A Prospective, Randomized, Open-label Clinical Trial to Assess the Safety and Immunogenicity of Simultaneous versus Sequential Administration of Tetanus Toxoid, Reduced Diphtheria Toxoid, and Acellular Pertussis Vaccine and Inactivated Influenza Vaccine in Pregnant Women – Pilot

Short Title: Safety of Simultaneous Tdap and IIV in Pregnant Women

Centers for Disease Control & Prevention
Clinical Immunization Safety Assessment (CISA) Project

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STATEMENT OF COMPLIANCE

- This trial will be conducted in compliance with the protocol, the International Conference on Harmonization (ICH) Guideline E6-Good Clinical Practice (GCP), and the applicable guidelines and regulatory requirements from the United States (US) Code of Federal Regulations (CFR), 45 CFR Part 46.
- All study personnel with subject contact have completed Human Subjects Protection Training.

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PROTOCOL SUMMARY

Title:	A Prospective, Randomized, Open-label Clinical Trial to Assess the Safety and Immunogenicity of Simultaneous versus Sequential Administration of tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis vaccine and inactivated influenza vaccine in Pregnant Women – Pilot
Phase:	Phase 4
Population:	80 adult pregnant women (18-45 years) at ≥ 26 weeks 0 days gestation through ≤ 32 weeks 0 days gestation who plan on receiving Tdap and IIV during the current pregnancy in accordance with the Advisory Committee on Immunization Practices (ACIP) and American College of Obstetricians & Gynecologists (ACOG) national recommendations.
Clinical Sites:	Two: Duke University (Lead); Cincinnati Children's Hospital Medical Center (Contributor)
Study Duration:	24 months (4 months to recruit/enroll (per flu season), maximum of 26 weeks to follow (5.5 months) (per flu season), 4.5 months to perform analysis and laboratory assays (per flu season))
Participant Duration:	Up to 23 weeks depending upon gestational age at enrollment and delivery
Description of Study Procedures:	This is a pilot, prospective, randomized, open-label clinical trial. During the study, pregnant women will be randomized (1:1) to receive co-administration of a single intramuscular (IM) 0.5 mL dose of US-licensed inactivated influenza vaccine (IIV) and a single intramuscular (IM) 0.5 mL dose of US-licensed Tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccine adsorbed (Tdap) or sequential administration of the vaccines (IIV followed by Tdap ~ 21 days later). Vaccines will be administered by licensed study personnel. Prior Tdap/Td/TT and influenza vaccine history will be verified by medical record review when possible. Injection-site (local) and systemic reaction data will be assessed on vaccination day and during the 7 days following vaccination using either identical web-based or paper diaries, depending on study participant preference. Maternal serum samples will be collected for antibody titers relevant to the Tdap and Influenza at time points that include: prior to vaccination(s), ~22 days post vaccination(s), and at

	delivery. Additionally, cord blood serum will be analyzed for the same antibody titers. Lastly, a subset of subjects will have infant blood collected prior to two-month vaccinations via heel stick. Pregnant women will be followed through delivery with comprehensive obstetric and neonatal outcomes obtained from medical record review.
Objectives:	Primary Objectives: • To assess proportions of reactogenicity events in pregnant women receiving Tdap and IIV simultaneously vs. sequentially • To assess maternal immune responses and cord blood antibody levels to pertussis antigens, tetanus and diphtheria toxoids, and influenza antigens in the women receiving Tdap and IIV simultaneously vs. sequentially • To assess the feasibility of conducting a larger clinical trial in pregnant women receiving Tdap and IIV simultaneously vs. sequentially Secondary Objectives: • To describe maternal and infant outcomes in pregnant women receiving Tdap and IIV simultaneously vs. sequentially • To evaluate clinical and histological chorioamnionitis in the women receiving Tdap and IIV simultaneously vs. sequentially • To build capacity to study immunogenicity of maternal vaccination in the CISA Project, in collaboration with the CDC laboratories. Exploratory Objectives: • To assess antibody levels to pertussis antigens at 2 months of life before receipt of pediatric diphtheria and tetanus toxoids and acellular pertussis vaccine adsorbed (DTaP) in infants born to women receiving Tdap and IIV simultaneously vs. sequentially
Outcome Measures:	Primary: • Proportions of injection-site and systemic reactions will be compared in simultaneous and sequential groups • Measurement of serum antibody levels to pertussis antigens (pertussis toxin, filamentous hemagglutinin

	and pertactin), diphtheria and tetanus toxoids, and influenza antigens in: • Maternal blood pre and post-vaccination in pregnant women • Maternal and infant cord blood obtained at delivery • Feasibility benchmarks to assess participant recruitment/retention and adequate, timely collection of data and biospecimens Secondary: • Proportions of maternal and infant outcomes, e.g. preterm birth, SGA, preeclampsia, • Proportions of clinical chorioamnionitis • Proportions of histologic chorioamnionitis on surgical pathology examination of placental tissue • Feasibility benchmarks for analyzing pertussis immunogenicity at the CDC laboratories: • Receipt of ≥95% of samples in testable condition and sufficient volume • Completion of testing of ≥95% of the testable samples Exploratory: • Measurement of serum antibody levels to pertussis antigens (pertussis toxin, filamentous hemagglutinin and pertactin in 2-month infant blood before receipt of pediatric DTaP
Estimated Time to Complete Enrollment:	Approximately 4 months for enrollment for both the 2016-17 and 2017-18 flu seasons

1 BACKGROUND

1.1 Background

The Advisory Committee on Immunization Practices (ACIP) and American College of Obstetricians & Gynecologists (ACOG) currently recommend two licensed vaccines for routine administration during pregnancy: inactivated influenza vaccine (IIV) for women who will be pregnant during influenza season and tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccine (Tdap) during every pregnancy.[1-5]

Pregnant women are at high risk for influenza-related morbidity and mortality due to changes in immunology and cardiorespiratory physiology. Influenza-infected pregnant women have higher rates of hospitalization, cardiopulmonary complications, and death compared to the general public.[6-11] Further, influenza is associated with adverse birth outcomes such as preterm birth and fetal demise.[12] Complications are even higher during pandemics, as seen in the 2009 outbreak when the preterm birth rate rose threefold among infected pregnant women and 5% of all related deaths occurred in pregnant women, yet they only encompass 1% of the total population.[13] The Advisory Committee on Immunization Practices (ACIP) and American College of Obstetricians & Gynecologists (ACOG) recommend inactivated influenza vaccine (IIV) for all women who will be pregnant during influenza season at any time during pregnancy.[3,4] Immunization of pregnant women is an effective way to prevent influenza in both mothers and their infants. Initial recommendations for IIV administration during pregnancy were extrapolated from the documented efficacy and safety of IIV in non-pregnant adults. Until the 2009 pandemic, there were only a few small-scale studies on the safety and immunogenicity of maternal influenza vaccination. Recent large cohort studies and randomized controlled trials have supported the presumed safety and effectiveness of IIV in pregnancy.[4,12,14-17]

Pertussis, a respiratory infection caused by *Bordetella pertussis* bacteria, has been on the rise in the US over the last three decades after many years of effective disease control and prevention.[18-20] Although increasing disease incidence has been documented among all age groups, pertussis has the greatest impact on infants <12 months of age as they are at greatest risk for hospitalization, subsequent complications and death.[18,19,21-23] Given that infants cannot be vaccinated against pertussis until 2 months of age and protection against pertussis is not established until after 6 months of age following at least 2 to 3 pediatric diphtheria and tetanus toxoids and acellular pertussis vaccine adsorbed (DTaP) vaccine doses,[24] alternative strategies are required for prevention of pertussis during infancy. After failed efforts to “cocoon” [25,26] infants or diminish their risk of exposure to pertussis by vaccinating adolescents and adults and demonstrated safety of antenatal Tdap administration in small studies, ACIP recommendations were updated in 2013 to administer Tdap vaccine during every pregnancy, preferable between 27 – 36 weeks gestation to maximize passive antibody transfer to the infant.[2]

