of adverse events specific to pregnancy, in pregnant women and their infants (as delineated in Section 9.2.4), description and comparison between those receiving BOOSTRIX and Td.
- Tolerability in pregnant women: Frequency and severity of solicited injection site and systemic reactogenicity events from study vaccination until 7 days following vaccination (Day 8).
- Tolerability in pregnant women: Frequency and severity of all unsolicited non-seriousAEs from day of study vaccination to Day 31, description and comparison between those receiving BOOSTRIX and Td.

- Safety in the infants: Frequency and severity of study vaccine-related serious adverse events, and all SAEs in infants from birth through 6 months of age, description and comparison between infants born to women vaccinated with BOOSTRIX and Td.

Immunogenicity:

- Infant humoral immunity: Geometric Mean Concentration (GMC) of serum IgG antibodies to PT as measured by Enzyme-Linked Immunosorbent Assay (ELISA) at birth between infants born to women vaccinated with BOOSTRIX and Td.

3.2.2. Secondary

Immunogenicity:

- Maternal humoral immunity: GMC of serum IgG antibodies to Tdap vaccine antigens (PT (Pertussis Toxin), FHA (Filamentous Hemagglutinin), PRN (Pertactin), tetanus, diphtheria) as measured by ELISA in pregnant women one month after receipt of BOOSTRIX or Td, at the time of delivery, and 6 months after delivery.
- Infant humoral immunity: GMC of serum IgG antibodies to Tdap vaccine antigens (PT, FHA, PRN, tetanus, diphtheria) as measured by ELISA at birth and prior to receipt of first DTwP (approximately 6 weeks of age) among infants born to women vaccinated with BOOSTRIX compared to Td.
- Placental antibody transfer: The geometric mean ratio (GMR) of maternal and infant-specific Tdap-specific antibodies (PT, FHA, PRN, tetanus, diphtheria) as measured by ELISA at delivery after intrapartum receipt of BOOSTRIX versus Td.
- Interference with infant responses among infants whose mothers received intrapartum BOOSTRIX compared to Td: GMC of antibodies to DTwP vaccine antigens (PT, FHA, PRN, Fimbriae 2 (FIM2), Fimbriae 3 (FIM3), tetanus, diphtheria) as measured by ELISA one month after the first dose of DTwP (~10 weeks of age, ½ of infants), one month after the third dose of DTwP vaccine (~18 weeks of age, ½ of infants), and at 6 months of age.

3.2.3. Exploratory

Immunogenicity:

- Maternal immunogenicity cofactors for maternal and neonatal anti-PT antibody responses following intrapartum BOOSTRIX: Maternal age (18-29, 30-39 years old), parity (primiparous vs. multiparous), GA at time of vaccination (14-17, 18-21, 22-26 weeks), GA at time of delivery (28-32, 33-36, 37 or more weeks), and infant birthweight as potential independent associations with PT GMC.
- Maternal secretory immunity: GMC of breast milk IgG and IgA antibodies to Tdap vaccine antigens (PT, FHA, PRN, tetanus, diphtheria) as measured by ELISA in women at the time of delivery, at 6 weeks after deliver, at 10 weeks (in ½ of women) or 18 weeks (in ½ of women) after delivery, and 6 months after delivery after intrapartum receipt of BOOSTRIX versus Td.
- Maternal cytokine responses: Cytokines produced by peripheral blood cells stimulated with DTwP vaccine antigens, as measured by multiplex assays, in women before and one month after receiving Tdap. Cytokines measured will include: Interferon (IFN)-γ, Inerleukin (IL)-10, IL-12p70, IL-12/IL-23p40, IL-13, IL-15, IL-16, IL-17A, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, Tumor Necrosis Factor (TNF)-α, and TNF-β.
- Infant cytokine responses: Cytokines produced by infant peripheral blood cells stimulated with DTwP antigens from infants whose mothers received intrapartum BOOSTRIX versus Td, measured by multiplex assays before the first dose (or umbilical cord blood), one month after the first dose of DTwP (~10 weeks of age, ½ of subjects), one month after the last dose of study vaccine (approximately 18 weeks, ½ of subjects), and at 6 months of age. Cytokines measured will include: IFN-γ, IL-10, IL-12p70, IL-12/IL-23p40, IL-13, IL-15, IL-16, IL-17A, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, TNF-α, and TNF-β.

4. STUDY DESIGN

This is a phase II, single center, randomized, double-blind, active-controlled study in which 200 healthy pregnant women, ages 18 through 39 years, inclusive, will be randomly allocated in a 2:1 ratio to receive either Tdap (BOOSTRIX*)* or Td at 14 0/7 weeks through 26 6/7 weeks estimated GA. After signing an informed consent form (ICF), all subjects will undergo a review of current and past medical history, current and prior medication use, and recent vaccination history. A physical examination performed as part of routine antenatal care or a study-specific brief exam may be used to determine eligibility. For the fetuses of pregnant subjects, GA will be established by ultrasound, whenever possible, in combination with date of last menstrual period (LMP), when available, and fundal height.

Eligibility will be confirmed, and the subject will be enrolled in the study. Each subject will receive prenatal care consistent with national guidelines and with local standards as practiced by clinicians caring for pregnant women and delivering neonates in the study area. In Mali, this includes hemoglobin, sickle cell test, Rh group, HIV, syphilis, toxoplasmosis, and rubella serology and urine glucose and protein at the first presentation for antenatal care, urine albumin and glucose at each subsequent visit and ultrasound at 22 and 32 weeks gestation.

At the time of randomization to vaccine allocation, women will also be randomized to a schedule of postpartum visits for specimen collection. All women-infant pairs will have specimens collected at birth, at 6 weeks after delivery, and at 6 months after delivery. In addition, women-infant pairs will be allocated in a 1:1 ratio to have specimen collections at one month following the first dose of DTwP (approximately 10 weeks of age), or at one month following the third dose of DTwP (approximately 18 weeks of age). Thus, pregnant subjects will typically have 7 clinic visits (only 6 if delivery occurs before the first visit after vaccination, on Day 31): 3 antenatal visits (Screening Visit, Study Day 1 and Study Day 31, unless delivery occurs first), 1 visit at the time of delivery, and 3 postpartum visits – at 6 weeks, at 10 or 18 weeks postpartum, and a final visit 6 months postpartum. The maternal subject will also undergo 2 home visits after vaccination to assess her health status (Days 4 and 8).

Infants born to the pregnant subjects will have 4 study visits: at birth, at approximately 6 weeks of age (day of first DTwP), at approximately 10 or 18 weeks of age (one month after first or third dose of DTwP, respectively), and at 6 months of age.

Blood and colostrum/breast milk samples for immunologic assessments will be collected as described below:

- Pregnant subject: approximately 30 mL of blood will be collected on Study Day 1 (prior to vaccination), Study Day 31 (one month after vaccination), at delivery, and at 6 months postpartum in all women.
- Infants: up to 30 mL of umbilical cord blood will be collected at delivery and 5 mL peripheral blood will be collected at 6 weeks (prior to first dose of DTwP), either the 10-

week or 18-week (per randomized study schedule), and 6 months of age in all infants. If cord blood cannot be obtained, 2-5 mL of peripheral blood will be collected by venipuncture within 72 hours of birth, before discharge, when possible. Cord blood is preferred over venipuncture.

- At delivery, and at the 6-week, either the 10-week or 18-week (per randomized study schedule), and the 6-month postpartum visit, a 10-20mL sample of colostrum or breast milk will be collected. When possible, the first breast milk or colostrum collection will occur while the mother and baby are admitted for delivery and postpartum care. When not possible, it may be collected any time within the first 4 days of life.

Safety will be monitored from the time of signing of the ICF until trial termination.
For additional details on study procedures and evaluations and study schedule by type of visit or study visits/days, see Sections 7 and 8 and Appendix A.

5. STUDY ENROLLMENT AND WITHDRAWAL

Subject Inclusion and Exclusion Criteria must be confirmed by a study clinician licensed to make medical diagnoses.
No exemptions are granted on Subject Inclusion/Exclusion Criteria in DMID-sponsored studies. Questions about eligibility will be directed toward the DMID Medical Officer.

5.1. Subject Inclusion Criteria

Prospective subjects must meet all of the following inclusion criteria to be considered eligible for enrollment:

1. Healthy pregnant woman 18-39 years of age, inclusive.

2. Singleton fetus, with estimated gestational age of 14 0/7 through 26 6/7 weeks gestation, inclusive, on the day of study vaccination.

3. Provide written consent after the nature of the study has been explained according to local regulatory requirements and prior to any study procedures.[1]

4. In good health as determined by medical history, targeted physical examination[2,3], vital signs[4,5,6], and clinical judgment of the investigator.

[1] Prior to obtaining individual informed consent for each subject, the investigators will obtain community consent by discussing the trial with all the appropriate local groups, as necessary, to obtain permission to approach the subjects. Written, informed consent for participation in the trial will be obtained by the investigators from all individual subjects. The consent forms will be written in French, the official language of Mali, and will be translated into Bambara, the most prevalent of the local languages, and recorded on audiotape.
[2] if indicated based on medical history, to evaluate acute or currently ongoing chronic medical diagnoses or conditions that would affect the assessment of eligibility and safety of subjects. Chronic medical diagnoses or conditions being actively managed must be within acceptable limits in the last 180 days. Any prescription change that is due to change of health care provider, insurance company, etc., or that is done for financial reasons, as long as in the same class of medication, will not be considered a deviation of this inclusion criterion. Any change in prescription medication due to improvement of a disease outcome, as determined by the site principal investigator or appropriate sub-investigator, will not be considered a deviation of this inclusion criterion. Subjects may be on chronic or as needed (prn) medications if, in the opinion of the site principal investigator or appropriate sub-investigator, they pose no additional risk to subject safety or assessment of reactogenicity and immunogenicity and do not indicate a worsening of medical diagnosis or condition. Similarly, medication changes subsequent to enrollment and the study vaccination are acceptable provided the subject is asymptomatic, condition stable, and there is no additional risk to the subject or interference with the evaluation of responses to the study vaccination.
[3] Physical examination performed as part of routine antenatal care of a study-specific brief exam may be used to determine eligibility.
[4] Oral temperature less than 37.8°C.
[5] Pulse 55 to 100 bpm, inclusive.
[6] Systolic blood pressure 90 to 140 mm Hg, inclusive. Diastolic blood pressure 55 to 90 mm Hg, inclusive.

5. Ability to comprehend and comply with all study procedures, as determined by the investigator determining eligibility, and availability for follow-up.

6. Willing to allow study staff to gather pertinent medical information, including pregnancy outcome data and medical information about her infant.

5.2. Subject Exclusion Criteria

Prospective subjects must not meet any of the following exclusion criteria to be considered eligible for enrollment:

1. History of illness or an ongoing illness that, in the opinion of the investigator, may pose additional risk to the subject or her fetus if she participates in the study.

2. Infection requiring systemic antibiotics or antiviral treatment within the 7 days prior to study vaccination.

3. Fever (oral temperature ≥ 37.8°C/100.0°F) or other acute illness within 3 days prior to study vaccination.[7]

4. Known active neoplastic disease[8], anticancer chemotherapy, or radiation therapy (cytotoxic) within 3 years prior to study vaccination.

5. History of any hematologic malignancy at any time.

6. A history of a serious adverse event following previous immunizations (e.g., Bell's Palsy, Guillain-Barre Syndrome, encephalopathy), or history of progressive neurologic disorders.

7. Known or suspected disease that impairs the immune system including known or suspected HIV infection or HIV-related disease.

8. Receipt of immunosuppressive therapy.[9]

9. Known hepatitis B or hepatitis C infection, by history or medical record.

[7] An acute illness which is nearly resolved with only minor residual symptoms remaining is allowable if, in the opinion of the site principal investigator or appropriate sub-investigator, the residual symptoms will not interfere with the ability to assess safety parameters as required by the protocol.
[8] excluding non-melanoma skin cancer.
[9] including long-term use of glucocorticoids: oral, inhaled, intranasal or parenteral prednisone ≥ 20 mg/day or equivalent for more than 2 weeks within the 30 days prior to enrollment. Use of topical corticosteroids is allowed.

10. Behavioral or cognitive impairment or psychiatric disease[10] that, in the opinion of the investigator, may interfere with the subject's ability to participate in the trial.

11. Have a history of alcohol or drug abuse within 5 years prior to study vaccination.[11]

12. Known hypersensitivity or allergy to any component of the study vaccine (formaldehyde, alum).

13. History of severe allergic reaction (e.g., anaphylaxis) after a previous dose of BOOSTRIX or any other vaccine directed against tetanus, diphtheria, or pertussis.

14. Receipt or planned receipt of any live licensed vaccine within 30 days before or after vaccination or any inactivated licensed vaccine within 14 days before or after vaccination.

15. Receipt of immunoglobulin (except RhoGAM, which is allowed) or other blood products within 90 days prior to study vaccination.

16. Receipt of an experimental agent or device within 30 days prior to vaccination, or the expected receipt of an experimental agent[12] (other than BOOSTRIX) during this trial-reporting period.

17. High risk for serious obstetrical complication (refer to ACOG Practice Bulletins for definitions, as necessary).[13]

18. Pregnant with a fetus with a known or suspected major congenital[14] anomaly or genetic abnormality.

19. Study personnel or immediate family members (brother, sister, child, parent) or the spouse of study personnel.

[10] includes hospitalization for psychiatric illness, suicide attempt, or confinement for danger to self or others within 10 years prior to study vaccination.
[11] that is believed by the site investigator to potentially interfere with the subject's ability to participate in the study.
[12] Experimental agents include vaccines, drugs, biologics, devices, blood products, and medications. Subjects who have received a licensed product, as a subject in a clinical trial, within 30 days prior to vaccination or who are expecting to enroll in such a trial during the study period will also be excluded. Observational studies, surveys, and other studies that do not involve experimental agents or devices are allowed.
[13] including the following: (a)gestational hypertension (well controlled history of essential or gestational hypertension, as evidenced by normal BPs as defined above, is allowed), (b)gestational diabetes not controlled by diet and exercise (the use of insulin or glyburide to control gDM, at the time of enrollment, is exclusionary), (c)current pre-eclampsia or eclampsia, (d)known current multiple gestation, (e)history of preterm delivery before EGA 35 weeks 0 days or current preterm labor, and/or (f)known intrauterine fetal growth restriction (defined as ultrasound confirmation of an estimated fetal weight that is less than the 10th percentile for gestational age).
[14] Congenital anomalies and definitions of major congenital anomalies are discussed in detail in Section 9.1 under Assessment of Safety.

5.3. Treatment Assignment Procedures

5.3.1. Randomization Procedures

Per International Council for Harmonisation (ICH) guideline E6: Good Clinical Practice (GCP), screening records will be kept at each participating VTEU site to document the reason why an individual was screened, but failed trial entry criteria. The reasons why individuals failed screening will be recorded in the Data Coordinating Center's (DCC) Advantage eClinical[SM] (Electronic Data Capture System).