The Centers for Disease Control and Prevention (CDC), American Academy of Pediatrics, and the Immunization Action Coalition all recommend that multiple inactivated vaccines can be administered at the same time, citing evidence that it does not diminish antibody response or increase reactogenicity.[27,28] Further, simultaneous administration of vaccines is more convenient and efficient for patients and providers. However, the simultaneous administration of vaccines during pregnancy such as IIV and Tdap has not been studied in a prospective manner to date. Funded by the CDC, the Vaccine Safety Datalink (VSD) assessed the safety of simultaneous Tdap and IIV in pregnant women using a large-linked database.[29] In the VSD study Tdap and IIV were

administered simultaneously in 8464 pregnancies and sequentially (in any order) in 28,380 pregnancies. The VSD study concluded that simultaneous administration of Tdap and IIV vaccines during pregnancy was not associated with a higher risk of medically attended adverse acute outcomes or birth outcomes compared with sequential vaccination. While this is certainly informative given the current paucity of data, it did not provide data on reactogenicity events that did not lead to a medical visit, such as maternal fever or injection-site pain. Another limitation of a retrospective study like the VSD analysis is that it did not allow for assessment of immunogenicity, or vaccine-induced antibody response and seroprotection against the vaccine-preventable disease. Some investigators on our study team participated in a randomized, placebo-controlled trial of Tdap given at 32 weeks gestation vs. immediately postpartum in which we followed infants for the first year of life. We found that antenatal Tdap resulted in high pertussis antibody levels in infants during the first 2 months of life and did not substantially affect infant immune responses to pediatric DTaP.[30] Such data have been used to support the ACIP recommendation to administer Tdap during every pregnancy. However, recent studies raise concerns about the impact on pertussis immunogenicity following co-administration of IIV and Tdap. Two studies in non-pregnant adults suggest that simultaneous vs. sequential administration (IIV followed by Tdap 4-6 weeks later) is safe and well-tolerated.[31,32] However, one of the studies demonstrated higher reports of injection-site pain after simultaneous administration.[31] Furthermore, both studies suggested a diminished antibody response to pertussis antigens following simultaneous administration of IIV and Tdap as compared to sequential dosing, and some investigators on our study team have demonstrated reduced antibody responses to influenza vaccine (as measured by hemagglutinin inhibition) in pregnant women compared to non-pregnant women.[33] This suggests that decreased immune responses may be exacerbated in pregnant women, though the clinical importance remains unclear in the adult population.[31,32] These findings raise questions about the potential impact of simultaneous administration during pregnancy. Given that the primary motivation for administering Tdap during pregnancy is to boost maternal antibodies against pertussis and attempt to achieve the highest possible antibody titer at the time of delivery, diminished anti-pertussis antibody production could result in a shorter duration of protection against pertussis in vulnerable young infants.

Another unanswered question in maternal immunization concerns a questionable small increased risk for clinical chorioamnionitis following both Tdap and IIV administration during pregnancy identified through VSD analyses.[15,34] At present, these findings remain unexplained as chorioamnionitis is a polymicrobial infection of the intra-amniotic cavity that affects 1-2% of term and 5-10% of preterm pregnancies and thus unlikely to result from a single vaccination given weeks to months earlier than delivery. [35,36] Further, the diagnosis was based on ICD-9 coding and not strict definitions of clinical chorioamnionitis combined with the lack of an increase in preterm birth which is highly intertwined with chorioamnionitis. While it is possible that these findings are due to chance, further evaluation of a possible association could help to resolve the issue.

1.2 Summary & Rationale

Given the limited data on the safety and immunogenicity of simultaneously administered IIV and Tdap during pregnancy, a prospective randomized clinical study could provide much-needed data on the maternal and infant risks and benefits. There are currently no published or registered randomized trials of simultaneous vs. sequential administration of IIV and Tdap during pregnancy. Such data would aid policymakers, clinicians, and pregnant women in making vaccination decisions during influenza season while

identification of a potential clinically-relevant reduction in pertussis antibody levels following simultaneous IIV and Tdap administration may warrant the consideration for altered guidelines for administration. Moreover, generation of data on simultaneous vaccination during pregnancy will likely be of great importance as the maternal immunization platform continues to develop and very possibly include vaccines against group B Streptococcus and respiratory syncytial virus.[37-39]

2 STUDY OBJECTIVES

Primary Objectives:

- To assess proportions of reactogenicity events in pregnant women receiving Tdap and IIV simultaneously vs. sequentially
- To assess maternal immune responses and cord blood antibody levels to pertussis antigens, tetanus and diphtheria toxoids, and influenza antigens in the women receiving Tdap and IIV simultaneously vs. sequentially
- To assess the feasibility of conducting a larger clinical trial in pregnant women receiving Tdap and IIV simultaneously vs. sequentially

Secondary Objectives:

- To describe maternal and infant outcomes in pregnant women receiving Tdap and IIV simultaneously vs. sequentially
- To evaluate clinical and histological chorioamnionitis in the women receiving Tdap and IIV simultaneously vs. sequentially
- To build capacity to study immunogenicity of maternal vaccination in the CISA Project, in collaboration with the CDC laboratories.

Exploratory Objectives:

- To assess antibody levels to pertussis antigens at 2 months of life before receipt of pediatric diphtheria and tetanus toxoids and acellular pertussis vaccine adsorbed (DTaP) in infants born to women receiving Tdap and IIV simultaneously vs. sequentially

2.1 Study Outcome Measures

2.1.1 Primary Outcome Measures:

- Proportions of injection-site and systemic reactions will be compared in simultaneous and sequential groups
- Measurement of serum antibody levels to pertussis antigens (pertussis toxin, filamentous hemagglutinin, and pertactin), diphtheria and tetanus toxoids, and influenza antigens in:
    - Maternal blood pre and post-vaccination in pregnant women
    - Maternal and infant cord blood obtained at delivery
- Feasibility benchmarks to assess participant recruitment/retention and adequate, timely collection of data and biospecimens

2.1.2 Secondary Outcome Measures:

- Proportions of maternal and infant outcomes, e.g. preterm birth, SGA, preeclampsia
- Proportions of clinical chorioamnionitis
- Proportions of histologic chorioamnionitis on surgical pathology examination of placental tissue
- Feasibility benchmarks for analyzing pertussis immunogenicity at the CDC laboratories:
    - Receipt of ≥95% of samples in testable condition and sufficient volume
    - Completion of testing of ≥95% of the testable samples

2.1.3 Exploratory Outcome Measures:

- Measurement of serum antibody levels to pertussis antigens (pertussis toxin, filamentous hemagglutinin and pertactin), in 2-month infant blood before receipt of pediatric DTaP

3 STUDY DESIGN

3.1 Main study design

This pilot study is a prospective, randomized open-label clinical trial of approximately 80 healthy pregnant women enrolled at Duke University Medical Center (Lead Contractor) and Cincinnati Children's Hospital (Contributing Contractor) who will be followed after they receive simultaneous or sequential single 0.5 mL intramuscular (IM) dose of US-licensed inactivated influenza vaccine (quadrivalent Fluzone) and single 0.5 mL intramuscular (IM) dose of US-licensed tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccine (Tdap, Boostrix). In each study participant, the Tdap and IIV vaccine that is administered will be produced by different manufacturers unless there are manufacturing issues or vaccine shortages that necessitate acquisition of Tdap and IIV from the same manufacturer.

Pregnant women 18-45 years of age and 26-32 weeks gestation will be enrolled over a period of approximately 4 months per flu season. The purpose of the study is to compare the rates of injection-site and systemic reactions and the immune response following simultaneous administration of IIV and Tdap or sequential administration of IIV followed by Tdap in pregnant women, and to assess rates of adverse pregnancy (maternal and infant) outcomes including, but not limited to, preterm birth, small for gestational age (SGA), and chorioamnionitis. Maternal and infant outcomes will be collected by medical record review from enrollment through hospital discharge following delivery.

3.2 Laboratory studies

3.2.1 Serologic studies – We will evaluate pre- and post-vaccination serologic responses in both simultaneous and sequential vaccination groups. A microsphere based multiplex antibody capture assay to determine levels of IgG to pertussis toxin, filamentous hemagglutinin, pertactin, and fimbrae (only for 2016-17 influenza season) in the serum will be performed by the laboratory at the CDC according to CDC protocol MPIR-4301. Tetanus and diphtheria toxoid IgG levels will be measured by an enzyme linked immunoassay (ELISA) at the Duke Human Vaccine Institute (Moody lab). In addition, IgG levels will be measured

for the for the specific viral strains included in the 2016-2017 and 2017-2018 quadrivalent IIV (depending upon vaccine availability) using hemagglutination inhibition assays at the Duke RBL Virology Unit. Venous blood (approximately 15 mL of blood) will be collected from each subject before and 21(+7) days after each vaccination. Additionally, maternal and cord blood (approximately 15mL of blood each) will be collected at delivery for serological analysis in both simultaneous and sequential vaccination groups. An assessment of placental antibody transfer (maternal:cord antibody ratio) will be determined. Prior to the 2-month infant vaccination visit, a blood sample (up to 0.5 mL) will be obtained via heel stick on a subset of 10 infants at each site (20 total). The blood sample will be used to form 5 dried blood spots (DBS) and sent to the CDC lab to determine levels of IgG to pertussis toxin, filamentous hemagglutinin, and pertactin.

3.2.2 Placental pathology – Routine placental pathology includes gross and microscopic evaluation of the placenta (including its weight), umbilical cord (including length, diameter, number of vessels, coiling, and insertion point) and fetal membrane characteristics. Histologic chorioamnionitis is diagnosed using standard hematoxylin and eosin (H & E) staining to identify inflammatory cells (neutrophils, lymphocytes, eosinophils, etc.) as infiltration of the chorionic plate by neutrophils is diagnostic of chorioamnionitis. Chorioamnionitis is staged as per the grading system described in Section 6.4 below.

3.2.3 Feasibility assessment - Feasibility for conducting a larger-scale study of simultaneous vs. sequential administration of IIV and Tdap in pregnancy will be assessed through pre-specified benchmarks for participant recruitment and retention, data collection, and maternal and infant biospecimen collection as described in Section 5.5 below.

3.2.4 Future studies - In addition to the specified analyses described thus far, there may be other tests or assays that have yet to be identified that may be important for interpreting our study findings or of relevance to maternal-infant health outcomes. Therefore, participants will be offered, through an opt-in/opt-out strategy to allow for the storage of any remaining blood (serum/plasma) after all specified analyses have been completed. Additional laboratory assays may test for antibodies against other bacteria or viruses, markers of inflammation, or used in research on the health of mothers and infants. Specimens banked for use in other studies will be linked to information (including identifying information) that participants provided to the study. Participants will not receive results of any future testing of their specimens.
In addition, participants will be offered, again through an opt-in/opt-out strategy, to allow study staff to contact them in the future to take part in other research studies.

3.2.5 Investigational New Drug (IND) Exemption – Upon completion of Duke University's "Checklist to Determine if an Investigational New Drug (IND) Application is Required" it was determined that the study meets criteria for IND exemption.

4 STUDY ENROLLMENT AND WITHDRAWAL

4.1 Subject Inclusion Criteria

Subjects who meet all of the following criteria will be eligible to participate in this interventional study.

1. Pregnant, as determined by medical history; 18 – 45 years of age inclusive
2. Intention of receiving Tdap and IIV vaccines based on ACIP recommendations
3. Willing to provide written informed consent prior to initiation of any study procedures
4. Singleton gestation ≥ 26 weeks 0 days gestation - ≤32 weeks 0 days gestation at the time of Visit 1 vaccination based on reconciliation of last menstrual period and ultrasound dating. Estimated due date (EDD) and Gestational Age (GA) - EDD will be based on reconciliation of a "sure" first day of the last menstrual period (LMP) and earliest dating ultrasound. If the LMP is uncertain, then the earliest dating ultrasound will be used to determine EDD and GA. If the ultrasound derived-EDD is in agreement with sure-LMP derived EDD (**Table 1**), then the LMP-derived EDD is used to determine GA. If the ultrasound derived EDD is not in agreement with the LMP-derived EDD, the ultrasound-derived EDD is used to determine GA.

Table 1. Ultrasound Parameters for Using Sure LMP to Determine Gestational Age

Gestational age at first ultrasound by LMP	Ultrasound agreement with LMP
≤ 19 weeks 6 days	± 7 days
20 weeks to 29 weeks 6 days	± 14 days
≥ 30 weeks	± 21 days

5. English or Spanish literate
6. Intention of being available for entire study period and complete all relevant study procedures, including follow-up phone calls and collection of delivery information.

4.2 Subject Exclusion Criteria

Subjects who meet any of the following criteria will not be eligible to participate in this study:

1.
    a. For subjects enrolling during the 2016-2017 influenza season: IIV/LAIV receipt during 2016-2017 influenza season prior to study enrollment
    b. For subjects enrolling during the 2017-2018 influenza season: IIV/LAIV or recombinant influenza vaccine (RIV) receipt during 2017-2018 influenza season prior to study enrollment
2. Tdap/Td/TT receipt during current pregnancy prior to study enrollment
3. Has immunosuppression as a result of an underlying illness or treatment, or use of anti-cancer chemotherapy or radiation therapy within the preceding 36 months.
4. Has an active neoplastic disease (excluding non-melanoma skin cancer), a history of any hematologic malignancy, current bleeding disorder, or taking anticoagulants (daily low dose aspirin may be acceptable).
5. Has a history of receiving immunoglobulin or other blood product (with exception of Rhogam) within the 3 months prior to enrollment in this study.
6. Known to have pre-existing diabetes mellitus or an autoimmune disorder.
7. Febrile illness within the last 24 hours or an oral temperature ≥ 100.4°F (≥ 38.0°C) prior to IIV or Tdap administration

8. Contraindication to IIV receipt including history of severe allergic reaction after a previous dose of any influenza vaccine; or to a vaccine component, including egg protein
9. Contraindication to Tdap receipt including history of severe allergic reaction after a previous dose of any tetanus toxoid-, diphtheria toxoid-, or pertussis antigen-containing vaccine or encephalopathy within 7 days of administration of a previous dose of a pertussis antigen-containing vaccine that is not attributable to another identifiable cause
10. Arthus-type hypersensitivity reaction following a prior dose of a tetanus toxoid-containing vaccine within the last 10 years
11. Any condition that may interfere with assessment of local injection site reactions, e.g. lymphadenectomy or obscuring tattoos
12. History of Guillain-Barré syndrome within 6 weeks of a prior dose of any tetanus toxoid-, diphtheria toxoid- or pertussis antigen-containing vaccine or influenza vaccine
13. Known or suspected impairment of immunologic function including infection with HIV, hepatitis B or C
14. Use of immunosuppressive or cytotoxic drugs except receipt of oral or parenteral (intravenous, subcutaneous or intramuscular) corticosteroids 30 or more days prior to enrollment. Persons who have used oral or parenteral corticosteroids within 12months prior to enrollment may be enrolled if the longest course of therapy was less than 14 consecutive days and no dose was given within 30 days of enrollment. Intraarticular, bursal, tendon, or epidural injections of corticosteroids are permissible if the most recent injection was 30 or more days prior to enrolment. Persons applying topically corticosteroid in either upper arm (i.e. injection site) may be enrolled 1 or more days after their therapy is completed. Corticosteroids administered topically at non-injection sites, by inhalation or intranasally are permissible
15. Receipt of any licensed vaccine within 14 days prior to study vaccination or planning receipt of any vaccines (except study vaccines) prior to Visit 7 follow up.
16. Receipt of live vaccine during current pregnancy.
17. High risk for preterm birth (active preterm labor, short cervix, cervical cerclage, receipt of antenatal corticosteroids for fetal lung maturity prior to Visit 1)
18. Antenatal ultrasound diagnosis of fetal growth restriction, defined as < 10^{th} percentile estimated fetal weight for gestational age
19. Known fetal congenital anomaly, e.g. genetic abnormality or malformation based on antenatal ultrasound
20. Any condition which, in the opinion of the investigators, may pose a health risk to the subject or interfere with the evaluation of the study objectives.
21. Anyone who is a relative of any research study personnel
22. Anyone who is an employee of any research study personnel
23. Anyone who is already enrolled or plans to enroll in another clinical trial with an investigational product. Co-enrollment in observational or behavioral intervention studies are allowed at any time.
24. Previous participation in the study.

4.3 Recruitment

Pregnant women at ≥ 26 weeks 0 day- ≤ 32 weeks 0 days gestation, 18-45 years old, who are planning to receive Tdap and IIV during their current pregnancy will be recruited at prenatal clinics whose patients deliver at Duke University Hospital or University of

Cincinnati Medical Center. Pregnant women will be pre-screened for eligibility via medical record review. The study will be introduced to a potential subject by a member of their care team. Potential subjects ≥ 20 weeks 0 days gestation will be approached by a member of the research team for study enrollment. Medical and obstetric history, including prior Tdap/Td/TT and influenza vaccine history, will be obtained via participant self-report with verification by chart review whenever feasible (including medical records, employee health records, immunization registry records, and pharmacy records). Study staff will attempt to recruit 10 infants born to women in this study from each site to assess infant pertussis antibody levels prior to 2-month vaccinations. To the extent feasible each site will preferentially recruit 5 infants for each assignment group (simultaneous vs. sequential).

4.4 Reasons for and Handling of Withdrawals

The following may be reason for study withdrawal:

- As deemed necessary by the principal investigator (PI).
- Subject withdrawal of consent.
- Loss to follow-up.
- Termination of the study by the sponsor.

Subjects may withdraw their consent for study participation at any time and for any reason, without penalty. Subjects who withdraw from the study prior to randomization vaccine will be replaced. Subjects who withdraw from the study after receiving vaccine will not be replaced. Every attempt should be made to collect all data specified by the protocol, including collection of pregnancy outcome/safety data via medical record review for subjects who request withdrawal from study interventions/procedures.