Once consented and upon entry of demographic data and confirmation of eligibility for this trial, the subject will be enrolled. Pregnant subjects will be randomly allocated to 1 of 2 treatment arms; 133 will be assigned to receive BOOSTRIX and 67 will be assigned to receive Td. No subject will receive more than one vaccination as part of enrollment in this study. All doses will be administered intramuscularly in the arm chosen by the subject.

At the time of enrollment subjects will also be assigned a study schedule. Women will be randomized in a 1:1 ratio for the third specimen collection after delivery/birth for mother/infant pairs at either one month following the first dose of DTwP (approximately 10 weeks of age) or one month following the third dose of DTwP (approximately 18 weeks of age).

When internet access allows, enrollment and randomization of subjects will be done online using the enrollment module of Advantage eClinical. A back-up randomization process will be in place in the event internet access is unavailable. The randomization schemes for assignment to treatment arm and assignment to study schedule will be prepared by statisticians at the DCC and included in the enrollment module for this trial. Advantage eClinical will assign each subject to a treatment arm and study schedule after the demographic and eligibility data have been entered into the system. Instructions for use of the enrollment module are included in the Advantage eClinical User's Guide. A designated individual or individuals at each participating VTEU site will be provided with a treatment code list for emergency unblinding purposes, which will be kept in a secure place.

Subjects who sign the informed consent form and are randomized but do not receive study vaccine may be replaced. Subjects who sign the informed consent form and are randomized and vaccinated, and subsequently withdraw or are withdrawn or terminated from this trial or are lost to follow-up, will not be replaced.

The randomization scheme for this trial is presented in Table 1.

Table 1: Treatment Arms and Study Vaccine to be Administered

	Treatment Arm		
Subject Group	**BOOSTRIX**	**Td**	**Total**
Pregnant women	133	67	200

5.3.2. Masking Procedures

This is a double-blind study. Pregnant subjects, investigators, study personnel performing any study-related assessments following study vaccine administration, and laboratory personnel performing antibody assays will be blinded to treatment assignments.

The randomization scheme will be generated by the DCC and provided to unblinded study personnel (i.e., research pharmacists or other trained/delegated personnel performing study vaccination preparations and unblinded study vaccine administrators) at each participating VTEU site or subcontracted site.

The unblinded study vaccine administrator is a study personnel member credentialed to administer vaccines and may also participate in dose preparation but will not be involved in study-related assessments or have subject contact for data collection following study vaccine administration, for pregnant women or their infants.

Data and Safety Monitoring Board (DSMB) may receive data in aggregate and presented by treatment arm (pregnant women, infants). The DSMB may also be provided with expected and observed rates of the solicited AEs in an unblinded fashion and may request the treatment assignment be unblinded for an individual subject if required for safety assessment. In the open session the DSMB may review data by subject group (pregnant women, infants) with all summaries for pregnant women and infants presented in aggregate over the treatment arms. In the closed session the DSMB may review unblinded data for the pregnant women and infant subject groups.

5.3.3. Reasons for Withdrawal and Discontinuation of Study Product Administration

Subjects may voluntarily withdraw their consent for study participation at any time without penalty or loss of benefits to which they are otherwise entitled.

An investigator may also withdraw a subject from receiving the study product for any reason. Follow-up safety evaluations will be conducted, if the subject agrees. If a subject withdraws or is withdrawn prior to completion of the study, the reason for this decision must be recorded in the data collection forms.

The reasons, might include, but are not limited to the following:

- Subject no longer meets eligibility criteria
- Subject meets individual halting criteria (reference to Section 9.5)
- Subject becomes noncompliant
- Medical disease or condition, or new clinical finding(s) for which continued participation, in the opinion of the investigator might compromise the safety of the subject, interfere with the subject's successful completion of this study, or interfere with the evaluation of responses
- Subject lost to follow-up
- Determined by a physician's discretion to require additional therapy not indicated in the protocol to ensure subject's health and well-being (or treatment failure, if applicable)
- New information becomes available that makes further participation unsafe or unwarranted.
- Termination of this trial
- Subject withdrawal of consent

The investigator should be explicit regarding study follow-up (e.g. safety follow-up) that might be carried out despite the fact the subject will not receive further study product. If the subject consents, every attempt will be made to follow all AEs through resolution. The procedures that collect safety data for the purposes of research must be inclusive in the original informed consent or the investigator may seek subsequent informed consent using an IRB/IEC-approved consent form with the revised procedures.
The investigator will inform the subject that already collected data will be retained and analyzed even if the subject withdraws from this study.

5.3.4. Handling of Withdrawals and Discontinuation of Administration

The primary reason for withdrawal from this trial will be recorded on the Study Status data collection form. Subjects will be encouraged to complete the Early Termination Visit. The Early Termination Visit procedures are listed in Section 7.6.
Every attempt will be made to follow all adverse events, including solicited injection site and systemic reactions, unsolicited non-serious adverse events, serious adverse events, and pregnancy outcomes, ongoing at the time of early withdrawal, through resolution as per applicable collection times defined for the specific type of adverse event.
In the case of subjects who fail to appear for a safety follow-up assessment, extensive effort (i.e., three documented contact attempts via phone calls, e-mails, home visits, etc., made on separate occasions) will be made to locate or recall them, or at least to determine their health status. These efforts will be documented in the subject's records.

Subjects who withdraw, or are withdrawn or terminated from this trial, or are lost to follow-up after signing the informed consent form, randomization, and receipt of study vaccine will not be replaced. Subjects who withdraw, or are withdrawn or terminated from this trial, or are lost to follow-up after signing the informed consent form and randomization but before receipt of study vaccine may be replaced.

5.3.5. Subject Replacement

Subjects who sign the informed consent form and are randomized but do not receive study vaccine may be replaced. Subjects who sign the informed consent form and are randomized and vaccinated, and subsequently withdraw or are withdrawn or terminated from this study or are lost to follow-up, will not be replaced. The proposed sample size for the trial accounts for a 15% rate of subject loss.

5.3.6. Termination of Study

Although the sponsor has every intention of completing this trial, it reserves the right to terminate this trial at any time for clinical or administrative reasons. Reasons for termination include, but are not limited to, study closure due to DSMB review and recommendation and at the discretion of DMID.

If the study is prematurely terminated by the sponsor, any regulatory authority, or the investigator for any reason, the investigator will promptly inform the study subjects and assure appropriate therapy or follow-up for the subjects, as necessary. The investigator will provide a detailed written explanation of the termination to the IRB/IEC.

6. STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

6.1. Study Product Description

BOOSTRIX

BOOSTRIX (Tetanus Toxoid, Reduced Diphtheria Toxoid, and Acellular Pertussis Vaccine, Adsorbed) is a noninfectious, sterile, vaccine for intramuscular administration. It contains tetanus toxoid, diphtheria toxoid, and pertussis antigens (inactivated PT, formaldehyde-treated FHA, and PRN). The antigens are the same as those in INFANRIX, but BOOSTRIX is formulated with reduced quantities of these antigens.

Tetanus toxin is produced by growing *Clostridium tetani* in a modified Latham medium derived from bovine casein. The diphtheria toxin is produced by growing *Corynebacterium diphtheriae* in Fenton medium containing a bovine extract. The bovine materials used in these extracts are sourced from countries which the United States Department of Agriculture (USDA) has determined neither have nor are at risk of bovine spongiform encephalopathy (BSE). Both toxins are detoxified with formaldehyde, concentrated by ultrafiltration, and purified by precipitation, dialysis, and sterile filtration.

The acellular pertussis antigens (PT, FHA, and PRN) are isolated from *Bordetella pertussis* culture grown in modified Stainer-Scholte liquid medium. PT and FHA are isolated from the fermentation broth; PRN is extracted from the cells by heat treatment and flocculation. The antigens are purified in successive chromatographic and precipitation steps. PT is detoxified using glutaraldehyde and formaldehyde. FHA and PRN are treated with formaldehyde.

Tetanus and diphtheria toxoid potency is determined by measuring the amount of neutralizing antitoxin in previously immunized guinea pigs. The potency of the acellular pertussis components (inactivated PT, formaldehyde-treated FHA, and PRN) is determined by ELISA on sera from previously immunized mice.

BOOSTRIX is manufactured by GSK and is approved for use in the US in persons 10 years and old and by the WHO for persons 4 years and older. In Mali, the vaccine is not approved by the national drug authority.

Tetanus and Diphtheria Toxoids Adsorbed (Td) Control Vaccine

The Td control vaccine is prequalified by WHO and will be provided by the Ministry of Health in Mali, as per standard of care. It is indicated for the active immunization of adults and children 7 years of age and older against diphtheria and tetanus. Vaccine manufacturer details will be provided in the protocol-specific Manual of Procedures (MOP).

6.1.1. Acquisition

BOOSTRIX will be provided by GSK Biologicals. Upon request by DMID, BOOSTRIX will be shipped to the following address:

DMID Clinical Materials Services (CMS)
Fisher BioServices
20439 Seneca Meadows Parkway
Germantown, MD 20876
Phone: 240-477-1350
Fax: 240-477-1360
Email: DMID.CMS@ThermoFisher.com

Study vaccine (BOOSTRIX) will be provided through the DMID CMS to the each VTEU participating subcontracted VTEU site prior to the start of this trial upon request and with prior approval from DMID. Should the site principal investigator require additional BOOSTRIX during this trial, further instructions on requesting study vaccine will be provided in the protocol-specific MOP.
Td control vaccine will be provided by Malian Expanded Program on Immunization (EPI) as per standard recommendations for vaccination of pregnant women.

6.1.2. Formulation, Packaging, and Labeling

BOOSTRIX

Formulation

Each 0.5-mL dose of vaccine is formulated to contain 5 Lf of tetanus toxoid, 2.5 Lf of diphtheria toxoid, 8 mcg of inactivated PT, 8 mcg of FHA, and 2.5 mcg of PRN (69 kiloDalton outer membrane protein). Each 0.5-mL dose contains aluminum hydroxide as adjuvant (not more than 0.39 mg aluminum by assay), 4.4 mg of sodium chloride, ≤100 mcg of residual formaldehyde, and ≤100 mcg of polysorbate 80 (Tween 80).

Storage

The vaccine must be stored at 2°C to 8°C (35°F to 46°F). Do not freeze.

Packaging and Labeling

The vaccine is supplied as 0.5 mL single-dose prefilled syringes. The tip caps of the prefilled syringes contain natural rubber latex; the plungers are not made with natural rubber latex.
The study product will be labeled according to manufacturer specifications and include the statement “Caution: New Drug – Limited by Federal Law to Investigational Use.”

Td Control Vaccine

Formulation

The vaccine contains purified tetanus and diphtheria toxoids, with a reduced dose of the diphtheria component. One dose of 0.5 mL has a potency of less than 30 IU of diphtheria toxoid, and not less than 40 IU of tetanus toxoid. The toxoids are adsorbed onto at least 1.5 mg aluminum phosphate. Thimerosal 0.1 mg/ml is used as a preservative.

Storage
The vaccine should be protected from light and must be stored at 2°C to 8°C (36°F to 46°F). Do not freeze.
Packaging
The vaccine is supplied as 10-dose vials of 5 mL.
Multi-dose vials of Td control vaccine from which one or more doses of vaccine have been removed during an immunization session may be used in subsequent immunization sessions for up to a maximum of 4 weeks, provided that all of the following conditions are met:

- The expiry date has not passed;
- The vaccines are stored under appropriate cold chain conditions;
- The vaccine vial septum has not been submerged in water;
- Aseptic technique has been used to withdraw all doses;
- The vaccine vial monitor, if attached, has not reached the discard point.

6.1.3. Product Storage and Stability

The temperature of the storage unit will be recorded manually daily (excluding non-business days and holidays as applicable), continuously monitored during the duration of this trial per each participating VTEU site's standard operating procedures, and documentation will be maintained. If the temperature fluctuates outside of the required range, the affected study product(s) will be quarantined at the correct storage temperature and labeled as 'Do Not Use' until further notice. The research pharmacist or other study staff member designated to oversee the storage of the study product, will alert the site principal investigator and study coordinator if the temperature fluctuates outside of the required range. In the event the temperature fluctuates outside of the required range, including accidental freezing or disruption of the cold chain, the affected study product(s) will not be administered without review and specific instructions from DMID. The site principal investigator or responsible person will notify the protocol principal investigator and immediately contact the DMID Product Support Team at DMIDProductSupportTeam@niaid.nih.gov for further instructions before any additional study vaccinations are administered. Based on the information collected, DMID and/or the manufacturer will determine whether the affected study product(s) can be used. If it cannot be used, the site will receive specific instructions on how to return the affected study product(s) to the DMID CMS or destroy it on site. Additional instructions for quarantine will be provided in the protocol-specific MOP.

6.2. Dosage, Preparation and Administration of Study Intervention/Investigational Product

See the Prescribing Information and protocol-specific MOP for detailed information on the preparation, labeling, storage, and administration of study vaccine for each treatment arm. Study

vaccine preparation will be performed by the participating VTEU site's (or subcontracted site's) research pharmacist or designee on the same day of study vaccine administration.
Visually inspect the study vaccines upon receipt and prior to use. If the study product(s) appear(s) to have been damaged, contaminated or discolored, contain visible particulate matter, or if there are any concerns regarding its integrity, do NOT use the affected study product(s). The affected study product(s) must be quarantined at 2°C to 8°C (35.6°F to 46.4°F) and labeled as 'Do Not Use' (until further notice). The site principal investigator or responsible person should immediately contact the DMID Product Support Team at DMIDProductSupportTeam@niaid.nih.gov and DMID Clinical Project Manager for further instructions before any additional study vaccinations are administered. Based on the information collected, DMID and/or the manufacturer will determine whether the affected study product(s) can be used. If it cannot be used, the site will receive specific instructions on how to return the affected study product(s) to the DMID CMS or destroy it on site. If the study vaccines are unusable, study personnel will use another from the study supply. Replacement study vaccine (BOOSTRIX only) may be requested by contacting DMID. Additional instructions for quarantine and DMID contact information are provided in the protocol-specific MOP.

6.2.1. BOOSTRIX

Dosage

BOOSTRIX will be administered intramuscularly as a single 0.5 mL dose.

Preparation

Visually inspect the BOOSTRIX vaccine upon receipt and prior to use. If the study product(s) appear(s) to have been damaged, contaminated or discolored, contain visible particulate matter, or if there are any concerns regarding its integrity, do NOT use the affected study product(s). The affected study product(s) must be quarantined at 2°C to 8°C (35.6°F to 46.4°F) and labeled as 'Do Not Use' (until further notice). The site principal investigator or responsible person should immediately contact the DMID Product Support Team at DMIDProductSupportTeam@niaid.nih.gov and DMID Clinical Project Manager for further instructions before any additional study vaccinations are administered.
No study vaccine preparation is required for the BOOSTRIX vaccine as it is contained in a prefilled syringe and the entire contents will be administered to subjects' pretreatment assignment. Prior to administration, shake vigorously to obtain a homogeneous, turbid, white suspension. Ensure that the plunger rod is firmly attached to the rubber stopper by turning the plunger clockwise until slight resistance is felt. Do not over tighten. Remove syringe LUER Tip-cap and needle cap. Attach needle by pressing and twisting in a clockwise rotation until secured to the syringe.

6.2.2. Td Control Vaccine

Dosage

Td control vaccine will be administered intramuscularly as a single 0.5 mL dose.

Preparation

The Td control vaccine vial should be shaken before use to homogenize the suspension. The Td control vaccine will be prepared by withdrawing sufficient volume of Td from multidose vial to administer a 0.5 mL dose. Further details on the handling of the multi-dose vials will be described in the protocol-specific MOP.

6.2.3. Administration

Study vaccine will be administered as a single 0.5 mL intramuscular (IM) injection into the deltoid muscle of the subject's preferred arm. The vaccine must NOT be administered intravenously, subcutaneously, or intradermally. The protocol-specific MOP contains additional information on how to administer IM injections.

Each dose of study vaccine will be administered as soon as possible, but no longer than 30 minutes after preparation. The prepared syringe must remain at room temperature until administered.

6.3. Modification of Study Intervention/Investigational Product for a Subject

There will be no dose modifications. All subjects will receive a single 0.5 mL dose of BOOSTRIX or Td control vaccine. For pregnant women, the dose will be administered between 14 0/7 weeks gestation and 26 6/7 weeks gestation.

6.4. Accountability Procedures for the Study Intervention/Investigational Product(s)

After receipt of the study vaccine, the site principal investigator is responsible for its distribution and disposition and has ultimate responsibility for study vaccine accountability. The site principal investigator may delegate distribution and disposition authority to each participating VTEU site's (or subcontracted site's) research pharmacist or designee. Each participating VTEU site's research pharmacist or other qualified delegated research staff member will be responsible for maintaining complete records and documentation of study vaccine receipt, accountability, dispensing, temperature and storage conditions, and final disposition of the study vaccine. All study vaccine, whether administered or not, will be documented on the appropriate study product accountability record or dispensing log. The sponsor's monitoring staff will verify each participating VTEU site's study product accountability records and dispensing logs per the site monitoring plan.

Used and unused study vaccine will be retained until monitored and released for disposition as applicable. This may occur on an ongoing basis for used study vaccine. Final disposition of the unused study vaccine will be determined by DMID and communicated to the subcontracted sites by the DMID Clinical Project Manager.

6.5. Assessment of Subject Compliance with Study Intervention/Investigational Product/Investigational Device

Subject compliance is not anticipated to be an issue. The study vaccine is given as one dose at a single visit according to treatment assignment, administered by an unblinded study vaccine administrator via IM injection. Administration will be documented on the appropriate data collection form and entered into the electronic Case Report Form (eCRF).

6.6. Concomitant Medications/Treatments

Subject receipt of any medications, therapies, or vaccines will be recorded on the appropriate data collection form. Concomitant medications will include all current medications and medications taken within the 90 days prior to signing the informed consent form until discharge after delivery for pregnant women. For infants, concomitant medications will be recorded from birth until the day of life 180 visit.

Medications/vaccinations reported in the eCRF are limited to those taken within 30 days prior to study vaccination through discharge after delivery for pregnant women and the day of life 180 visit for infants. Participants cannot plan to receive a live vaccine within 30 days following study vaccination, nor should they plan to receive an inactivated vaccine within 14 days following study vaccination. Prescription medications, vaccinations, over-the-counter drugs, herbals, vitamins, and supplements will be recorded. Use of any new post-study vaccination medication that is not considered standard of care for conditions associated with pregnancy should prompt evaluation for the presence of a new diagnosis or condition.

Medications that might interfere with the evaluation of the investigational product should be avoided unless necessary. Medications in this category include the prohibited medications per the Subject Exclusion Criteria (see Section 5.2). In addition, the site principal investigator or sub-investigator may identify other medications that should not be used due to a risk to subject safety or assessment of reactogenicity and immunogenicity.

7. STUDY SCHEDULE

Complete study schedule details listed by type of visit are described below. Refer also to Sections 4, 5, and 8 and Appendix A.

7.1. Recruitment

We will enroll 200 pregnant women in their first and second trimesters, 18 to 39 years old, inclusive. The infants of the pregnant subjects will be followed from birth to 6 months of age. Subjects must be in good health and meet all eligibility criteria. They will be enrolled at a referral health center of Commune I with prenatal and labor/delivery capabilities in Bamako, Mali. The target population will reflect the community at large. Estimated time to complete enrollment in this study is approximately 2 months of startup activities and 6 months of enrollment from first to last enrollee joining. Means of recruitment may include flyers, word of mouth, and discussions about the study in the clinics, community, and health care clinic outreach. The Malian research team may also use group meetings to explain the nature of the study to prospective subjects, prior to engaging in the individual informed consent process. The local IRB will approve all materials prior to use.

Subject Inclusion and Exclusion Criteria will be assessed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572. This clinician will either be the site principal investigator or be delegated by the site principal investigator to perform this role.

No exemptions are granted on Subject Inclusion/Exclusion Criteria in DMID-sponsored studies. Questions about eligibility should be directed toward the DMID Medical Officer.

7.2. Screening

Screening Visit, Day -30 to Day 1: Clinic Visit, Pregnant Women (Visit 00 or V00)

Potential subjects will be screened for eligibility within 30 days prior to the administration of the study vaccination. The following activities will be performed:

- If not performed at the time of recruitment, subjects will be provided with a description of the study (purpose and study procedures) and asked to read and sign the informed consent form. The informed consent form will be signed prior to performing any study procedures, including any screening procedures.
- Eligibility criteria will be reviewed with prospective subjects.
- Complete medical history, including vaccination history, will be obtained by chart review and supplemented by interview of the subject to evaluate for eligibility.
- All concomitant medications and vaccinations, as determined by chart review and subject interview, taken within 90 days prior to signing the informed consent form, will be

reviewed with subjects. Medications/vaccinations reported in the eCRF are limited to those taken within 30 days prior to study vaccination.

- Obtain vital signs (oral temperature, pulse, blood pressure), height, and weight. Subjects will be asked not to eat or drink anything hot or cold, or smoke within 10 minutes prior to taking an oral temperature.
- A targeted physical examination may be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator, if indicated based on review of complete medical history. For pregnant women, an examination performed by her own obstetrical clinician may also provide data when a targeted exam is called for.
- An ultrasound will be performed to date the pregnancy.
- Subjects will be given a research identification card that includes a photograph.

7.3. Enrollment/Baseline

Enrollment and Vaccination Visit, Day 1: Clinic Visit, Pregnant Women (Visit 01 or V01)

- Reconfirm subject's willingness to participate prior to performing any study procedures, including administration of the study vaccination.
- Obtain informed consent (if screening and enrollment visits have been combined).
- Review eligibility criteria with subjects prior to the study vaccination to ensure eligibility.
- Complete medical history and any updates obtained by interview and/or review of medical charts of subjects since the screening visit will be reviewed with subjects prior to the study vaccination to ensure eligibility.
- All concomitant medications and vaccinations will be reviewed with subjects prior to the study vaccination for accuracy and completeness. Any new concomitant medications/vaccinations taken since the screening visit will be reviewed with subjects and used in the assessment for eligibility, prior to the study vaccination. Medications reported in the eCRF are limited to those taken within 30 days prior to the study vaccination. Subjects will be reminded they should not plan to receive any live or inactivated vaccine within 30 or 14 days, respectively, after study vaccination.
- Vital signs, namely oral temperature, pulse, and blood pressure, will be obtained prior to the study vaccination. Vital signs assessed on Day 1 prior to the study vaccination will be considered as baseline. Subjects will be asked not to eat or drink anything hot or cold, or smoke within 10 minutes prior to taking an oral temperature.

- Height and weight will be obtained, if not done on screening visit.
- A targeted physical examination may be performed prior to the study vaccination by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator, if indicated based on review of complete medical history and any updates obtained by interview of subjects or medical chart review since the screening visit. For pregnant women, an examination performed by her own obstetrical clinician may also provide data when a targeted exam is called for.
- Approximately 30 mL of venous blood will be collected immediately prior to the study vaccination for baseline serology and cytokine assays.
- Subjects will be enrolled in Advantage eClinical and assigned to treatment arm (BOOSTRIX or Td) and post-partum study schedule.
- Pre-administration solicited event assessments will be performed prior to study vaccination to establish baseline. Subjects will then receive a single dose of study vaccine via IM injection into the deltoid muscle of the subject preferred arm. The site of injection (right or left arm) and time of administration will be recorded on the appropriate data collection form. Subjects will be observed in the clinic for at least 30 minutes after the study vaccination. The study vaccination site will be examined, post-administration solicited event assessments will be performed, and any AE/SAEs will be assessed and recorded on the appropriate data collection form prior to discharge from the clinic.
- Subjects' home addresses will be recorded so study personnel may complete follow-up home visits.
- Subjects will be instructed to notify the study center if they develop any severe reactions after the study vaccination. If the site principal investigator or appropriate sub-investigator deems the reaction severe enough, s/he will give further instructions on the proper course of action, including a return to the clinic for immediate evaluation if appropriate.
- It is possible the screening and enrollment visits may occur at the same time.

7.4. Follow-up

Follow-up visits are scheduled in reference to the study vaccination date (Day 1) as indicated for each visit window. The types of follow-up visits are described below as home visits, and clinic visits. See Appendix A for specific study visits/days and study procedures and evaluations to be conducted at each follow-up visit. At any time during the follow-up period of pregnant women, study personnel may review her medical chart to gather information about the current pregnancy that is relevant to the conduct of this study. If a woman experiences a stillbirth, all specimen

collection will stop, however the mother will be followed for safety until 6 months after the stillbirth.

7.4.1. Clinic or Home Visits

These visits will occur in person at the clinic or may be conducted by trained field officers at the participants' homes.

7.4.1.1. Post Study Vaccination Follow-up Visit, Day 4 (±1 d): Home Visit, Pregnant Women (Visit 02 or V02)

- This visit will take place in the clinic or alternatively by trained field workers (FWs).
- Study personnel will obtain interim medical history, including an assessment for new medical conditions by interview of subjects and note any changes since the previous visit.
- Study personnel will review solicited AEs with subjects and assess and record all solicited data, AE/SAEs, unsolicited AEs, and concomitant medications/vaccinations on the appropriate data collection forms. FWs will be trained on the assessment of local and systemic reactogenicity. If the subject reports an AE that is either not included among the solicited AEs or is a grade 3 solicited AE, then the FW will contact a study clinician. The study clinician will either visit the participant at home or the participant will be referred to the study health center based on the FW's assessment.

7.4.1.2. Post Study Vaccination Follow-up Visit, Day 8 (+3 d): Home Visit, Pregnant Women (Visit 03 or V03)

- This visit will take place in the clinic or alternatively by trained FWs.
- Study personnel will obtain interim medical history, including an assessment for new medical conditions, by interview of subjects and note any changes since the previous visit.
- Study personnel will review solicited AEs with subjects and assess and record all solicited data, AE/SAEs, unsolicited AEs, and concomitant medications/vaccinations on the appropriate data collection forms. FWs will be trained on the assessment of local and systemic reactogenicity. If the subject reports an AE that is either not included among the solicited AEs or is a grade 3 solicited AE, then the FW will contact a study clinician. The study clinician will either visit the participant at home or the participant will be referred to the study health center based on the FW's assessment.

7.4.2. Clinic Visits

7.4.2.1. Immunology and Safety Follow-up Visit, 30 Days Post Study Vaccination Visit, Day 31 (±4 d): Clinic Visit, Pregnant Women (Visit 04 or V04)

- Study personnel will reconfirm subject's willingness to participate prior to performing any study procedures. They will also obtain an interim medical history, including an assessment for new medical conditions by interview of subjects and note any changes since the previous clinic visit, home visit, or phone call. Study personnel will confirm that all eligibility criteria continue to be met.
- Study personnel will review the solicited AEs with subjects, if not already reviewed and recorded during previous visits, and assess and record all new AE/unsolicited AEs/SAEs and concomitant medications/vaccinations on the appropriate data collection forms.
- Vital signs, namely oral temperature, pulse, and blood pressure will be obtained. Subjects will be asked not to eat or drink anything hot or cold, or smoke within 10 minutes prior to taking an oral temperature.
- A targeted physical examination may be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator, if indicated based on review of interim medical history. An examination performed by subject's own obstetrical clinician may also provide data when a targeted exam is called for.
- The study vaccination site will be examined.
- Approximately 30 mL of venous blood will be collected for post-vaccination serology and cytokine assays.

7.4.2.2. Subsequent Follow-up Visits

7.4.2.2.1. Labor and Delivery Visit, Birth (+3 d): Clinic Visit, Pregnant Women and Infants (Visit 05 or V05)

- Obtain interim medical history, including an assessment for new medical conditions by interview of subjects and note any changes since the previous clinic visit. Study personnel will solicit and record all new SAEs (as well as AEs (solicited and unsolicited)) and concomitant medications/vaccinations if less than 30 days post vaccination) on the appropriate data collection form.
- Vital signs, namely oral temperature, pulse, and blood pressure, will be obtained. Subjects will be asked not to eat or drink anything hot or cold, or smoke within 10 minutes prior to taking an oral temperature. Vital signs recorded for the mother as part of

her delivery record may be used in lieu of study personnel performing vital sign measurements.

- A targeted physical examination may be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator, if indicated based on review of interim medical history. An examination performed by the subject's own obstetrical clinician may also provide data when a targeted exam is called for.
- On admission for parturition, 30 mL of blood will be drawn from the pregnant woman for serology. When possible, this blood will be drawn at the time of admission labs.
- After delivery, study personnel will review the study procedures required for the infant going forward, add the infant's name to the informed consent form, and ask the woman to acknowledge her infant's participation as well as her continuing participation by signing and dating the form again.
- If possible, 20- 30 mL of cord blood will be collected at the time of delivery. If cord blood cannot be obtained, 2-5 mL of peripheral blood will be collected by venipuncture within 72 hours of birth, before discharge, when possible. Cord blood is preferred over venipuncture.
- Study personnel will record birth outcomes on the appropriate data collection form.
- Colostrum or breast milk, 10-20 mL, will be collected from the mother for serology. In Mali, women are often discharged on the same day as delivery and lactation is not always established. Thus, the first collection may occur on any of the first 4 days of life of the infant.
- The newborn infant will be enrolled into the study at the time of birth. A targeted physical examination may be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator, if indicated based on review of interim medical history. A physical exam performed by the infant's doctor may also provide information, if required, for documentation.
- Axillary temperature, heart rate, respiratory rate, head circumference, length, and weight will be collected from the medical chart of the infant. If not performed as part of routine care, these measurements, except weight may be taken by qualified members of the research team within 7 days of life. Weights will only be recorded when obtained at birth.
- For infants, study personnel will solicit and record on the appropriate data collection form infant AEs, SAEs, and concomitant medications/vaccinations until the day of life 180

visit. Only medically attended AEs will be recorded. Events associated with routine newborn care and health maintenance visits will not be recorded.

- To collect cord blood and to perform physical examination of the mother and newborn at delivery, an on-call team comprised of a study physician and nurse will be posted at the recruitment site close to the delivery room. This team will collaborate with the local on-call team. The team will refer to the study ID or consent form to confirm the identification of the participant.