4.5 Termination of Study

This study may be terminated for safety concerns of the principal investigators from the Lead or Contributing sites, CDC, or participating IRBs.

5 STUDY SCHEDULE, PROCEDURES, & EVALUATIONS

5.1 Schedule of events

Pregnant women meeting the proposed eligibility criteria (Section 4.1-4.2) will be recruited. Written informed consent (Appendix I) will be obtained from study participants prior to conducting any study procedures. **Table 2** describes the proposed schedule of study visits with further details below.

Table 2: Study Visit Schedule

Procedure	Screening Visit* Day -28 to 1	Visit 1 Day 1	Visit 2 Day 4 ± 1	Visit 3 Day 9 + 3	Visit 4 Day 22 + 7	Visit 5 Day 25 – 32 ± 1	Visit 6 Day 30 – 37 + 3	Visit 7 Day 43 -50 + 7	Visit 8 Delivery	Visit 9 Infant Blood Draw	Unscheduled Visit
Type of contact	Chart Abstraction/ Clinic	Clinic	Phone/ Email/ Text	Phone/ Email/ Text	Clinic	Phone/ Email/ Text	Phone/ Email/ Text	Clinic/ Phone/Email/ Text	Hospital/ Chart Abstraction	Clinic	Clinic
Informed consent & Medical Release of Information	X									X[e]	
Review Eligibility Criteria	X	X									
Review eligibility criteria for Tdap vaccination					X[a]						
Data Collection [b]	X	X	X	X	X	X	X	X	X		X
Influenza and Td/TT/Tdap Vaccination History	X										
Vital signs (temperature, heart rate, respiratory rate and blood pressure) [c]		X			X[a]						X

Collection of Cord Blood & Placenta									X		
Randomization		X									
Vaccination		X			X[a]						
Dispense and review instructions for Memory aid supplies & (REDCap or paper)		X			X						
Complete Memory aid form (REDCap or paper)		X	X	X	X	X	X				
Reactogenicity (local & systemic)			X	X		X	X				
Concomitant medications	X	X	X	X	X	X	X	X	X		X
Obtain solicited & unsolicited adverse events		X	X	X	X	X	X				X
Obtain medically-attended events and serious adverse events		X	X	X	X	X	X	X	X		X
Infant Data Collection[d]										X	
Infant Blood Sample (DBS)										X	

*** Screening visit may occur simultaneously with Visit 1**

[a] Procedures for subjects in sequential group only

[b] Data collection includes demographic information, medical and obstetric history, current pregnancy status. Changes in baseline medical status such as worsening or new onset condition will be capture as adverse events

[c] Vital signs measured prior to vaccinations during Visit 1 and Visit 4

[d] Data collection includes pertussis-specific medical history

[e] Informed consent to be completed prior to Visit 9 blood draw

Screening Visit, Study Day -28 to 1 (Chart Abstraction & Clinic Visit – may occur simultaneously with Visit 1)

- Obtain written informed consent (Appendix I) and release of medical record information
- Review and confirm study eligibility
- Obtain information on preferred method of contact for follow-up (telephone, email or reminder)
- Obtain demographic data
- Obtain month/year of prior history of influenza (IIV or LAIV) vaccines for the past 2 years
- Obtain month/year of any prior history of Tdap
- Obtain month/year of prior history of Td/TT vaccines in the past 10 years
- Obtain medical and obstetric history and current pregnancy status
- Obtain current concomitant medication use

Visit 1, Study Day 1 (Vaccination Day) - Screening, Enrollment, and Randomization (Clinic Visit)

- If Screening Visit occurred prior to Visit 1, review and confirm eligibility and all data collected during Screening Visit
- Obtain vital signs including oral temperature prior to vaccination
- Obtain 15mL blood sample prior to vaccination for serologic analysis (Section 6)
- Randomize study participant to simultaneous or sequential vaccine administration (Section 5.2.1)
- Administer assigned study products – Trained, licensed research staff will administer either IIV alone or IIV + Tdap as described in Section 5.2.3. Ensure participants receive corresponding Vaccine Information Sheets (VIS) during visit.
- Dispense memory aid (Appendix B and C), thermometer, and ruler (in order to standardize measurements). Review instructions for use of thermometer, ruler, and

memory aid (Appendix A) completion.
- Confirm preferred method of contact for follow-up (telephone, text or email reminder)
- Confirm date of next scheduled prenatal appointment
- Record any AEs and SAEs during post-vaccination monitoring

Visit 2, Study Day 4 (window Days 3 – 5) Phone Call/Email/SMS Text Follow-Up
Study staff will contact study participants (Appendix F) to review the memory aid data and record any adverse events (AEs), serious AEs (SAEs), concomitant medications, and current medical/pregnancy status as described in Section 5.1. Participants will be reminded that they will be contacted again after Day 8.
For participants who choose to utilize REDCap web-based system for entry of memory aid information, study staff will review REDCap system to confirm data capture and assess for any AEs, SAEs, and concomitant medications. Subjects who chose to utilize REDCap will be called if they have not entered their information to remind them to enter their information.

Visit 3, Study Day 9 (window Days 9 – 12) Phone Call/Email/SMS Text Follow-Up
Study staff will contact study participants (Appendix F) to review the memory aid data and record any AEs, SAEs, concomitant medications, and current medical/pregnancy status as described in Section 5.1. Participants who are lost to follow-up by phone contact will continue to be called on a weekly basis until approximately Day 45 following vaccination for safety assessment. Study staff will also attempt to follow up with participants at subsequent scheduled prenatal appointments which should be within 2-4 weeks from enrollment visit based on routine prenatal care guidelines.

For participants who choose to utilize REDCAP web-based system for entry of memory aid information, study staff will review REDCAP system to confirm data capture and assess for any AEs, SAEs, and concomitant medications. Participants who are lost to follow-up by phone contact will continue to be called on a weekly basis until approximately Day 45 following vaccination for safety assessment.

Visit 4, Study Day 22 (window Days 22 – 29) Clinic Visit
- For study participants in the sequential study group, review exclusion criteria listed in section 4.2 for Tdap vaccination
- Obtain vital signs including oral temperature prior to vaccination for study participants in the sequential group.
- Study staff will review the memory aid data and record any AEs, SAEs, concomitant medications, and current medical/pregnancy status as described in Section 5.1
- Obtain 15mL blood sample for serologic analysis (Section 6). Study participants in sequential group to have blood sample obtained prior to vaccination.
- For subjects in the sequential study group, trained, licensed research staff will administer Tdap as described in Section 5.2.3. Ensure participants receive Tdap Vaccine Information Sheets (VIS) during visit.
- Confirm subjects still have previously dispensed thermometer and ruler; if not, provide new supplies. Review instructions for use of thermometer, ruler, and memory aid (Appendix A) completion. All subjects will complete the memory aid (Appendix D and E) again to allow for the collection of “mock” data in the simultaneous group for comparison with the sequential group. Simultaneous group will only provide information for systemic reactions and oral temperature.

- Confirm preferred method of contact for follow-up (telephone or email reminder)
- Confirm date of next scheduled prenatal appointment

Visit 5, Study Day 25 - 32 (window Days 24 – 33) Phone Call/Email/SMS Text Follow-Up

Study staff will contact study participants (Appendix F) to review the memory aid data and record any AEs, SAEs, concomitant medications, and current medical/pregnancy status as described in Section 5.1. Participants will be reminded that they will be contacted again after Day 29. Participants who are lost to follow-up by phone contact will continue to be called on a weekly basis following vaccination until approximately Day 45 for safety assessment. Study staff will also attempt to follow up with participants at subsequent scheduled prenatal appointments which should be within 2-4 weeks from enrollment visit based on routine prenatal care guidelines.

Visit 6, Study Day 30 - 37 (window Days 30 – 40) Phone Call/Email/SMS Text Follow-Up

Study staff will contact study participants (Appendix F) to review the memory aid data and record any AEs, SAEs, concomitant medications, and current medical/pregnancy status as described in Section 5.1. Participants who are lost to follow-up by phone contact will continue to be called on a weekly basis until approximately Day 45 following vaccination for safety assessment. Study staff will also approach participants at follow-up prenatal appointments which should be within 2-4 weeks based on routine prenatal care guidelines.

Visit 7, Study Day 43 - 50 (window Days 43 – 57) Clinic Visit for sequential group, Phone Call/Email/Text for simultaneous group

- This review will be done by phone call/email/text for study participants (Appendix F) in the simultaneous group and in clinic for participants in the sequential group. Study staff will review the memory aid data and record any AEs, SAEs, concomitant medications, and current medical/pregnancy status as described in Section 5.1. Obtain 15mL blood sample for serologic analysis for sequential group only (Section 6)

Visit 8, Delivery Visit, Medical Record Review/Phone Call/Hospital

Study staff will screen the inpatient census for the site-specific Labor & Delivery/Birthing Centers daily to identify study participants admitted for delivery. Electronic medical records will be comprehensively reviewed for detailed information about maternal health events during pregnancy and maternal and infant outcomes as defined by the American College of Obstetrics & Gynecology's REVITALIZE Obstetric Data Definitions.[40] Concomitant medications during delivery hospitalization will be limited to those that are relevant to pregnancy outcomes of interest, e.g. antenatal corticosteroids, or tocolytics in the setting of preterm labor/preterm birth, magnesium in the setting of preeclampsia, antibiotics in the setting of Group Beta Streptococcus (GBS) prophylaxis or chorioamnionitis, etc. Maternal blood and infant cord blood will be collected at this visit. Placentas will be sent for clinical pathology examination.

In order to capture and follow all participants, as a separate plan of follow up, study participants who are not identified via medical record review at delivery will be contacted by phone approximately 2 weeks after their estimated due date to determine if delivery

occurred elsewhere. If so, maternal and infant medical records will be requested from the delivery hospital for data collection.

Visit 9, Infant Follow up Clinic Visit
At Visit 9, infants must be ≥ 6weeks and ≤ 12 weeks of age and not have received any dose of DTaP. Study staff will obtain a blood sample via heel stick (up to 0.5 mL) prior to the infant receiving 2-month vaccination series to collect 5 dried blood spots (DBS). Study staff will recruit 10 infants born to women in this study from each site and will attempt to preferentially recruit 5 infants for each assignment group (simultaneous vs. sequential) at each site. Dried blood spot cards will be sent to the CDC for testing.

Additionally, study staff will collect information about pertussis symptoms, diagnosis and exposure in the infants. The study staff will also assess to ensure the child does not have an increased risk for bleeding or infection after a heel stick.

Unscheduled Clinic Visit

- Unscheduled visits would be conducted for study participants with severe local or systemic reactions with assessment including vital signs, direct measurement of the extent of local reactions, and photographs of local reactions. Providers will be informed of the reactions in their patients and direct care as needed. Study staff will review the memory aid data (if applicable) and record any AEs, SAEs, concomitant medications, and current medical/pregnancy status as described in Section 5.1.

5.2 Treatment Assignment Procedures
This is a pilot, open-label, prospective, randomized study involving pregnant women who are to receive Tdap and IIV vaccines. The specific vaccines administered are based on availability from manufacturers prior to the 2016-2017 and 2017-2018 influenza seasons.

5.2.1 Randomization
Participants will be randomized (1:1) to receive either IIV and Tdap simultaneously or sequentially (IIV followed by Tdap) using a permuted block randomization scheme stratified by Lead and Contributing sites. The project statistician at Duke University will generate permuted block randomization schemes which will be uploaded to REDCap. The randomization schedule will not be available to the study staff, so the next randomization allocation will not be known before randomization occurs. Following confirmation of study eligibility criteria during Visit 1, participant randomization will be through REDCap with treatment allocation recorded on the CRF.

5.2.2 Blinding
This study will be open label and study staff and subjects will not be blinded. Laboratory and pathology staff will be blinded to treatment arm assignment.

5.2.3 Vaccine Supply, Storage, and Administration
In order to ensure adherence to study randomization assignment, US licensed IIV and Tdap vaccines will be administered as study procedures. Licensed Tdap and IIV (single dose vials or prefilled syringes) will be purchased for study administration and maintained at the study sites in a research-specific medication refrigerator at 2-8°C in accordance with package insert guidelines. Vaccine brand will be uniform across sites for both Tdap and IIV. The vaccine brands used for the study will be Boostrix® (GSK) [Tdap vaccine] and Fluzone Quadrivalent® (Sanofi) [influenza vaccine].

Pregnant women meeting eligibility criteria for the study will either receive 1) a single intramuscular (IM) 0.5 mL dose of IIV vaccine and single IM 0.5 mL dose of Tdap vaccine (simultaneous vaccination group) or 2) a single IM 0.5 mL dose of IIV vaccine followed by a single IM 0.5 mL dose of Tdap vaccine 22-29 days later (sequential vaccination group). Both vaccines will be administered by a licensed provider (RN, NP, PA, MD) following randomization assignment within the electronic data capture system. The study team will ensure that the vaccines will not be administered in the same arm. The vaccine manufacturer, lot number, expiration date, site of administration, and date/time of administration will be recorded on the case report form (CRF) and on the study product accountability log. Following administration, used study syringes will be disposed of according to standard operating procedure. Emergency management supplies will be available for initial treatment of an allergic reaction if needed.

The vaccine injection site(s) will be reviewed prior to subject leaving the clinic at least 20 minutes after vaccination takes place, which is consistent with current ACIP recommendations that individuals be observed for 15 minutes post-vaccination.[41] The site(s) will be scored for evidence of local reactogenicity as listed below in **Tables 3 and 4**.

5.3 Reactogenicity Assessment

Frequency and occurrence of local and systemic reactogenicity, solicited and unsolicited AEs, SAEs, concomitant medication use, and unscheduled medical care will be assessed immediately after vaccination (day 1) through post-vaccination Day 8 (day 1-8) using a standard memory aid (Appendix B-E). At the time of study enrollment, participants will be given an oral digital thermometer and instructed on completing the memory aid to document oral temperatures and post-injection symptoms, daily. A study staff member will review each portion of the memory aid with the subject in order to familiarize the subject with the form. The subject will be given the choice of either entering their data into REDCap daily or utilizing a traditional paper memory aid. Subjects will be instructed to notify study staff promptly using the 24-hour contact number provided in the memory aid in the event of any severe (Grade 3) local or systemic reactions.

Beginning on the evening of Study Visit 1 (Day 1) following vaccination, participants will record their oral temperature using the study-supplied thermometer, the occurrence of AEs, and concomitant medication use for the next 7 days (Day 1 – 8). Temperature will be recorded at roughly the same time each day. If a temperature ≥ 100.0°F (37.8°C) is recorded, a second measurement will be taken within 20 minutes. If more than one temperature is taken on the same day, the highest temperature should be recorded. Solicited adverse events (local and systemic), which will be classified as mild, moderate, or severe as described in **Tables 3 and 4**, will be reviewed and recorded during Visits 2-3 and 5-6.

Each day, women will record reactogenicity data either on paper or directly into the REDCap system through 7 days post-vaccination. All subjects will be provided with a paper memory aid (Appendix B-E) to record their reactogenicity data for ease of recall when entering into REDCap or recalling to research staff over the phone. We have selected a reactogenicity period of vaccination day and the next seven days based on pre-licensure studies of Tdap and IIV vaccines in adults and adolescents which suggest

that the onset of solicited, local adverse events is highest during days 1 through 4 post-vaccination. Moreover, pre-licensure data indicate a low incidence of late onset reactions (> 3 days post-vaccination). [42] Contemporary evaluations of booster doses of both licensed Tdap vaccines (i.e., Boostrix and Adacel) have used either 4- or 7-day periods for assessments of reactogenicity citing that most local and systemic reactions occur within the first 48 hours after vaccination. [43]

Women using REDCap will receive an email soliciting their record of events (daily link is sent via email for subjects to complete). The REDCap system provides real-time reports regarding who has responded. Local and systemic reactogenicity will be measured as described in **Tables 3** and **4** and subjects will contact the study team if they have any severe solicited symptoms or experience an SAE. AEs and SAEs will be reviewed in REDCap or by phone follow-up. For severe symptoms in REDCap, study team will follow-up with the subject by phone to gather more information about the symptoms.

Table 3: Injection-site Reactogenicity

Symptom	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Pain (without touching)	Noticeable but does not interfere with activity	Interferes with activity but did not necessitate medical visit or absenteeism	Prevents daily activity and/or resulted in medical visit and/or absenteeism
Pain upon touching (Tenderness)	Noticeable but does not interfere with activity	Interferes with activity but did not necessitate medical visit or absenteeism	Prevents daily activity and/or resulted in medical visit and/or absenteeism
Swelling (Induration)	<10 mm	10-34 mm	>=35 mm
Redness (Erythema)	<10 mm	10-34 mm	>=35 mm

Participants will also be queried during Visits 2-3 and Visits 5-6 on common post-vaccination local and systemic symptoms as described in **Tables 3 and 4**.