7.4.2.2.2. 6 Weeks Postpartum Safety Follow-up Visit, Birth + Day 42 (±5 d): Clinic Visit, Postpartum Women and Infants (Visit 06 or V06)

- Obtain interim medical history of mother and infant, including an assessment for new medical conditions by interview of subjects and note any changes since the previous clinic visit. Study personnel will solicit and record all new congenital anomalies and SAEs on the appropriate data collection form.
- Study personnel will record infant concomitant medications on the appropriate data collection form.
- A targeted physical examination may be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator, if indicated based on review of interim medical history. A targeted physical exam may be performed on the mother, infant, or both.
- 5 mL of infant venous blood will be collected for serology and cytokine assessments.
- 10-20 mL breast milk will be collected from the mother for antibody measurements.
- Study personnel will collect infant length, weight, and head circumference as well as birth outcomes on the appropriate data collection form.

7.4.2.2.3. 10 or 18 Weeks Postpartum Safety Follow-up Visit, Birth + Day 70 (±5 d) or Birth + Day 130 (±5 d): Clinic Visit, Postpartum Women and Infants (Visit 07 or V07)

- Obtain interim medical history of mother and infant, including an assessment for new medical conditions by interview of subjects and note any changes since the previous clinic visit. Study personnel will solicit and record all new congenital anomalies and SAEs on the appropriate data collection form.
- Study personnel will record infant concomitant medications on the appropriate data collection form.

- A targeted physical examination may be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator, if indicated based on review of interim medical history. A targeted physical exam may be performed on the mother, infant, or both.
- 5 mL of infant venous blood will be collected for serology and cytokine assessments prior to receipt of DTwP.
- 10-20 mL breast milk will be collected from the mother for antibody measurements.
- Study personnel will collect infant length, weight, and head circumference as well as birth outcomes on the appropriate data collection form.

7.5. Final Study Visit

6 months Postpartum Safety Follow-up Visit, Birth + Day 180 (±7 d): Clinic Visit, Postpartum Women and Infants (Visit 08 or V08)

- Obtain interim medical history of mother and infant. Study personnel will solicit and record all new congenital anomalies and SAEs on the appropriate data collection form.
- Study personnel will record infant concomitant medications on the appropriate data collection form.
- A targeted physical examination may be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator, if indicated based on review of interim medical history. A targeted physical exam may be performed on the mother, infant, or both.
- 5 mL of infant venous blood will be collected for serology assessments.
- Approximately 30 mL of venous blood will be collected from the mother for post-vaccination serology and cytokine assays.
- 10-20 mL breast milk will be collected from the mother for antibody measurements.
- Study personnel will collect infant length, weight, and head circumference as well as birth outcomes on the appropriate data collection form.
- Study personnel will discharge mother and infant from study.

7.6. Early Termination Visit (if needed)

The following activities will be performed at the early termination visit for subjects who withdraw, or are withdrawn or terminated from this study:

- Obtain interim medical history, including an assessment for new medical conditions by interview of subjects and review of medical charts, if applicable, and note any changes since the previous clinic visit or phone call.

- Obtain vital signs: heart rate, blood pressure, and temperature. Subjects will be asked not to eat or drink anything hot or cold, or smoke within 10 minutes prior to taking an oral temperature.
- Study personnel will collect infant length, weight, and head circumference as well as birth outcomes on the appropriate data collection form.
- Review solicited AEs (if within 8 days after the study vaccination or if after the 8-day period but the AE information had not previously been collected).
- All concomitant medications/vaccinations will be recorded on the appropriate data collection form (if within the period of collection of concomitant medications).
- Review adverse events (if within the collection period for AEs).
- Review serious adverse events, if within the collection period for SAEs.
- A targeted physical examination may be performed on the mother and/or infant by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator, if indicated based on review of interim medical history.
- Examine study vaccination site (if within 30 days after the study vaccination).
- Record birth outcomes, if applicable.

7.7. Unscheduled Visit (if needed)

Unscheduled visits may occur at any time during this study. Any of the following activities may be performed:

- Obtain interim medical history, including an assessment for new medical conditions by interview of subjects and review of medical charts, if applicable, and note any changes since the previous clinic visit or phone call.
- Obtain vital signs: heart rate, blood pressure, and temperature. Subjects will be asked not to eat or drink anything hot or cold, or smoke within 10 minutes prior to taking an oral temperature.
- Study personnel will collect infant length, weight, and head circumference as well as birth outcomes on the appropriate data collection form.
- Review solicited AEs (if within 8 days after the study vaccination or if after the 8-day period but the AEs had not previously been collected).
- Review concomitant medications/vaccinations (if within the collection period for concomitant medications).

- Review adverse events (if within the collection period for AEs).
- Review serious adverse events, if within the collection period for SAEs.
- A targeted physical examination may be performed on the mother and/or infant by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator, if indicated based on review of interim medical history.
- Examine study vaccination site (if within 30 days after the study vaccination).
- Record birth outcomes, if relevant.

8. STUDY PROCEDURES/EVALUATIONS

8.1. Clinical Evaluations

Complete medical history to determine eligibility will be obtained by review of the medical record supplemented by interview for pregnant women. The information will be obtained at the screening visit and will be updated on Day 1, prior to study vaccination. Subjects and records, when available, will be queried regarding a history of significant medical disorders of the head, eyes, ears, nose, throat, mouth, cardiovascular system, lungs, gastrointestinal tract, liver, pancreas, kidney, urologic system, nervous system, blood, lymph nodes, endocrine system, musculoskeletal system, skin, and genital/reproductive tract. A history of any allergies, cancer, immunodeficiency, psychiatric illness, substance abuse, and autoimmune disease will be solicited. At follow-up visits, an interim medical history will be obtained by interview of the subjects and review of the medical chart of pregnant women when needed, noting any changes since the previous clinic visit or phone call. The interim medical history will include an assessment for new medical conditions and symptoms.

Medications history (concomitant medications) will include a review of all current medications through the intervals described in Section 6.6 (Concomitant Medications/Treatments). Prescription and over-the-counter drugs will be included as well as vaccines, herbals, vitamins, and supplements. Use of any new medication should prompt evaluation for the presence of a new diagnosis, unless the medication is an expected medication in the care of a pregnant woman. Examples of medications used in the care of pregnant women that would not prompt evaluation for a new diagnosis would be prenatal vitamins for all women and RhoGAM for Rh(D)-negative women. Assessment of eligibility will include a review of all permitted and prohibited medications per the Subject Inclusion and Exclusion Criteria. In addition, the site principal investigator or appropriate sub-investigator may identify other medications that should not be used due to a risk to subject safety or assessment of reactogenicity and immunogenicity.

At the screening visit, a targeted physical examination, if indicated based on subject's complete medical history, may be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. For pregnant women, information from a physical exam performed by the subject's clinician may also be used. On Day 1, prior to the vaccination, and as needed at follow-up visits after vaccination, a targeted physical examination may be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator, if indicated based on the subject's interim medical history. Targeted physical examinations are performed to investigate further symptoms reported by the subject that may have relevance to the study.

Vital signs (oral temperature, pulse, and blood pressure) will be collected on Day 1 prior to the administration of the study treatment and on subsequent visits as denoted in the study schedule.

Vital signs assessed on Day 1 prior to study vaccination will be considered as baseline. Subjects will be asked to not eat or drink anything hot or cold, or smoke within the 10 minutes prior to taking an oral temperature.

Solicited event assessments will include an assessment of solicited adverse events occurring from the time of vaccination (Day 1) through Day 8. These assessments include injection site (local) reactions of ecchymosis (bruising), erythema (redness), induration (hardness)/edema (swelling), pain, and tenderness as well as systemic reactions including fever, feverishness (chills/shivering/sweating), fatigue (tiredness), malaise (general unwell feeling), myalgia (body aches/muscular pain exclusive of the injection site), arthralgia (joint pain exclusive of the injection site), headache, nausea, and allergic reaction (pruritus/urticaria). Pre-administration solicited systemic event assessments will be performed prior to study vaccination to establish baseline, then the study vaccination will be given.

Subjects will be observed in the clinic for at least 30 minutes after the study vaccination. The study vaccination site will be examined, post-administration solicited event assessments will be performed, and any AE/SAEs will be assessed and recorded on the appropriate data collection form prior to discharge from the clinic. A field worker will visit all subjects on Days 4 and 8 to collect information on solicited adverse events and any AE/SAEs. The study vaccination site will also be examined on Day 31.

8.2. Laboratory Evaluations

8.2.1. Clinical Laboratory Evaluations

Clinical screening laboratory parameters are not included in the routine evaluation for eligibility. If the medical record or history reveals laboratory abnormalities that might preclude eligibility or affect subject safety or the investigator's ability to assess the safety, tolerability, or immunogenicity of the vaccine, the investigator may use that information to determine eligibility.

8.2.2. Special Assays or Procedures

Immunogenicity

Laboratory assays to determine serum antibody concentrations to Tdap antigens will be performed at VTEU site laboratories using qualified ELISAs. Cytokines produced by peripheral blood cells from vaccinated mothers and infants stimulated with DTwP antigens will be measured using commercial and validated multiplex technology. Subjects who withdraw early will have assays run on available samples.

Breast Milk and Colostrum: The exploratory measurement of antibody to Tdap antigens in colostrum/breast milk will be performed at VTEU site laboratories using qualified ELISAs. These assays have not been validated for colostrum/ breast milk.

Laboratory personnel performing all assays will be blinded as to the allocation arm of pregnant subjects (Tdap vs. Td), study visit number, and whether the samples are from adult or infant subjects. The volume of venous blood and colostrum/breast milk to be collected for assays is presented in Table 2.

Table 2: Specimen Volumes (mL) for Pregnant Women and Infants

Study Visit Number	**V00**	**V01**	**V02**	**V03**	**V04**	**V05**	**V06**	**V07**	**V08**	**Total**
Study Day (pregnant women)	**Screen**	**D1**	**D4±1d**	**D8+3d**	**D31±4d**	**Birth+3d**	**Birth+D42±5d** *6 weeks post partum*	**Birth+D70±5d or D130±5d** *10 or 18 weeks post partum**	**Birth+ D180±7d** *6 months post partum*	
Study Day in relation to delivery	**pre**	**pre**	**pre**	**pre**	**pre**	**1**	**42**	**70-130**	**180**	
Study vaccination		X								
Pregnant women blood		30[&]	--	--	30	30	--	--	30	120 mL
Umbilical cord blood			--	--		20-30	--		--	20-30 mL
Infant blood			--	--		5[#]	5[%]	5[%]	5	15 mL (20 mL, max)[#]
Colostrum or breast milk			---	--		10-20	10-20	10-20	10-20	40-80 mL

[#]2-5 mL of infant blood will be taken by phlebotomy during the delivery admission only if cord blood cannot be obtained.
*One-half of woman-infant pairs will be randomized to the 10 week time point and one-half to the 18 week.
[&]Prior to receipt of tetanus-containing vaccine.
[%]Prior to receipt of DTwP.

8.2.3. Specimen Preparation, Handling, and Shipping

8.2.3.1. Instructions for Specimen Preparation, Handling, and Storage

Instructions for specimen preparation, handling, and storage are included in the protocol-specific MOP, as appropriate.

8.2.3.2. Specimen Shipment

Specimen shipment will occur at intervals during the course of this trial following all applicable International Air Transport Association (IATA) requirements and according to the specifics for storage temperature and documentation as detailed in the protocol-specific MOP, as appropriate. Further instructions for specimen shipment will be included in the protocol-specific MOP, as appropriate.

9. ASSESSMENT OF SAFETY

9.1. Specification of Safety Parameters

Safety will be assessed by the frequency and severity of the following:

1) Study vaccine-related Serious Adverse Events occurring from the time of the study vaccination through the visit 6 months postpartum (180 days of life for the infant visit) for pregnant subjects and infants. All SAEs during this timeframe will be assessed and documented.
2) Adverse Events related to pregnancy and the infant as delineated below in Section 9.2.4. For the pregnant woman/mother: pregnancy loss, vaginal bleeding (antepartum or postpartum), post-abortal endometritis/salpingitis, preterm rupture of membranes, preterm contractions/labor/delivery, poor fetal growth, hypertension/pre-eclampsia/eclampsia, chorioamnionitis, postpartum endometritis, gestational diabetes mellitus. For the infant: preterm birth, low birth weight, neonatal complications, and congenital anomalies.
3) Solicited Adverse Events – reactogenicity events occurring from the time of study vaccination through 7 days after study vaccination (Day 8) or until resolution.
    a. Injection site reactions including ecchymosis (bruising), erythema (redness), induration (hardness)/edema(swelling), pain, and tenderness.
    b. Systemic reactions including fever (days 4 and 8 only during home visits), feverishness (chills/shivering/sweating), fatigue (tiredness), malaise (general unwell feeling), myalgia (body aches/muscular pain exclusive of the injection site), arthralgia (joint pain exclusive of the injection site), headache, nausea, and allergic reaction (pruritus/urticaria).
4) Unsolicited Non-Serious Adverse Events – all non-serious unsolicited adverse events will be assessed and documented, and all study vaccine-related non-serious adverse events occurring from the time of study vaccination through approximately 30 days after study vaccination (the Day 31 visit). For pregnant women who deliver before Day 31, unsolicited adverse events will be recorded until delivery.
5) Major Congenital Anomalies
   Congenital Anomalies: In this study, we will report as SAEs all major congenital anomalies known prior to a mother/infant dyad completing the study. These outcomes must occur following maternal vaccination during gestation or the perinatal period (within the first 5 days after delivery) but the diagnosis may be confirmed anytime during participation. We define “major congenital anomaly” as “defects that are present at birth and that have surgical, medical, or serious cosmetic significance.” [33]

   The following list of problems are among the conditions reported in the US National Birth Defects registry and would be deemed as examples of major congenital anomalies:

anencephaly, spina bifida, encephalocele, anophthalmia, microphthalmia, cyanotic congenital heart diseases, cleft lip or palate, tracheoesophageal fistula, rectal stenosis, major limb deformities including shortening, gastroschisis, omphalocele, and diaphragmatic hernia. Some problems that are congenital anomalies but would be considered minor include most simple birthmarks, single transverse palmar creases, clinodactyly, umbilical hernias, post-axial polydactyly, ankyloglossia, and epicanthal folds.

Positional deformations, such as molding or overriding cranial sutures, metatarsus adductus, tibial torsion, and genu varum are not to be recorded as major congenital anomalies. Normal variants, such as minor anomalies of the pinna, supernumerary nipples, and penile torsion are also not major congenital anomalies. If major congenital anomalies are found prenatally, before enrollment, women will be excluded from participation. Many minor congenital anomalies will typically only be noted at or soon after birth.

9.2. Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

9.2.1. Adverse Events

Adverse Event:

ICH E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. FDA defines an AE as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study recipient presenting for medical care, or upon review by a study monitor.