Participants using the paper diary will be contacted at Visits 2-3 and Visits 5-7 to review and document the information recorded in their diary. They will also be encouraged to report unsolicited adverse events in an open-ended question format, e.g. “How are you doing? Are you having any medical or clinical problems? If so, please tell me about them.” Participants who report severe solicited adverse events or express any concern about symptoms/unsolicited events will be encouraged to follow up with their ObGyn or primary care provider. Study staff will assist with coordination of referral appointments as necessary. Medical records will be obtained and reviewed for any unscheduled medical appointment through post-vaccination Day 8. The same procedures for solicited adverse events will follow the second vaccination for the sequential group and be performed around the same time period for the simultaneous group in a “mock” fashion (Visits 4-6, with the exception of solicited injection site reaction). After Visit 4, solicited injection site reactions will not be recorded by participants in the simultaneous group. After the reactogenicity assessment period, only medically-attended adverse events will be collected.

Table 4: System Reactogenicity			
Systemic	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Oral Temperature (°C/°F)**	≥ 37.8 - ≤ 38.7° C ≥ 100.0 - < 102.0 °F	≥ 38.8 - ≤ 39.4° C ≥ 102.0 - ≤ 103.0° F	≥ 39.5° C ≥ 103.1° F
Fatigue (Malaise)	Noticeable but does not interfere with activity	Interferes with activity but did not necessitate medical visit or absenteeism	Prevents daily activity and/or resulted in medical visit and/or absenteeism
Body Aches (Myalgia)	Noticeable but does not interfere with activity	Interferes with activity but did not necessitate medical visit or absenteeism	Prevents daily activity and/or resulted in medical visit and/or absenteeism
Headache	Noticeable but does not interfere with activity	Interferes with activity but did not necessitate medical visit or absenteeism	Prevents daily activity and/or resulted in medical visit and/or absenteeism
Chills/shivering (Feverishness)	Noticeable but does not interfere with activity	Interferes with activity but did not necessitate medical visit or absenteeism	Prevents daily activity and/or resulted in medical visit and/or absenteeism
Joint Pain (Arthralgia)	Noticeable but does not interfere with activity	Interferes with activity but did not necessitate medical visit or absenteeism	Prevents daily activity and/or resulted in medical visit and/or absenteeism
Nausea	Noticeable but does not interfere with activity	Interferes with activity but did not necessitate medical visit or absenteeism	Prevents daily activity and/or resulted in medical visit and/or absenteeism
Vomiting	Noticeable but does not interfere with activity	Interferes with activity but did not necessitate medical visit or absenteeism	Prevents daily activity and/or resulted in medical visit and/or absenteeism
Diarrhea	Noticeable but does not interfere with activity	Interferes with activity but did not necessitate medical visit or absenteeism	Prevents daily activity and/or resulted in medical visit and/or absenteeism
Abdominal Pain	Noticeable but does not interfere with activity	Interferes with activity but did not necessitate medical visit or absenteeism	Prevents daily activity and/or resulted in medical visit and/ or absenteeism
Body Rash	Noticeable but does not interfere with activity	Interferes with activity but did not necessitate medical visit or absenteeism	Prevents daily activity and/or resulted in medical visit and/ or absenteeism

*** Oral temperature, no recent hot/cold beverages or smoking*

5.4 Safety Assessment

Both IIV and Tdap administration for pregnant women are recommendations for routine clinical care during pregnancy such that we do not anticipate having a significant issue with SAEs. However, we will monitor study participants for medically-attended events and SAEs during the protocol-defined surveillance period of vaccination through delivery. Subjects will be followed for SAEs up to the 6-week postpartum visit. Significant infant complications or SAEs identified during the delivery visit will be followed up to the 2 month Well Child Visit.

An SAE is defined as an AE that meets one of the following conditions:

- Results in death (maternal, fetal, or infant) during the study period

- Is life-threatening (defined as immediate risk of death at the time of the event)
- Requires inpatient hospitalization prolongation of hospitalization during the study period (other than routine hospital admission for labor & delivery)
- Results in congenital anomaly or birth defect
- Results in a persistent or significant disability/incapacity
- Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above. 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.

SAEs will be reported to the CDC, Duke, and Cincinnati Children's IRBs within 48 hours of study staff awareness of the event. If indicated, SAEs will be reported to the CDC's Vaccine Adverse Event Reporting system (VAERS).

Given that this is a pilot study involving vaccines that are included as part of routine clinical care, there will not be a designated data safety monitoring board for this pilot study. If deemed necessary, we will designate an independent safety monitor with relevant expertise, in collaboration with the CDC.

5.5 Biospecimens Collection & Handling

5.5.1 Serum

Maternal and cord blood specimens will be collected during study visits as described in **Table 1**. All blood samples will be collected, processed, and frozen according to the standard operating procedures and not contain personal identifiers. Serum aliquots will be frozen and stored at each site until planned laboratory analyses. A portion of serum aliquots from each subject's blood draw visits will be sent to the CDC for Pertussis assays once all study samples have been collected for all subjects. Remaining frozen serum aliquots will be stored and then shipped to the lead site for influenza, tetanus, and diphtheria assays. Please refer to the study's manual of operations for specifics of sample shipping to CDC and Duke University.

The Study site(s) will process and ship these samples to CDC using standard procedures, in accordance with specimen transport policies (http://www.cdc.gov/laboratory/specimen-submission/shipping-packing.html). The study site(s) will use the CDC File Accessioning Form to send the specimen data via e-mail to Peter Browning (isk8@cdc.gov) or designee. All samples will be barcode labelled and contain a unique ID and date and time of sample collection. No personal identifiers will be included on the samples or CDC File Accessioning Form.

Any unused serum samples will be returned to the study site(s) after all assays have been completed.

5.5.2 Placental Tissue

The study team will work with labor and delivery staff to ensure placentas from study subjects are sent for pathological evaluation for chorioamnionitis. The results from the pathology reports will be entered into REDCap.

Following delivery of all subjects, placental histological slides will be over-read for histological diagnosis of chorioamnionitis. Over-reading will be performed by a single board-certified pathologist with expertise in placental pathology at Duke University. The pathologist will be blinded to study details including randomization arm assignment. Chorioamnionitis will be assessed using the grading scale described in **Table 5**. If a clinically-significant discrepancy is noted between the original pathology report regarding chorioamnionitis and the over-read by the blinded pathologist, then another independent pathologist will review the pathology slides in question to allow for adjudication of the discrepant results.

5.5.3 Infant Dried Blood Spots

The study team will obtain a blood sample via heel stick on a subset of 10 infants at each site (20 total) to collect 5 dried blood spots to determine levels of IgG to pertussis toxin, filamentous hemagglutinin, and pertactin (the pertussis antigens in the Boostrix® vaccine) at the CDC. DBS punch cards will be adequately dried with desiccant provided by the CDC and shipped to the CDC for pertussis testing. CDC pertussis laboratory will destroy unused DBS after all assays and the study have been completed.

6 LABORATORY ANALYSES

6.1 Pertussis Laboratory Analysis

Quantification of antibodies against pertussis toxin (PTx), pertactin (PRN), filamentous hemagglutinin (FHA), and fimbrial antigen 2/3 (Fim2/3) in a microsphere based multiplex antibody capture assay according to CDC protocol MPIR-4301. Antibody to fimbrial antigen 2/3 will only be done on samples collected in 2016-2017 influenza season. All specimens will be run in duplicate by 2 independent operators. Briefly, each of the 5 antigens are conjugated to spectrally distinct fluorescent microspheres (Luminex XMAP™, Austin, TX). The microspheres are incubated with serum for 40 minutes at room temperature, washed, then incubated for 20 minutes with a fluorescently labelled anti-human antibody. The beads are then washed again and read using a Luminex instrument. Antibody levels are quantified by interpolation to a WHO reference standard. Samples that read higher than the useable range of the reference standard are repeated at a higher starting dilution. The independent results are compared, and if the coefficient of variation (CV) is below 30% the mean is reported. If the CV is greater than 30%, up to 2 additional runs are performed and the median is reported.

6.2 Tetanus and Diphtheria Laboratory Analysis

Quantification of antibodies against diphtheria and tetanus toxoids will be performed by ELISA using kits containing an internal standard (AbCam, Cambridge, MA) per the manufacturer's directions. Briefly, wells pre-coated with diphtheria or tetanus antigen are incubated with dilution media as a blank, control specimens that contain a defined amount of anti-diphtheria or anti-tetanus antibody, or an unknown test sample. All specimens are run in duplicate. Primary antibody is allowed to bind for 1 hour at 37°C, plates washed, and secondary antibody allowed to bind for 30 min at room temperature.

Plates are washed again and substrate added; this is incubated for 15 min at room temperature in the dark, followed by reading on a microtiter plate reader at 450 nm. The values from replicate wells are averaged, the background of blank wells subtracted from all other wells, and a dilution curve created using the known controls. Optical density readings are then compared to the standard curve and an absolute value for binding antibodies in international units is calculated. For specimens that have optical density readings higher than the standard curve range, a dilution of the serum/plasma sample is assessed in a repeat assay.