AEs, including solicited local (injection site) and systemic (subjective and quantitative) reactions, will be captured on the appropriate data collection form and eCRF. Information to be collected for unsolicited non-serious AEs includes event description, date of onset, assessment of severity, relationship to study product and alternate etiology (assessed only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as an investigator), date of resolution, seriousness and outcome. FWs will be trained on the assessment of local and systemic reactogenicity. If the subject reports an AE that is either not included among the solicited AEs or is a grade 3 solicited AE, then the FW will contact a study clinician. The study

clinician will either visit the participant at home or the participant will be referred to the study health center based on the FW's assessment. AEs occurring during the trial collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

Any medical condition that is present at the time that the subject is screened will be considered as baseline and not reported as an AE. However, if the severity of any pre-existing medical condition increases, it should be recorded as an AE.

All AEs must be graded for severity and relationship to study product (see definitions). Adverse events characterized as intermittent require documentation of onset and duration of each episode. The start and stop date of each reported AE will be recorded on the appropriate data collection form and eCRF.

Severity of Event: AEs will be assessed by the investigator using a protocol-defined grading system (toxicity table included as an appendix). For events not included in the protocol-defined grading system, the following guidelines will be used to quantify severity:

- Mild (Grade 1): Events require minimal or no treatment and do not interfere with the subject's daily activities.
- Moderate (Grade 2): Events result in a low level of inconvenience or concern with therapeutic measures. Moderate events may cause some interference with functioning and daily activities.
- Severe (Grade 3): Events interrupt the subject's daily activities and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

Changes in the severity of an AE should be documented to allow an assessment of the duration of the event at each level of intensity to be performed. Adverse events characterized as intermittent require documentation of onset and duration of each episode.

Relationship to Study Products: The assessment of the relationship of an AE to the administration of study product is made only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as an investigator based on all available information at the time of the completion of the eCRF. Whether the AE is related or not, is not a factor in determining what is or is not reported in this trial. If there is any doubt as to whether a clinical observation is an AE, the event should be reported.

In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.
- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

9.2.2. Reactogenicity (for Vaccine Studies and Some Therapeutic Trials)

Reactogenicity events are AEs that are common and known to occur following administration of this type of study vaccine. The following Toxicity Grading Scales will be used to grade solicited local (injection site) and systemic (subjective and quantitative) reactions:

Table 3: Local (Injection Site) Reactogenicity Grading

Local (Injection Site) Reaction	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Pain	Does not interfere with daily activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with daily activity	Any use of narcotic pain reliever or prevents daily activity
Tenderness	Discomfort only to deep palpation	Discomfort to light palpation or movement	Significant discomfort to any touch or movement
Ecchymosis (Bruising)*	No interference with daily activity	Some interference with daily activity not requiring medical intervention	Prevents daily activity and requires medical intervention
Erythema (Redness)*	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity
Induration (Hardness)/Edema (Swelling)*	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity

Ecchymosis (bruising), erythema (redness), and induration (hardness)/edema (swelling) as analyzed by measurement will be graded as follows:

Table 4: Local (Injection Site) Reactogenicity Measurements

Local (Injection Site) Reaction	Small	Medium	Large
Ecchymosis (Bruising)*	1-50 mm	51-100 mm	> 100 mm
Erythema (Redness)*	1-50 mm	51-100 mm	> 100 mm
Induration (Hardness)/Edema (Swelling)*	1-50 mm	51-100 mm	> 100 mm

*Will not be used as halting criteria.

In addition to grading the measured local reactions at the greatest single diameter, the measurements will be recorded as continuous variables.

Table 5: Subjective Systemic Reactogenicity Grading

Systemic (Subjective)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Feverishness (Chills/Shivering/Sweating)	No interference with daily activity	Some interference with daily activity not requiring medical intervention	Prevents daily activity and requires medical intervention
Fatigue (Tiredness)	No interference with daily activity	Some interference with daily activity	Significant; prevents daily activity
Malaise (General Unwell Feeling)	No interference with daily activity	Some interference with daily activity not requiring medical intervention	Prevents daily activity and requires medical intervention
Myalgia (Body Aches/Muscular Pain)*	No interference with daily activity	Some interference with daily activity	Significant; prevents daily activity
Arthralgia (Joint Pain)*	No interference with daily activity	Some interference with daily activity not requiring medical intervention	Prevents daily activity and requires medical intervention
Headache	No interference with daily activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with daily activity	Significant; any use of narcotic pain reliever or prevents daily activity
Nausea	No interference with daily activity	Some interference with daily activity	Prevents daily activities
Allergic Reaction	Pruritus without rash	Localized urticaria OR requires oral therapy	Generalized urticaria; angioedema OR anaphylaxis OR requires epinephrine

*Not at injection site.

Oral temperature[#] will be graded as follows:

Table 6: Quantitative Systemic Reactogenicity Grading

Systemic (Quantitative)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Fever* - oral[†]	38.0 – 38.4°C 100.4 – 101.1°F	38.5 – 38.9°C 101.2 – 102.0°F	> 38.9°C ≥ 102.1°F

[#]Oral temperature assessed on Day 1 prior to study vaccination will be considered as baseline.
*A fever can be considered not related to the study product if an alternative etiology can be documented.
†Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.

9.2.3. Additional Adverse Event Severity Grading

Pulse and blood pressure[#] will be graded as follows:

Table 7: Pulse and Blood Pressure Severity Grading

Physiologic Parameter	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Bradycardia - beats per minute	50 – 54 or 45-50 if baseline <60 bpm	45 – 49 or 40-44 if baseline <60 bpm	< 45 or <40 if baseline <60 bpm
Tachycardia - beats per minute	101 – 115	116 – 130	> 130 or ventricular dysrhythmias
Hypotension (systolic) mm Hg	85-89	80-84	< 80
Hypertension (systolic) mm Hg	141-150	151-160	> 160
Hypertension (diastolic) mm Hg	91-95	96-109	> 109

[#]Pulse and blood pressure assessed on Day 1 prior to the study vaccination will be considered as baseline.

9.2.4. Grading of Specific Adverse Events in the Pregnant Women and their Infants

Table 8: Maternal and Infant Adverse Events

Maternal Adverse Events (Adverse Events during Pregnancy)				
Parameter	**Normal**	**Grade 1**	**Grade 2**	**Grade 3**
Pregnancy loss (Pregnancy does not result in a live birth) **Spontaneous abortion or miscarriage in the first or second trimester of gestation** **Fetal death at or after 20 weeks of gestation (stillbirth)**	None	N/A	N/A	Spontaneous abortion or miscarriage
Bleeding (vaginal) during pregnancy prior to the onset of labor	None	Spotting or bleeding less than menses	Bleeding like menses or heavier, no intervention indicated	Profuse bleeding with dizziness or orthostatic hypotension, transfusion indicated
Postpartum hemorrhage	EBL[c] ≤500 cc for vaginal delivery or ≤1000 cc after CS or reported as normal	EBL 501-1000 for vaginal delivery or 1001-1500 for CS or reported as slightly increased	EBL >1000 for vaginal delivery or >1500 for CS, with or without mild dizziness, no transfusion required	Hemorrhage at a level for which transfusion of packed cells or any amount of other blood components is indicated
Postabortal endometritis/salpingitis	None	Low grade fever and uterine tenderness, resolved with oral antibiotics	Moderate symptoms, requiring ≤3 days of parenteral antibiotics	Severe symptoms requiring >3 days of IV antibiotics or development of TOA
Preterm rupture of membranes	None	N/A	Preterm rupture but not resulting in delivery before 37 weeks gestation	Preterm rupture resulting in delivery before 37 weeks gestation

Preterm contractions /labor/delivery[e]	None Braxton Hicks contractions (as early as second trimester)	Preterm contractions which resolve without medical intervention	Preterm contractions with cervical change which result in medical intervention but not resulting in preterm delivery	Delivery before 37 weeks gestation
Poor fetal growth If infant is small for gestational age on newborn exam: report as NEONATAL OUTCOME based on birth weight (see below)	At or above 10th percentile	Fetal growth <10th percentile but ≥3rd percentile for gestational age by ultrasound	N/A	Fetal growth <3rd percentile for gestational age by ultrasound
Hypertension, preeclampsia/eclampsia	None	Pregnancy induced hypertension	Preeclampsia	HELLP syndrome, or eclampsia,
Chorioamnionitis	None	Fever (38°C–38.4°C or 100.4°F–100.9°F) with two or more: FHR[e] >160 BPM[f], maternal HR >120, uterine tenderness between contractions or purulent AF or preterm labor	Same as Grade 1 plus fever 38.5°C–40°C or 101°F–104°F	Criteria for Grade 2 plus fetal distress or fever >40°C or 104°F
Postpartum endometritis	None	Low grade fever and uterine tenderness, resolved with oral antibiotics	Moderate symptoms, treated by ≤3 days of parenteral antibiotics	Severe symptoms treated with >3 days of IV antibiotics, addition of heparin, or operative intervention
Gestational diabetes mellitus	N/A	New onset controlled by diet	New onset with initiation of medical therapy	Uncontrolled despite treatment modification
Clinical AE, pregnancy-related, NOT identified elsewhere in this AE Grading Table	None	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities

Mode of delivery[f] (normal spontaneous vaginal delivery, induced delivery, assisted vaginal delivery, Caesarean Section)	**Not reported as AE/SAE**. Mode of delivery is a procedure and should not be reported or graded as an AE/SAE. The reason for selecting the mode of delivery (for example: Caesarean Section for **severe preeclampsia**) should be reported and graded as appropriate.

Infant Adverse Events
(Neonatal and Infant Events)

Parameter	Normal	Grade 1	Grade 2	Grade 3
Preterm birth[g]	Born after 36 6/7 weeks gestation	Late preterm: 34 to 36 6/7 weeks gestation	Early preterm: 32 to 33 6/7 weeks gestation	Very or extremely preterm: ≤ 31 6/7 weeks gestation
Birth weight (grams)[g] (Small, appropriate, large for gestational age) Report separately poor fetal growth (as above)	Birth weight ≥2500		Low birth weight: 1501-2500	Very low birth weight ≤1500
Neonatal complications in a term infant	Normal term infant discharged home with mother after uncomplicated delivery and nursery course	Transient signs and symptoms requiring **no intervention**, and resolved spontaneously[h]	Signs/symptoms requiring **intervention**[i] but **discharged home with mother (± 2 days)**, or close to it	Required NICU for more than 1 week, and/or interventions (including surgery) leading to prolonged hospitalization (report SAE)[j]
Clinical AE in the newborn NOT identified elsewhere in this AE Grading Table (postmaturity[l]**)**	None	Symptoms causing no or minimal interference with usual functional activities	Symptoms causing greater than minimal interference with function and activities	Symptoms causing inability to perform functions or activities
Congenital anomalies/ birth defects[m]	Variants of normal and minor anomalies are NOT reported, graded or followed as AE, or SAEs. They are collected in the database as part of the medical examination and reported in the Final Study Report.	N/A	N/A	Major congenital anomalies are reported as SAEs.

Abbreviations and definitions:

[a] Please refer to 'Definitions of Safety Parameters' for regulatory definition of 'life threatening' that requires reporting of SAE.

[b] Even if pregnancy loss does not require obstetrical care, and is a natural outcome of non-viable conception, psychological events around the event require recognition especially in research subjects.

[c] EBL: Estimated blood loss.

[d] TOA: Tubo-ovarian abscess.

[e] For preterm delivery, report two AEs: one for mother and one for infant. Unless specified otherwise in the protocol, only one neonatal AE will be reported for all preterm birth-related problems. Additional AEs will be reported only if infant has unexpected AEs: surgery, congenital anomalies, etc.

[f] Examples of complications of delivery that would meet AE/SAE reporting are: maternal death, stillbirth, bleeding placenta previa or abruption that required emergency delivery, etc.
[g] Preterm birth and low birth weight carry different risks and will be reported separately (or twice if an infant is both preterm and low birth weight). If gestational age is not known, only birth weight will be reported. Infants will be plotted on the appropriate growth scale for the population being studied and percentage of 'appropriate', 'large', or 'small for gestational age' infants will be reported in the final study report.
[h] Examples: transient tachypnea, hypothermia, cephalo-hematoma, bruising. Admitted to normal nursery or observed for less than one day in NICU/SCN.
[i] Examples: admission to NICU/SCN for physiological causes like hyperbilirubinemia requiring phototherapy, hypoglycemia, suspected but ruled out congenial infection, oxygen via nasal cannula, or NCPAP.
[j] Examples: confirmed neonatal infection: bacterial, viral, fungal sepsis, meconium aspiration syndrome on respirator, severe laboratory, or/and clinical signs and symptoms.
[k] Examples: perinatal asphyxia: 10 min Apgar scores below 5, shock, critical neonatal laboratory values, etc.
[l] Post maturity carries unique, but still not clearly defined risks. Currently, this category should be used to evaluate signs and symptoms related to post-maturity.
[m] Investigators should consult the protocol and/or the MOP for a list of 'major congenital anomalies' and report them as SAE. Minor anomalies (also listed in the protocol and/or MOP) are structural findings that do not have surgical, medical or cosmetic importance. Minor physical features that typically occur in more than 4% of newborn infants are considered 'variants' of normal, while 'minor anomalies' occur in less than 4% of infants. If in doubt: review referenced articles in Rasmussen et al Clin Infect Dis 2014 p S 428ff[29] and/or consult DMID.
AE: Adverse Events
CS: Cesarean Section
HELLP syndrome: Group of symptoms that occur in pregnant women who have: hemolysis (H); elevated liver enzymes (EL); low platelet count (LP).

9.2.5. Serious Adverse Events

Serious Adverse Event (SAE): An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the site principal investigator or sponsor, it results in any of the following outcomes:

- Death,
- a life-threatening adverse event*,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect¥.
- Important medical events that may not result in death, be life-threatening, or require hospitalizations may be considered serious when, based upon appropriate medical judgment they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

*Life-threatening adverse event. An adverse event is considered "life-threatening" if, in the view of either the site principal investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event that, had it occurred in a more severe form, might have caused death.

¥Congenital anomalies will be reported as described under Section 9.1.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator. FWs will be trained on the assessment of local and systemic reactogenicity. If the subject reports an AE that is either not included among the solicited AEs or is a grade 3 solicited AE, then the FW will contact a study clinician. The study clinician will either visit the participant at home or the participant will be referred to the study health center based on the FW's assessment.
- Recorded on the appropriate SAE data collection form and eCRF.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Reviewed and evaluated by an Independent Safety Monitor (ISM) (as deemed necessary per Section 9.6.1), the DSMB (periodic review unless related), DMID, and the IRB, as required by local reporting guidelines.

9.2.6. Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

The site principal investigator or appropriate sub-investigator is responsible for recording all AE/SAEs that are observed or reported during this trial, as described in this protocol, regardless of the relationship to study product. AE/SAEs, or abnormal clinical findings will be collected, assessed, documented, reported, and followed appropriately.

9.3. Reporting Procedures

Solicited injection site and systemic reactogenicity events for pregnant women will be documented and reported from the time of each study vaccination through 7 days after the study vaccination.

Unsolicited non-serious AEs for pregnant women will be documented and reported from the time of study vaccination through approximately 30 days after the study vaccination (Day 31 or delivery, whichever occurs first, for pregnant women).

SAEs among pregnant women will be documented and reported from the time of study vaccination through 6 months postpartum.

For infants, there are no solicited events. AEs and SAEs will be recorded from birth until the 180 days of life for the infant visit. Events that are not medically attended, or that are a part of routine infant health maintenance, will not be reported.

9.3.1. Serious Adverse Events

SAEs will be followed until resolution even if this extends beyond the study-reporting period. Resolution of an AE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

Any AE that meets a protocol-defined serious criterion must be submitted immediately (within 24 hours of site awareness) on an SAE form to the DMID Pharmacovigilance Group, at the following address:

DMID Pharmacovigilance Group
Clinical Research Operations and Management Support (CROMS)
6500 Rock Spring Dr. Suite 650
Bethesda, MD 20817, USA
SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)
SAE FAX Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)
SAE Email Address: PVG@dmidcroms.com

In addition to the SAE form, select SAE data fields must also be entered into the DCC's Advantage eClinical. Please see the protocol-specific MOP for details regarding this procedure. Other supporting documentation of the event may be requested by the DMID Pharmacovigilance Group and should be provided as soon as possible.

The site will send a copy of the SAE report(s) to the ISM (as deemed necessary per Section 9.6.1) when they are provided to the DMID Pharmacovigilance Group. The DMID Medical Monitor and DMID Clinical Project Manager will be notified of the SAE by the DMID Pharmacovigilance Group. The DMID Medical Monitor will review and assess the SAE for regulatory reporting and potential impact on study subject safety and protocol conduct.

At any time after completion of the study, if the site principal investigator or appropriate sub-investigator becomes aware of an SAE that is suspected to be related to study product, the site principal investigator or appropriate sub-investigator will report the event to the DMID Pharmacovigilance Group.

9.3.2. Regulatory Reporting for Studies Conducted Under DMID-Sponsored IND

Following notification from the investigator, DMID, the Investigational New Drug (IND) sponsor, will report any suspected adverse reaction that is both serious and unexpected. DMID will report an adverse event as a suspected adverse reaction only if there is evidence to suggest a

causal relationship between the drug and the adverse event. DMID will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its IND(s) or under any principal investigator's IND(s)) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting as specified in 21 CFR Part 312.32. DMID will also notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information. Relevant follow up information to an IND safety report will be submitted as soon as the information is available. Upon request from FDA, DMID will submit to FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

All serious events designated as "not related" to study product(s), will be reported to the FDA at least annually in a summary format.

9.4. Type and Duration of Follow-up of Subjects after Adverse Events

Non-serious AEs will be collected, assessed, and followed through resolution from the time of study vaccination through approximately 30 days after study vaccination. For pregnant women, this period extends from vaccination until Day 31 or delivery, whichever occurs earlier. All unresolved non-serious AEs will be followed through resolution even if this extends beyond the study-reporting period.

SAEs and new-onset chronic medical conditions will be collected, assessed, and followed from the time of study vaccination through resolution even if this extends beyond the study-reporting period. The reporting period extends from vaccination until 6 months postpartum (180 days of life for the infant visit) for pregnant women.

Resolution of an AE/SAE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

Follow-up procedures, evaluations, and outcomes will be recorded on the appropriate data collection form.

9.5. Halting Rules

For pregnant women, further enrollment and study vaccinations will be halted for DSMB review/recommendation if any of the following are reported:

- Any subject experiences ulceration, abscess, or necrosis at the injection site related to study product administration.
- Any subject experiences laryngospasm, bronchospasm, or anaphylaxis within 1 day after administration of study product that is considered related to study product.

- Two or more subjects experience generalized urticaria within 3 days after administration of study product that is considered related to study product.
- Any subject experiences a study vaccine-related SAE from the time of study vaccination through subject's last study visit.
- Any subject experiences acute weakness of limbs and/or cranial nerve innervated muscles (description of potential signal of GBS) after administration of study vaccine.

For pregnant women, this study will also be halted for DSMB review/recommendation if, within 7 days after administration of the study vaccination, any of the following occurs:

- 5% or more of subjects (with a minimum of 5 subjects) who received study vaccine experience the same severe (Grade 3) study vaccine-related injection site reaction.
- 5% or more of subjects (with a minimum of 5 subjects) who received study vaccine experience the same severe (Grade 3) study vaccine-related subjective systemic reaction with the exception of allergic reaction, for which the severity (grade) is corroborated by study personnel.
- 5% or more of subjects (with a minimum of 5 subjects) who received study vaccine experience the same severe (Grade 3) study vaccine-related quantitative systemic reaction.
- 5% or more of subjects (with a minimum of 5 subjects) who received study vaccine experience the same severe (Grade 3) pregnancy-related AEs, as found in Section 9.2.4, that are deemed study-vaccine related.

Grading scales for solicited local (injection site) and systemic (subjective and quantitative) reactions are included in Section 9.2.2. Solicited injection site measurements will not be used to halt the study.

If any of the halting rules are met following any subject receipt of study vaccination, then this study will not continue with the remaining enrollments or study vaccinations without a review by and recommendation from the DSMB to proceed.

DMID retains the authority to suspend additional enrollment and study interventions/ administration of study product during the entire study, as applicable.

9.6. Safety Oversight (ISM plus SMC or DSMB)

9.6.1. Independent Safety Monitor (ISM)

The ISM is a physician with relevant expertise whose primary responsibility is to provide independent safety monitoring in a timely fashion. Participation is for the duration of the trial and is a voluntary position that does not receive payment. The ISM must meet the requirements of the NIAID conflict of interest policy.

The ISM:

- Will have experience in obstetrics.
- Is in close proximity to the study site and has the authority and ability to readily access subject records in real time.
- May be a member of the participating institution's staff but preferably be from a different organizational group within the institution.
- Should not be in a direct supervisory relationship with the investigator.
- Should have no direct involvement in the conduct of the study.

The ISM will:

- Sign a Conflict of Interest (COI) certification at the time they are asked to participate and provide updates to this information as needed.
- Receive reports of Serious Adverse Events (SAEs) deemed related to the study from the site investigator and will be notified by email when DMID is notified of the SAE.
- Receive reports from the site investigator of any subject who experiences acute weakness of limbs and/or cranial nerve innervated muscles (description of potential signal of GBS) after administration of study vaccine.
- Evaluate the SAE and report their clinical assessment to DMID, through DMID-CROMS SOCS in a timely manner using the attached report form and email the report to DMID-CROMS SOCS.
- Communicate with the investigator at the participating site as needed.
- Review additional safety related events at the request of DMID.
- Provide additional information to DMID and/or the DSMB by teleconference as requested.

9.6.2. Data and Safety Monitoring Board (DSMB)

Safety oversight will be conducted by a DSMB that is an independent group of experts that monitors subject safety and advises DMID. The DSMB members will be separate and independent of study personnel participating in this trial and should not have scientific, financial, or other conflicts of interest related to this trial. The DSMB will consist of members with appropriate expertise to contribute to the interpretation of the data from this trial.

The DSMB will operate under the rules of a DMID-approved charter that will be written at the organizational meeting of the DSMB. Procedures for DSMB reviews/meetings will be defined in the charter. Reports may include enrollment and demographic information, medical history, concomitant medications, physical assessments, clinical laboratory values, dosing compliance,

and solicited and unsolicited AE/SAEs. The DSMB will review SAEs on a regular basis and ad hoc during this trial. The DMID Medical Monitor and the ISM (as deemed necessary per Section 9.6.1) will be responsible for reviewing SAEs in real time.

Additional data may be requested by the DSMB, and interim statistical reports may be generated as deemed necessary and appropriate by DMID. The DSMB may receive data in aggregate and presented by treatment arm (pregnant women, infants). The DSMB may also be provided with expected and observed rates of the solicited AEs in an unblinded fashion and may request the treatment assignment be unblinded for an individual subject if required for safety assessment.

The DSMB will review grouped and unblinded data in the closed session only. As an outcome of each review/meeting, the DSMB will make a recommendation as to the advisability of proceeding with study vaccinations (as applicable), and to continue, modify, or terminate this trial.

The DSMB will conduct the following reviews:

- When safety data collected through Day 8 is available for 25% of pregnant study subjects.
- Ad hoc when a halting rule is met or DMID/DSMB chair may convene an ad hoc meeting if there are immediate concerns regarding observations during this trial.
- Final review meeting: 6 to 8 months after clinical database lock to review the cumulative unblinded data for this trial. The data will be provided in a standard summary format. The DSMB may be asked to provide recommendations in response to questions posed by DMID.

10. CLINICAL MONITORING

10.1. Site Monitoring Plan

Site monitoring is conducted to ensure that the human subjects' protections, study and laboratory procedures, study intervention administration, and data collection processes are of high quality and meet sponsor, ICH/GCP guidelines, and applicable regulations, and that this trial is conducted in accordance with the protocol, protocol-specific MOP, and applicable sponsor standard operating procedures. DMID, the sponsoring agency, or its designee will conduct site-monitoring visits as detailed in the clinical monitoring plan.

Site visits will be made at standard intervals as defined by DMID and may be made more frequently as directed by DMID. Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, eCRFs, informed consent forms, medical and laboratory reports, and protocol and GCP compliance. Site monitors will have access to each participating VTEU site, study personnel, and all study documentation according to the DMID-approved site monitoring plan. Study monitors will meet with site principal investigators to discuss any problems and actions to be taken and document visit findings and discussions.

11. STATISTICAL CONSIDERATIONS

The primary goals of this study are to assess the safety and tolerability of BOOSTRIX when administered to pregnant women, as well as to determine the maternal and infant immune response. Additional goals include evaluating the effect of maternal antibody on primary infant immune responses to DTwP, identifying cofactors associated with infant and maternal immune responses and determining immune responses in colostrum/breast milk.

11.1. Study Hypotheses

BOOSTRIX will be safe and well tolerated in pregnant women and will lead to PT levels that are higher in the pregnant women and their infants as compared to pregnant women vaccinated with a Td vaccine and their infants. This is one of the first studies of maternal Tdap vaccination in a low resource setting in Africa, and where infants received whole cell, rather than acellular pertussis vaccinations. Thus, this study is intended to obtain preliminary estimates of immune response in this setting; it is not designed to test a formal hypothesis.

11.2. Sample Size Considerations

11.2.1. Study Design

This is a phase II, randomized, double-blind, active-controlled study in which 200 healthy pregnant women, ages 18 through 39 years, inclusive, will be randomly allocated in a 2:1 ratio to receive either Tdap (BOOSTRIX) or Td at 14 0/7 weeks through 26 6/7 weeks estimated GA. After signing an ICF, all subjects will undergo a review of current and past medical history, current and prior medication use, and recent and relevant vaccination history. All pregnant subjects will have a physical examination as part of routine antenatal care. For the fetuses of pregnant subjects, GA will be established by ultrasound, whenever possible, in combination with date of LMP, when available, and fundal height. Subjects will be enrolled, vaccinated, and followed for safety, tolerability, and immune responses as described in this protocol.

11.2.2. Study Population

200 healthy pregnant women, ages 18 through 39 years, inclusive, who meet all eligibility criteria, and their infants.

Based on the accrual rate for similar studies, it seems reasonable to expect that the sites will be able to enroll this study in a timely fashion. Prior experience suggests up to 15% of subjects may be excluded from the per protocol analysis for the primary immunogenicity outcome either because they were lost-to-follow-up or otherwise do not have data available following the study vaccination, or because they had a protocol deviation requiring their exclusion from the per protocol analysis.

11.2.3. Sample Size

The study is planned to enroll 200 pregnant women (133 BOOSTRIX, 67 Td).

This will be one of the first studies of maternal Tdap vaccination in an African, low resource setting, and where infants received whole cell, rather than acellular pertussis vaccinations. Thus, this study is intended to obtain preliminary estimates of immune response in this setting; it is not designed to test a specific null hypothesis. The tables below describe the power to detect safety events and the precision available for estimating immune response for the planned sample size. Table 9 indicates the probability of observing one or more safety event, such as solicited injection site or systemic symptom, or an unsolicited adverse event of a particular type within a treatment arm.

Table 9: Power (%) to Detect Safety Events

Event Frequency	N = 133	N = 67
≥0.01% Rare	1.3	<1
≥0.1% Uncommon	12.5	6.5
≥1% Common	73.7	49.0
≥10% Very Common	100.0	99.9

In addition to safety, as a co-primary objective, the study will assess serum PT antibody at birth among infants whose mothers received a single dose of BOOSTRIX, and as secondary and exploratory objectives, the study will assess maternal and infant humoral response to all vaccine antigens. Immune response will be measured by ELISA. Table 10 presents the precision (95% CI) available for estimating GMC of ELISA titers against the vaccine component using assumptions for GMC and standard deviation (SD) for maternal and infant response to Tdap from Munoz et al. [13] and infant response post third dose of primary DTwP vaccine series from Ladhani et al. [34]

Table 10: Precision for Estimating GMC of ELISA Antibody Response

	GMC	SD (log-scale)	95% CI Estimate			
			N = 133		N=67	
Maternal ELISA Antibodies at Delivery following maternal Tdap						
PT	51	0.90	43.7	59.5	41.0	63.5
FHA	184.8	0.73	163.1	209.3	154.8	220.6
PRN	184.5	1.45	143.8	236.7	129.5	262.9
FIM	1485.7	1.17	1214.8	1817.0	1115.8	1978.2
Infant ELISA Antibodies at Birth following maternal Tdap						
PT	68.8	0.78	60.2	78.7	56.8	83.3
FHA	234.2	0.67	208.7	262.8	198.8	276.0
PRN	219	1.38	172.9	277.4	156.5	306.5
FIM	1867	1.22	1514.7	2301.3	1386.7	2513.7
Infant Response post 3rd Dose DTwP following maternal Tdap						
PT	28.8	0.68	25.6	32.3	24.4	34.0
FHA	25.5	0.60	23.0	28.3	22.0	29.5
FIM	113.9	0.81	99.1	130.9	93.5	138.8
Infant Response post 3rd Dose DTwP (no maternal vaccination)						
PT	43.2	0.65	38.6	48.3	36.9	50.6
FHA	41.1	0.66	36.7	46.1	35.0	48.3
FIM	224.9	0.97	190.2	265.9	177.2	285.4

Table 11 presents the minimum difference in infant PT antibody GMC at birth between the Td and Tdap (BOOSTRIX) vaccination arms that would be detectable with 80% power. The assumed GMC and SD used for these calculations are based on the PT antibody GMC and SD observed at birth in infants whose mother received either Tdap or placebo vaccination during pregnancy, reported in Munoz et al. [13]. The calculations are based on a two-sided two sample T-test to compare means, with alpha = 0.05.

Table 11: Minimum Detectable Difference in Infant PT Antibody GMC at Birth Comparing Infants Born to Mothers Vaccinated with BOOSTRIX versus Td

Td	Tdap (BOOSTRIX)	Assumed GMC (BOOSTRIX)	Maximum GMC Detectable with Power = 0.8 (Td)	Minimum Detectable Difference (BOOSTRIX - Td)
N=67	N=133	50.0	33.1	16.9
		60.0	40.4	19.6
		70.0	44.7	25.3
		80.0	49.4	30.6
		90.0	60.3	29.7

11.3. Planned Interim Analyses

Interim analyses would only be used to terminate this trial in the event that unanticipated safety events deemed to be of sufficient concern to require such action by the sponsor. A DSMB will be convened by DMID to review study progress and subject, clinical, safety, and reactogenicity data.