6.3 Influenza Laboratory Analysis

We intend to utilize the 2016-2017 and 2017-2018 quadrivalent Fluzone (Sanofi) as the IIV study product. As such, reference wild-type, reassortant, or vaccine virus strains representative of the specific viral antigens included in the 2016-2017 and 2017-2018 influenza vaccines will be used to evaluate the relative levels of all four influenza strain-specific antibodies in maternal serum pre- and post-vaccination (60 subjects, 240 samples total). To accomplish these activities all patient samples will be interrogated for influenza antibodies against the strains of interest using the influenza hemagglutination inhibition assay (HI). This assay is considered the "gold-standard" measure by which to evaluate seroconversion/seroprotection in response to seasonal influenza vaccination. This assay will be performed in accordance with the RBL Virology Unit's fully optimized and approved SOP (RVUSOP004 Influenza HI of Serum Samples). Briefly, test samples will be assayed by HAI as duplicate 2-fold dilution series starting at 1:10. Serum dilutions are then incubated with a concentration of virus verified to possess a known potential for red blood cell (RBC) agglutination. The presence of virus-specific antibodies is visualized via incubation of the virus-serum mixture with a RBC solution; the endpoint titer for a given dilution series is then expressed as the reciprocal of the final dilution in which complete HI is observed. By convention, seronegative samples are defined as having an endpoint HI titer < 1:40 and seropositive samples as having an endpoint titer of ≥ 1:40; and seroconversion as a 4-fold change in endpoint titer relative to pre-immunization baseline or a change from <1:10 to ≥1:40.

6.4 Placental pathology and Chorioamnionitis Analysis

Placentas from subjects will be sent to the surgical pathology department at each site in accordance with standard clinical care following delivery (sent in-total in a specimen container with formalin for fixation). Routine placental pathology will be performed and pathologists will be unaware of study group at the time of placental evaluation. Routine pathology reporting includes gross and microscopic evaluation of the placenta including weight, umbilical cord including length, diameter, number of vessels, coiling, and insertion point, fetal membrane characteristics.

Histologic chorioamnionitis is diagnosed using standard hematoxylin and eosin (H & E) staining to identify inflammatory cells (neutrophils, lymphocytes, eosinophils, etc.) as infiltration of the chorionic plate by neutrophils is diagnostic of chorioamnionitis. Chorioamnionitis is staged as per the grading system described below.

Table 5: Histologic Chorioamnionitis Grading Scheme

Stage 1 – Mild	Stage 2 - Moderate	Stage 3 - Severe
Acute subchorioamnionitis or acute chorioamnionitis	Acute chorioamnionitis	Necrotizing chorioamnionitis

Characterized by neutrophils in subchorionic fibrin or interface between decidua and chorion	Neutrophils in connective tissue plane between chorion and amnion	Necrosis, amnion sloughing, thickening of amnion basement membrane and neutrophilic karyorrhexis, Multifocal abscesses may be present as well as funisitis (identification of neutrophils in the umbilical cord connective tissue)

For microscopic evaluation of chorioamnionitis, we use the 2003 Pediatric Pathology Society consensus recommendations to diagnose and stage chorioamnionitis (Redline, Raymond W., et al. "Amniotic infection syndrome: nosology and reproducibility of placental reaction patterns." *Pediatric and Developmental Pathology* 6.5 (2003): 435-448.).

7 STATISTICAL CONSIDERATIONS

In collaboration with the CDC and Cincinnati sites, the research team at Duke will oversee the statistical analysis. This pilot study is not designed based on statistical considerations regarding power/sample size in relation to group comparisons. Therefore, descriptive statistics and confidence intervals for continuous variables will be presented (e.g., mean, standard deviation, median, interquartile range, and confidence boundaries). Categorical variables will be summarized with frequencies, percentages, and exact confidence boundaries.

For analysis, data will reside on a secure Duke server maintained by Duke Health Technology Solutions (DHTS). For the study, a database will be developed and a dataset for the study without personal identifiers will be made available to the CDC upon request.

7.1 Analysis Plan

7.1.1 Study Populations – There will be two study populations for this study. The Intent-to-Treat (ITT) and Safety populations. The ITT Population includes any participant that was enrolled and randomized into the study. The Safety Population is a subset of the ITT Population excluding those participants that never received the study intervention (either the influenza or Tdap vaccine). Statistical analyses will be performed for both study populations, or the ITT Population only if no participants are excluded for the Safety Population.

7.1.2 Primary Objective 1 - To assess proportions of reactogenicity events in pregnant women receiving Tdap and IIV simultaneously vs. sequentially

Reactogenicity will be assessed for 7 days following each vaccination visit, as described in Section 5.3. The proportion of women and a 95% exact binomial confidence interval will be presented by treatment group, symptom, and grade level (mild/moderate/severe) for women with injection site reactogenicity within 7 days post-vaccination. This will be presented for each of the four reactogenicity outcomes, using the highest grade reported per woman. A 95% confidence interval of the difference in proportions between the treatment groups will also be presented. Systemic reactogenicity will be presented in a similar fashion. For adverse events, the proportion of women and a 95% exact binomial

confidence interval of adverse events, as well as the total number of adverse events, will be presented by treatment group, severity, and relatedness

7.1.3 Primary Objective 2 - To assess maternal immune responses and cord blood antibody levels to pertussis antigens, tetanus and diphtheria toxoids, and influenza antigens in the women receiving Tdap and IIV simultaneously vs. sequentially

7.1.3.1 **Pertussis antigens -** Geometric mean titers (GMTs) will be calculated along with 95% confidence intervals for the two treatment groups for each pertussis antigen tested. A 95% confidence interval of the difference in GMTs between the treatment groups will also be presented**.**

7.1.3.2 **Tetanus and diphtheria antigens -** The proportion of subjects with seroprotection, defined as ≥ 0.1 IU/mL post-vaccination for both tetanus and diphtheria ELISAs, in the two treatment groups will be presented along with 95% exact binomial confidence intervals. Ninety-five percent confidence intervals of these differences in proportions between the treatment groups will also be presented. Given there is no acceptable definition of seroconversion with these assays, we will evaluate the data to derive a potential post hoc definition for seroconversion.

7.1.3.3 **Influenza antigens** - The proportion of subjects with seroprotection (pre- and post-immunization) and seroconversion (4-fold change in endpoint titer relative to pre-immunization baseline or a change from <1:10 to ≥1:40) in the two treatment groups will be presented along with 95% exact binomial confidence intervals. A 95% confidence interval of the difference in proportions between the treatment groups will also be presented. The GMTs for each influenza antigen and 95% confidence boundaries will be presented for both treatment groups. A 95% confidence interval of the ratios in GMTs between the treatment groups will also be presented.

Outcomes listed in Primary Objective 2 may be analyzed using a regression model to adjust for covariates such as BMI or race. This will be performed as a secondary analysis if normality assumptions for model based analyses are met.

7.1.4 Primary Objective 3 – To assess the feasibility of conducting a larger clinical trial in pregnant women receiving Tdap and IIV simultaneously vs. sequentially

Feasibility will be assessed based on the following benchmarks for recruitment and retention in **Table 6,** as well as adequate and timely collection of data and biospecimens.

Table 6: Proposed Feasibility Benchmarks

Benchmark	Completion Target
Recruitment and randomization	30-50* subjects during 4 month enrollment period
Retention	98% completion of all in-person and delivery visits
Data collection	≥ 95% collection of reactogenicity data
Biospecimen collection – maternal	≥ 95% collection of pre/post-vaccination blood samples

Biospecimen collection – delivery	≥ 95% collection of maternal blood, umbilical cord blood, and placental pathology

*enrollment target will depend upon whether additional enrollment occurs at the Cincinnati Site

7.1.5 Secondary Objective 1 – To describe maternal and infant outcomes in pregnant women receiving Tdap and IIV simultaneously vs. sequentially

The proportion and 95% exact binomial confidence interval will be presented by treatment group for adverse birth outcomes such as preterm birth, low birthweight and SGA. A 95% confidence interval of the difference in proportions between the treatment groups will also be presented.

7.1.6 Secondary Objective 2 – To evaluate clinical and histological chorioamnionitis in the women receiving Tdap and IIV simultaneously vs. sequentially

The proportion and 95% exact binomial confidence interval will be presented by treatment group for histological and clinical chorioamnionitis. A 95% confidence interval of the difference in proportions between the treatment groups will also be presented.

7.1.7 Secondary Objective 3 – To build capacity to study immunogenicity of maternal vaccination in the CISA Project, in collaboration with the CDC lab as described in **Table 7**.