Cumulative safety information, study status, and primary endpoint results may be presented at a public forum in a blinded manner or presented as summaries aggregated by study arm at the discretion of the sponsor while the primary study is ongoing. While the primary study is ongoing no data will be released that is unblinding at an individual subject level, and caution will be taken to ensure that data summarized by treatment arm does not identify the treatment assignment of any individual subject. Any ad-hoc analyses, jointly developed by the SDCC and/or University of Maryland, will be executed by the SDCC as needed.

None of the interim analyses will include any formal statistical hypothesis testing; therefore, p-value adjustment will not be made to any analyses.

11.3.1. Interim Safety Review

An interim safety review will occur when safety data collected through Day 8 is available for 25% of pregnant study subjects. Reports for interim safety reviews will include data for pregnant participants, and any infants born at the time of data cutoff. Summaries may include enrollment and demographic information, medical history, concomitant medications, physical assessments, and solicited and unsolicited AE/SAEs. Additional data may be requested by the DSMB as described in Section 9.6.2.

This trial will be monitored to determine if any of the halting rules described in Section 9.5 are met.

11.3.2. Interim Immunogenicity or Efficacy Review

An interim immunogenicity review is not planned.

11.4. Final Analysis Plan

A formal statistical analysis plan will be developed and finalized prior to database lock.

11.4.1. Analysis Populations

The Safety Analysis population includes all pregnant women who received the study vaccination, and all infants born during the study.

The maternal intent-to-treat (ITT) population for immunogenicity analyses includes all pregnant women who received the study vaccination.

The maternal per-protocol (PP) population for immunogenicity analyses includes all subjects in the ITT subset with the following exclusions:

- Data from all available visits for subjects:
  - Found to be ineligible at baseline.
  - Without results from the baseline visits.
  - Without results from at least one post-vaccination visit.
- Data from all visits subsequent to major protocol deviations, such as:
  - Receipt of non-study licensed vaccine within the pre-specified exclusion windows (Section 5.2).
  - Receipt of immunosuppressive therapy (e.g., corticosteroids) within 30 days prior to or after study vaccination.
- Data from any visit that occurs substantially out of window.

The infant ITT population for immunogenicity analyses includes all infants born to women who received the study vaccination.

The infant PP population for *primary and secondary* immunogenicity analyses includes all infants in the ITT subset who have a blood sample from the perinatal period (cord blood or drawn blood within the 72 hours after birth).

The infant PP population for *exploratory* immunogenicity analyses includes all infants in the ITT subset with the following exclusions:

- Infants who did not receive DTwP within appropriate window.
- Infants who received any product that would impact immune response.
- Infants with blood samples captured substantially out of window.

In the case of mis-randomization, subjects will be analyzed according to the study product actually received for all analysis populations.

11.4.2. Safety Data

Maternal safety and tolerability, and fetal/infant outcomes will be summarized for the Safety Analysis Population.

Solicited AEs will be summarized by severity on days 4 and 8 after the study vaccination and as the maximum severity over the 8 days. Additionally, solicited AEs will be analyzed by taking the most severe response over the follow-up period, dichotomizing into a binary variable (none versus mild, moderate, or severe) and using standard techniques, such as exact confidence intervals, to summarize the proportion of subjects reporting each symptom, any injection site symptom, and any systemic symptom.

Unsolicited AEs will be coded by Medical Dictionary for Regulatory Activities (MedDRA®) for preferred term and system organ class (SOC). The number of SAEs is expected to be small in this trial, so these events will be reported by detailed listings showing the event description, MedDRA® preferred term and SOC, date of study vaccination, event date, severity, relatedness, and outcome for each event. Non-serious unsolicited AEs will be summarized as number and percentage of subjects reporting at least one event in each MedDRA® preferred term and SOC, tabulated by severity and relationship to study product. Additionally, the proportion of subjects and exact 95% confidence intervals of AEs in aggregate and by MedDRA® categories will be computed.

The proportion of subjects reporting solicited or unsolicited AEs may be compared between treatment arms using Fisher's exact test. The number of untoward fetal outcomes, perinatal complications, and major congenital abnormalities is expected to be small and so these events will be reported by a detailed listing including the event description, MedDRA® preferred term and SOC, relevant dates (study vaccination and AEs), severity, relatedness, and outcome for each event. If more than 5% of pregnant women experience untoward fetal outcomes, perinatal complications, or infants born with major congenital abnormalities, the proportion of subjects with such events will be compared between the BOOSTRIX and Td treatment arms using Fisher's Exact test.

11.4.3. Immunogenicity Data (ELISA)

Immunogenicity data summaries and analysis for pregnant women will be presented for the ITT and the PP populations. Immunogenicity data summaries and analysis for infants will be presented for the infant ITT and infant PP populations.

Immune responses will be reported for antibodies against each of the antigens in the vaccine and at each time point for each subject group- pregnant women receiving BOOSTRIX, pregnant women receiving Td, and the infants of women who received BOOSTRIX or Td while pregnant. GMC and corresponding 95% confidence intervals will be reported.

For each antigen, GMC will be compared between treatment arms using a t-test on log-transformed ELISA titers.

The level of antibody transplacentally transferred will be summarized as the Geometric Mean Ratio (GMR), with corresponding 95% confidence interval, of mother's concentration to infant's concentration for all mother-infant pairs for which both the mother and infant have data at the applicable time point (delivery/birth).
Graphical presentations of immune response may include reverse cumulative distribution (RCD) curves, and longitudinal presentations of GMTs.
To evaluate the association with of maternal immunogenicity cofactors, Maternal and Infant anti-PT ELISA response will be summarized stratified by maternal age (18-29, 30-39 years old), parity (primiparous vs. multiparous), GA at time of vaccination (14-17, 18-21, 22-26 weeks), GA at time of delivery (28-32, 33-36, 37 or more weeks), and infant birthweight, and these cofactors will be evaluated in regression modeling.
Antibody responses in breast milk/colostrum will be evaluated in a similar way to above.
Additional exploratory analyses, including analysis of cytokine response will be described in the statistical analysis plan.

11.4.4. Missing Values and Outliers

All attempts will be made to collect all data per protocol. As missing data are expected to be minimal, no imputation will be performed for missing values. Any data point that appears to be erroneous or inexplicable based on clinical judgment will be investigated as a possible outlier. If data points are identified as outliers, sensitivity analyses will be performed to examine the impact of including or excluding the outliers. Any substantive differences in these analyses will be reported.

12. SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Each participating VTEU site (or subcontracted 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. Each participating VTEU site will permit authorized representatives of the DMID, its designees, and appropriate regulatory agencies to examine (and when required by applicable law) to copy clinical trial records for the purposes of quality assurance reviews, audits, monitoring and evaluation of the trial safety and progress. These representatives will be permitted access to all source data, which include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and subject files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in this clinical trial. Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial.

Interview of subjects is sufficient for obtaining medical history. For pregnant women, it is expected that certain source data, such as gestational age and diagnosis of high risk obstetrical conditions, will be extracted from the antenatal record. In the event of the need to obtain information for the evaluation of eligibility or safety, the research team may, with the permission of the subject via the consent process, obtain that information from the medical chart.

13. QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted site quality management plan, each participating VTEU site and its subcontractors are responsible for conducting routine quality assurance (QA) and quality control (QC) activities to internally monitor study progress and protocol compliance. The site principal investigator will provide direct access to all study-related sites, source data/data collection forms, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. The site principal investigator will ensure all study personnel are appropriately trained and applicable documentations are maintained on site.

The DCC will implement quality control procedures beginning with the data entry system and generate data quality control checks that will be run on the database. Any missing data or data anomalies will be communicated to the site(s) for clarification and resolution.

DMID-designated clinical monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, ICH/GCP guidelines, and the applicable regulatory requirements. Clinical monitoring reports will be submitted to DMID.

14. ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1. Ethical Standard

The site principal investigator will ensure that this trial is conducted in full conformity with the principles of the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research (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, as applicable. The site principal investigator will also ensure conformity with ICH E6 Good Clinical Practice, and applicable federal regulations, guidance, and guidelines for Good Clinical Practice and Clinical Trials with humans.

14.2. Institutional Review Board

Each site principal investigator will obtain IRB approval for this protocol to be conducted at his/her research site(s) and send supporting documentation to the DMID before initiating recruitment of subjects. The investigator will submit applicable information to the IRB/IEC on which it relies for the review, to conduct the review in accordance with 45 CFR 46, ICH E6 GCP, and as applicable, 21 CFR 56 (Institutional Review Boards) and 21 CFR 50 (Protection of Human Subjects), other federal, state, and local regulations. The IRB/IEC must be registered with the Office for Human Research Protections (OHRP) [*OHRP-only* or *OHRP/FDA*] as applicable to the research. DMID must receive the documentation that verifies IRB/IEC-approval for this protocol, associated informed consent documents, and upon request any recruitment material and handouts or surveys intended for the subjects, prior to the recruitment and enrollment of subjects.

Any amendments to the protocol or consent materials will be approved by the IRB/IEC before they are implemented. IRB/IEC review and approval will occur at least annually throughout the enrollment and follow-up of subjects and may cease if annual review is no longer required by applicable regulations. The investigator will notify the IRB/IEC of deviations from the protocol and reportable SAEs, as applicable to the IRB/IEC policy.

Each institution engaged in this research will hold a current Federalwide Assurance (FWA) issued by OHRP for federally funded research.

14.3. Informed Consent Process

Specifically, prior to obtaining individual informed consent for each subject, the investigators will discuss the trial with all the appropriate community associations and leaders, as necessary, to obtain permission to approach the prospective subjects. After community permission is granted, written, informed consent for participation in the trial will be obtained by the investigators from all individual subjects. The consent forms will be written in English; French, the official

language of Mali; and Bambara, the most prevalent of the local Malian languages. The consent documents will be recorded on audiotape, in order to allow Malian prospective subjects who may not be able to read English, French, or Bambara to hear the consent document verbatim in the language of their choice.

The site principal investigator will choose subjects in accordance with the eligibility criteria detailed in Section 5. Before any study procedures are performed, subjects must sign (or, as is customary in Mali, consent may be indicated using a fingerprint) an informed consent form that complies with the requirements of 21 CFR Part 50 and 45 CFR 46 and the local IRB.

Informed consent is a process that is initiated prior to an individual agreeing to participate in a trial and continues throughout the individual's trial participation. Before any study procedures are performed, including screening of subjects for eligibility, subjects will receive a comprehensive explanation of the proposed study procedures and study interventions/products, including the nature and risks of this trial, alternate therapies, any known AEs, the investigational status of the components, and the other elements that are part of obtaining proper informed consent. Subjects will also receive a detailed explanation of the proposed use and disclosure of their protected health information, including specifically their serum samples. Subjects will be allowed sufficient time to consider participation in this trial, after having the nature and risks of this trial explained to them and have the opportunity to discuss this trial with their family, friends, or others or think about it prior to agreeing to participate.

The consent process of the pregnant subject will also include permission for inclusion of the infant in the postpartum phase of the study. After participants have given birth, study personnel will review the infant participation with them. After this review, participants will reaffirm their consent for their infants to take part in the post-partum phase of the study by signing the applicable page on the consent form. If this page is not signed, it is assumed that consent is not given and the infant will not participate in the post-partum phase of the study. Separate permission from the father of the infant will not be required, unless individual IRBs or Research Ethics Committees require it. Subject understanding of the study is determined by the research team member who is performing the informed consent process by asking the subject questions and by discussing the study with the subject. No formal Evaluation to Sign Consent or Comprehension Questionnaire will be used.

For illiterate subjects, the consent document will be provided on audiotape with verbatim reading of the consent document in the language understandable to the subject. For illiterate pregnant women, even if the father of the baby is literate, the pregnant woman will provide permission. Whenever possible, the father of the baby will be involved in the decision, but his

signature/fingerprint to confirm permission for the infant participation will not be required, unless the local IRB/Research Ethics Committee requires it.

The consent process for illiterate subjects will require that an impartial witness is present during the consent process and that the witness signs and dates the consent form. The witness must speak the language in which consent is obtained and must not be a key personnel member in the study.

Informed consent forms describing in detail the study interventions/products, study procedures, risks, and possible benefits will be given to subjects. The informed consent form will not include any exculpatory statements. Informed consent forms will be IRB-approved and prospective subjects will be asked to read and review the appropriate document. Upon reviewing the appropriate document, the site principal investigator (or designee) will explain the research study to prospective subjects and answer any questions that may arise. Prospective subjects, if willing to join the study, will sign or fingerprint the informed consent form. Written documentation of the informed consent process will occur prior to starting any study procedures or interventions, including administering study product.

DMID will provide the site principal investigator, in writing, any new information that significantly impacts subject risk of receiving the investigational product. This new information will be communicated by the site principal investigator to subjects who consent to participate in this trial in accordance with IRB requirements. The informed consent document will be updated, and subjects will be re-consented per IRB requirements, if necessary.

Study personnel may employ IRB-approved recruitment efforts prior to obtaining subject consent; however, before any study procedures are performed to determine protocol eligibility, an informed consent form must be signed or fingerprinted. Subjects will be given a copy of all their signed or fingerprinted informed consent forms.

The process of confirming on-going willingness to participate in the study will be documented on templated source documentation records. Subjects will be asked at visits through the study about their willingness to continue to provide consent for participation.

The rights and welfare of subjects will be protected by emphasizing to subjects that the quality of their medical care will not be adversely affected if they decline to participate in or withdraw from this trial.

14.3.1. Informed Consent/Assent Process (in Case of a Minor)

N/A

14.4. Exclusion of Women, Minorities, and Children (Special Populations)

This trial will be inclusive of all persons who meet the Subject Inclusion and Exclusion Criteria, regardless of religion, or ethnic background. Men are excluded because the study involves the evaluation of a vaccine for pregnant women. Given that this is the first formal study of BOOSTRIX in pregnant women in Mali, adolescent women who are pregnant will not be enrolled, only women 18 to 39 years old. In addition, women over age 39 years are being excluded because of the small increased risk of adverse pregnancy and fetal/infant outcomes in older women.

14.5. Subject Confidentiality

Subjects will have code numbers and will not be identified by name. Subject confidentiality is strictly held in trust by each participating VTEU site principal investigator, their study personnel, the sponsor(s), and their agents. This confidentiality is extended to cover testing of biological samples, in addition to the clinical information relating to subjects.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning this trial or the data will be released to any unauthorized third party without prior written approval of the DMID and the subject. Subject confidentiality will be maintained when study results are published or discussed in conferences.

All information provided by the sponsor and all data and information generated by each participating VTEU site as part of this trial (other than a subject's medical records) will be kept confidential by the site principal investigator and other study personnel to the extent permitted by law. This information and data will not be used by the site principal investigator or other study personnel for any purpose other than conducting this trial. These restrictions do not apply to the following: (1) information which becomes publicly available through no fault of the site principal investigator or other study personnel; (2) information which is necessary to disclose in confidence to an IRB solely for the evaluation of this trial; (3) information which is necessary to disclose in order to provide appropriate medical care to a trial subject; or (4) study results which may be published as described in Section 16.

The study monitor, applicable regulatory authorities, such as the FDA, or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the site principal investigator. This includes, but is not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this trial. Each participating VTEU site will permit access to such records.

To protect privacy, we have received a Certificate of Confidentiality. With this Certificate, the researchers cannot be forced to release information that may identify the research subject, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The researchers will use the Certificate to resist any demands for information that would identify the subject, except as explained below.

The Certificate cannot be used to resist a demand for information from personnel of the United States (US) Government that is used for auditing or evaluation of federally funded projects, like this study, or for information that must be released in order to meet the requirements of the US Food and Drug Administration (FDA).

A Certificate of Confidentiality does not prevent the subject from voluntarily releasing information about themselves or their involvement in this research. If any person or agency obtains a written consent to receive research information, then the researchers may not use the Certificate to withhold that information.

The Certificate of Confidentiality does not prevent the researchers from reporting without the subject's consent, information that would identify the subject as a participant in the research project regarding matters that must be legally reported including: child and elder abuse, sexual abuse, or wanting to harm themselves or others.

The Certificate of Confidentiality applies only under US law and does not apply to Malian courts.

14.6. Study Discontinuation

If the study is prematurely terminated by the sponsor, any regulatory authority, or the investigator for any reason, the investigator will promptly inform the study subjects and assure appropriate therapy or follow-up for the subjects, as necessary. The investigator will provide a detailed written explanation of the termination to the IRB/IEC. If any subject's private information will continue to be collected for this study, the IRB/IEC must approve a consent form with the study procedures, any risks and discomforts, and applicable elements, and the investigator or designee will re-consent the subjects as approved by the IRB/IEC.
If this trial is discontinued, subjects who sign the informed consent form, and are randomized and vaccinated will continue to be followed for safety assessments. No further study vaccinations will be administered.

14.7. Costs, Subject Compensation, and Research Related Injuries

There is no cost to subjects for taking part in this trial.
Subjects may be compensated for their participation in this trial. Compensation will be in accordance with the local IRB's policies and procedures, and subject to IRB approval.
If it is determined by a participating VTEU site and the site principal investigator that an injury occurred to a subject as a direct result of the tests or treatments that are done for this trial, then referrals to appropriate health care facilities will be provided to the subject. Study personnel will try to reduce, control, and treat any complications from this trial. Immediate medical treatment may be provided by a participating VTEU site, such as giving emergency medications to stop immediate allergic reactions to the study vaccine. No financial compensation will be provided to

the subject by a participating VTEU site or the sponsor for any injury suffered due to participation in this trial.

14.8. Future Use of Stored Specimens and Data

Subjects will be asked for their permission/refusal to allow storage of their remaining samples for possible use in future research studies, such as examining additional immunological assessments or testing for antibodies against other viruses or bacteria. This permission is recorded on the consent document but separate from the consent for inclusion in the trial itself. These residual samples will be stored for 15 years at a central clinical storage facility and may be shared with investigators at each participating VTEU site and with other investigators at other institutions. The samples will not be sold or used directly for production of any commercial product. No human genetic tests will be performed on samples. Each sample will be encoded (labeled) only with a barcode and a unique tracking number to protect subject confidentiality. If agreements regarding specimen transfer between institutions are required, such as Materials Transfer Agreements, the terms of those agreements will not contradict this protocol or the consent documents, and investigators will comply with the agreements.

There are no benefits to subjects in the collection, storage, and subsequent use of their specimens for future research. Reports about future research done with subject's samples will NOT be kept in their health records.

Subjects may be given the option to decide if they want their samples to be used for future research or have their samples destroyed at the end of this trial. The subject's decision can be changed at any time prior to the end of this trial by notifying the study doctors or nurses. However, if the subject originally consents to future use and subsequently changes her decision, any data from a previously collected sample may still be used for this research.

15. DATA HANDLING AND RECORD KEEPING

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

Data collection forms will be derived from the eCRF and provided by the DCC to record and maintain data for each subject enrolled in this trial. All data collection forms will be completed in a neat, legible manner to ensure accurate interpretation of data. Black or blue ink will be used to ensure clarity of reproduced copies. When making a change or correction, study staff members will cross out the original entry with a single line and initial and date the change. Study staff members will not erase, overwrite, or use correction fluid or tape on the original.

Data reported in the eCRF derived from the data collection forms will be consistent with the data collection forms or the discrepancies will be explained.

The sponsor and/or its designee will provide guidance to site principal investigators and other study personnel on making corrections to the data collection forms and eCRF.

15.1. Data Management Responsibilities

All data collection forms and laboratory reports will be reviewed by the clinical team and data entry personnel, who will ensure that they are accurate and complete. Adverse events will be recorded on the appropriate data collection form, assessed for severity and relationship, and reviewed by the site principal investigator or appropriate sub-investigator.

Data collection is the responsibility of the study personnel at each participating VTEU site under the supervision of the respective site principal investigator. During this trial, the site principal investigator will maintain complete and accurate documentation for this trial.

The DCC for this trial will be responsible for data management, quality review, analysis, and reporting of the trial data.

15.2. Data Capture Methods

Clinical (including, but not limited to, AE/SAEs, concomitant medications, medical history, and physical assessments), reactogenicity, and immunogenicity data will be entered into a 21 CFR 11-compliant Internet Data Entry System provided by the DCC. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical and reactogenicity data will be entered directly from the data collection forms completed by the study personnel.

15.3. Types of Data

Data for this trial will include clinical, safety, and outcome measures (e.g., clinical laboratory values, reactogenicity, and immunogenicity data).

15.4. Timing/Reports

Clinical, safety, reactogenicity and immunogenicity data will be analyzed once the last subject and last infant born to a pregnant subject completes the final visit. Analyses will occur after all data have been entered, cleaned, and monitored, and the database locked. These analyses will be made available to the sponsor for planning subsequent trials and to the clinical investigators for publication. These analyses will not be used to make any decisions concerning the conduct of this trial.

The clinical study report (CSR) will be completed when all primary safety endpoint data and all primary immunogenicity endpoint data are available. Any available data from the secondary and exploratory immunogenicity endpoints may also be included. The CSR will be amended as additional secondary and exploratory immunogenicity endpoint data become available.

Interim statistical reports may be generated as deemed necessary and appropriate by DMID.

Safety and immunogenicity summary reports may be generated for the DSMB.

Cumulative safety information, study status, and primary endpoint results may be presented at a public forum in a blinded manner or presented as summaries aggregated by study arm at the discretion of the sponsor while the primary study is ongoing. While the primary study is ongoing no data will be released that is unblinding at an individual subject level, and caution will be taken to ensure that data summarized by treatment arm does not identify the treatment assignment of any individual subject. Any ad-hoc analyses, jointly developed by the SDCC and/or University of Maryland, will be executed by the SDCC as needed. Publication of manuscripts may occur at the discretion of the sponsor in accordance with DMID's Expanded Distribution of Clinical Research Endpoint Data Policy.

15.5. Study Records Retention

Study documents will be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents will be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

The participating VTEU site must contact DMID for authorization prior to the destruction of any study records. Informed consent forms that include future use sections will be maintained as long as the sample exists.

15.6. Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or protocol-specific MOP requirements. The noncompliance may be either on the part of the subject, the site

principal investigator, or other study personnel. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with the following sections of ICH E6- 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 principal investigator and other trial personnel to use continuous vigilance to identify and report deviations within five working days of identification of the protocol deviation, or within five working days of the scheduled protocol-required activity. All deviations will be promptly reported to DMID, via the DCC's Advantage eClinical.

All protocol deviations, as defined above, will be addressed in study subject data collection forms. A completed copy of the DMID Protocol Deviation Form will be maintained in the Regulatory File as well as in the subject's chart. Protocol deviations will be sent to the local IRB/IEC per its guidelines. The site principal investigator and other study personnel are responsible for knowing and adhering to their IRB requirements.

16. PUBLICATION POLICY

All investigators funded by the NIH must submit or have submitted for them to the National Library of Medicine's PubMed Central (http://www.ncbi.nlm.nih.gov/pmc/) an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication. The NIH Public Access Policy ensures the public has access to the published results of NIH-funded research. It requires investigators to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. Further, the policy stipulates that these papers must be accessible to the public on PubMed Central no later than 12 months after publication.

Refer to:

- NIH Public Access Policy, http://publicaccess.nih.gov/
- NIH Office of Extramural Research (OER) Grants and Funding, http://grants.nih.gov/grants/oer.htm

As of January 2018, all clinical trials supported by the NIH must be registered on ClinicalTrials.gov, no later than 21 days after the enrollment of the first subject. Results of all clinical trials supported by the NIH, generally, need to be submitted no later than 12 months following the primary completion date. A delay of up to 2 years is available for trials that meet certain criteria and have applied for certification of delayed posting.

As part of the result posting a copy of this protocol (and its amendments) and a copy of the Statistical Analysis Plan will be posted on ClincialTrials.gov.

For this trial the responsible party is DMID which will register the trial and post results.

The responsible party does not plan to request certification of delayed posting.

Refer to:

- Public Law 110-85, Section 801, Clinical Trial Databases
- 42CFR11
- NIH NOT-OD-16-149

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Appendix A

Study Visit Type	**Recruitment**	**Screening**	**Enrollment/ Vaccination**	**Clinic or Home Visit**	**Clinic or Home Visit**	**Follow-up**	**Follow-up**	**Follow-up**	**Follow-up**	**Follow-up**	**Early Termination**	**Unscheduled**
Study Visit Number	**R˜**	**VOO**	**V01**	**V02**	**V03**	**V04**	**V05**	**V06**	**V07**	**V08**		
Study Day	**1st & 2nd Trimesters**	**-30 to 1**	**D1**	**D4±1**	**D8±3d**	**D31±4d**	**Birth + 3d**	**Birth + 42d±5d**	**Birth + 70d±5d or 130d±5d**	**Birth + 180d±7d**		
EGA or Infant Age	**10-26 weeks**	**14-26 weeks**	**14-26 weeks**	**15-27 weeks**	**15-27 weeks**	**19-31 weeks**	**Birth**	**1.5 months**	**2 5 or 4.5 months**	**6 months**		
Introduction to Study, Recruiting	X	X[m]	X[m]									
Obtain Informed Consent∞		X	X[m¬]									
Review Eligibility Criteria		X	X[m¬]			X¬						
Medical History[@]		X	X[m¬]	X¬	X¬	X¬	X¬	X¬˜	X¬˜	X¬˜	X¬	X¬
Concomitant Medications[√@]		X	X[m¬]	X¬	X¬	X¬	X!	X˜	X˜	X˜	X!	X!
Vaccination History[@]		X	X[m¬]	X¬	X¬	X¬	X!					
Vital Signs[$] (Oral Temperature[%], Pulse, and BP)		X	X[†]			X	X				X	X
Height and Weight		X	X[m]									
Targeted Physical Exam {}		X	X [m¬†#]			X	X	X	X	X	X	X
Ultrasound to Date Pregnancy		X										
Venous Blood Collection from Mother (30 mL)			X[†]			X	X			X		
Cord Blood (up to 30 mL)							X					
Venous Blood from Infant (5 mL)							X[&]	X	X	X		
Obtain Breastmilk Sample from Mother (10-20 mL)							X[ψ]	X	X	X		
Pre-Administration Solicited Event Assessments			X[†]									
Randomize and Vaccinate Mother			X									
30-minute Evaluation After Study Vaccination			X									

Study Visit Type	Recruitment	Screening	Enrollment/ Vaccination	Clinic or Home Visit	Clinic or Home Visit	Follow-up	Follow-up	Follow-up	Follow-up	Follow-up	Early Termination	Unscheduled
Study Visit Number	R~	VOO	V01	V02	V03	V04	V05	V06	V07	V08		
Study Day	1st & 2nd Trimesters	-30 to 1	D1	D4±1	D8±3d	D31±4d	Birth + 3d	Birth + 42d±5d	Birth + 70d±5d or 130d±5d	Birth + 180d±7d		
EGA or Infant Age	10-26 weeks	14-26 weeks	14-26 weeks	15-27 weeks	15-27 weeks	19-31 weeks	Birth	1.5 months	2 5 or 4.5 months	6 months		
Subject AE instruction			X									
Examine Study Vaccination Site			X			X					X[!]	X[m!]
Post-Administration Solicited Event Assessments			X	X	X						X[×]	X[×]
Record Mother AEs and SAEs			X[/]	X	X	X	X*	X*	X*	X*	X*	X*
Record Infant AEs and SAEs[+]							X	X	X	X	X	X
Record Birth Outcomes							X	X	X	X	X	X
Infant Vital Signs , Head Circumference, Length, and Weight							X[Y]	X[Ω]	X[Ω]	X[Ω]	X[Ω]	X[Ω]
Infant Targeted Physical Exam, if indicated							X	X	X	X	X	X

~Recruitment and screening visits are not entered into Advantage eClinical.
∞Prior to study procedures. In Mali, a research ID card with photo may be given to subjects.
&Venous blood for infant within 72 hrs. of birth only if no cord blood obtained.
[m]If not obtained in previous visit.
†Prior to study vaccination.
¬Review/confirm information or activity in subjects previously consented and screened/enrolled. This includes review of the pregnant woman's medical chart to obtain information about this pregnancy that may be relevant to the conduct of this study.
^Review results of all clinical screening or safety laboratory evaluations.
@Complete medical history, medication use, and vaccination history by medical record review and interview of subjects to be obtained prior to study vaccination (screening or Day 1) and interim medical history, medication use, and vaccination history by interview of subjects to be obtained at follow-up visits.
√All current medications and vaccinations taken within 90 days prior to vaccination will be reviewed, but only those taken within 30 days prior to vaccination and through discharge after delivery for pregnant women will be recorded in Advantage eClinical.
%Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.
/If AE or SAE occurs post-vaccination.

{}Targeted physical examination, if indicated based on review of complete or interim medical history. An examination performed by a pregnant woman's own obstetrical clinician may also provide data when a targeted exam is called for. An exam should be performed for both the mother and infant if indicated for pregnant women on and after birth.
$Vital signs assessed on Day 1 (Visit 01) prior to the study vaccination will be considered as baseline and used to determine eligibility. For the birth visit, vital signs recorded for the mother as part of her delivery record may be used in lieu of study personnel performing vital sign measurements.
#A physical examination performed as part of routine antenatal care or a study-specific brief exam may be used.
!If within 30 days post vaccination.
× If within 8 days post vaccination.
*SAEs and new-chronic onset medical conditions only after Day 31 or delivery, whichever occurs earlier.
+Only medically attended AEs will be recorded.
Ψ The first colostrum collection may occur within the first 4 days of life of the infant.
° Infant vital signs will include axillary temperature, heart rate, and respiratory rate.
ɣ If not performed as routine care, these measurements, except for weight, may be taken by qualified members of the research team within 7 days of infant life. Weights will only be recorded when obtained at birth.
~ Procedures will be performed on both mother and infant.
Ω Only infant height, weight, and head circumference will be recorded at these visits.