7.1.8 Exploratory Objective 1 - To assess antibody levels to pertussis antigens at 2 months of life in infants born to women receiving Tdap and IIV simultaneously vs. sequentially

Geometric mean titers (GMTs) will be calculated along with 95% confidence intervals for the two treatment groups for each pertussis antigen tested. A 95% confidence interval of the ratio in GMTs between the treatment groups will also be presented as well as for the infant cord blood versus the 2-month blood draw.

Table 7: Feasibility Benchmarks for CDC lab

Benchmark	Completion Target
Quality of blood specimens submitted to the CDC lab for serologic response to pertussis antigen	Receipt of ≥95% of blood samples in testable condition
Quantity of blood specimens submitted to the CDC lab for serologic response to pertussis antigen	Receipt of ≥95% of blood samples with sufficient volume for testing
Completion of testing	Completion of testing of ≥95% of testable blood samples.

7.2 Data Management

The amount of data that will be collected for the proposed project will be substantial and will require a sophisticated, practical and flexible system that can accommodate different modes of data collection and several separate linked surveys. The novel Vanderbilt-designed resource developed specifically for online collection of research information,

the Research Electronic Data Capture (REDCap) platform, will be used to design study forms, including the reaction forms, and short customized questionnaires to collect information from study subjects. This system will be used by Duke for data management. All electronic linkages will fulfill regulations for protection of human subjects and requirements to minimize the risk of breach of confidentiality. After initial set-up, the work load required for electronic data collection will be substantially reduced (description of REDCap resources below).[44] Duke investigators have previously used the REDCap system for collection and analysis of large quantities of data. Participants will be given the option to fill out their reactogenicity diary either directly in the REDCap system or on paper. Participants who choose the paper form will receive phone calls (Visits 2, 3, 5, and 6) to collect the information recorded on their diary card, which will then be entered by study personnel onto REDCap. Participants who choose to enter the information directly into REDCap will receive phone calls if they fail to enter their information. All study-related documents containing protected health information, e.g. enrollment logs, case report forms, memory aids (Appendix B-E) completed by study participants, will be maintained in secure research offices at Duke, which are accessible to research staff only.

The study team will utilize a secure, encrypted, file transfer method for sharing study documents and data with the CDC Pertussis laboratory and study teams. No personal identifiers will be included in any shared documents or datasets.

7.2.1 Research Electronic Data Capture (REDCap)

Investigators within the NIH-funded Clinical and Translational Research Unit at Vanderbilt have developed REDCap (http://project-redcap.org/), to collect and manage data for diverse clinical and translational research studies. REDCap was designed around the concept of giving research teams an easy method to specify project needs and rapidly develop secure, web-based applications for collection, management and sharing of research data. REDCap accomplishes these key functions through use of a single study metadata table referenced by presentation-level operational modules. Based on this abstracted programming model, databases are developed in an efficient manner with little resource investment beyond the creation of a single data dictionary. The concept of metadata-driven application development is well established, and the critical factor for successful data collection lies in creating a simple workflow methodology allowing research teams to autonomously develop study-related metadata in an efficient manner.[44] Of particular interest for this project, a subcomponent of REDCap, the REDCap Survey is designed for studies where data are collected directly from the research participant. This will be used with the web-based reaction forms that will be completed by the study subjects. Both products include secure institutional data hosting and include full audit-trails in compliance with HIPAA security requirements. The REDCap Consortium is comprised of 647 active institutional, including CCHMC. The REDCap currently supports 68,000 projects with over 89,000 users spanning numerous research focus areas across the consortium. The current project will use this software application for the design of electronic forms to collect information from study participants, to link the baseline data, sample collection date, and laboratory results in an automated database family, to perform data cleaning and data quality assurance efficiently, and to design an analytical dataset for the analysis of the project data.

Data will be entered into the REDCap database by members of the study team, from Duke and Cincinnati using the paper case report forms utilized to record data collected

as part of study procedures. Study investigators will be responsible for assuring that all paper records are securely stored according to the requirements of their IRBs. The study investigators will be responsible for assuring the accuracy of the data entered from the paper forms into REDCap. Only the assigned identifiers will be used in REDCap. Therefore, personal health identifiers will not appear in the REDCap database.

In order to perform data cleaning and data quality assurance efficiently, numerous built-in filters and checks for consistency of the data including range and limit checks, branching logic and pull down menus to limit choices for categorical variables to a pre-specified list will be implemented and performed automatically to minimize data entry error. The data will be randomly sampled and checked against source records on a regular basis. The data and related analytical datasets will also be stored at the lead and contributing sites with secured password-protected computers. Coded data without personal identifiers will be made available to the CDC and transferred using a secure transfer method as described in Section 7.2.

7.2.2 CDC Laboratory Management

The CDC lab will send results back to Duke University using secure data transfer methods. CDC will send any remaining serum samples back to the site(s) after completing the pertussis testing or disposed at the request of the Sponsor. CDC pertussis laboratory will destroy unused DBS after all assays and the study have been completed. CDC laboratory staff will be blinded to the vaccination status of the samples being tested.

7.3 Role of the CDC Investigators in the Project

This study is funded by a CDC contract with Duke University and Cincinnati as Task Orders in the CISA Project Contract; the National Vaccine Program Office (NVPO) has also provided funding. The Duke University PI (Geeta Swamy) will oversee the study in partnership with the Cincinnati PI (Elizabeth Schlaudecker). The NVPO and CDC staff will collaborate with both sites to develop the protocol, conduct the study, ensure the study is aligned with US Department of Health and Human Services (CDC and NVPO) public health priorities, and analyze the data and disseminate the results. CDC may receive access to coded data not containing any directly identifying information. The CDC lab will conduct pertussis immunogenicity testing for the maternal serological samples and infant DBS collected in the study.

8 HUMAN SUBJECTS

8.1 Human Subjects Involvement, Characteristics, and Design

Duke and Cincinnati investigators will be responsible for submitting the protocol, informed consent (Appendix I), memory aids (Appendix B-E), recruitment letters, flyers (Appendix G-H), and any written or verbally conveyed materials (Appendix A and F) specific to this project to their institutional review boards. CDC staff will be responsible for submitting materials to the CDC IRB for review and approval.

To facilitate subject recruitment at the practices, we will request a waiver of consent and HIPAA authorization for ascertainment (identification, selection) and/or recruitment of potential subjects while recording identifiable private health information (PHI) prior to obtaining the subject's consent. This information will be obtained from review of the

electronic scheduling and medical record systems in the clinics in order to determine eligibility for study enrollment. We will review information only the minimum amount of information necessary to determine eligibility, i.e. date of birth, current pregnancy status, pregnancy history, medical and surgical history, ultrasounds pertaining to current pregnancy, and recent laboratory test results. The PHI collected prior to consent will be used to recruit and screen only. Use of PHI in this manner involves no more than minimal risk to subjects and no information will leave the study sites.

Continuing reviews will be submitted to the IRBs on an annual basis. Protocol deviations or concerns about study integrity will be reported promptly to the overseeing IRB in accordance with institutional requirements.

8.2 Sources of Material

Medical history and immunization history will be obtained from the medical record and from patient report. Demographic information will be obtained from the medical record and patient report. Subjects will record solicited adverse reactogenicity events and any medical intervention sought on the day of and 7 days following vaccinations (or 7 days following mock vaccination) on the memory aid (Appendix B-E). Memory aid information will be reported to the study team during a telephone call or in the web-based REDCap system. The research staff will assess one or more of the following: weight, height, temperature, blood pressure, and pulse.

8.3 Potential Risks and Benefits

Tdap and IIV are FDA licensed vaccines approved for use in non-pregnant individuals and are not contraindicated for use in pregnant women. Both vaccines are standard clinical practice and recommended by the CDC and ACOG during pregnancy. Participants will be provided with the CDC Vaccine Information Statements (VIS) for IIV and Tdap.

IIV risks include minor problems such as soreness, redness, swelling, or pain where the shot was given, hoarseness, sore, red or itchy eyes, cough, fever, aches, headache, itching, fatigue, all of which usually occur within 1-2 days of vaccination and are self-limiting. Syncope (fainting) can occur in association with administration of injectable vaccines. More serious problems including a small increased risk of Guillain-Barré Syndrome estimated at 1 or 2 additional cases per million people vaccinated. This is much lower than the risk of severe complications from influenza infection, which can be prevented by IIV. In addition, any medication can cause a severe allergic reaction, or anaphylaxis, which is estimated at ~ 1 in a million doses of IIV (http://www.cdc.gov/vaccines/hcp/vis/vis-statements/tdap.html and http://www.cdc.gov/vaccines/hcp/vis/vis-statements/flu.html).

Tdap risks include mild problems such as redness, swelling, and pain where the shot was given, redness or swelling where the shot was given, fever, chills, sore joints, headache, body aches, fatigue, nausea, vomiting, diarrhea, stomach ache, rash, and swollen glands, all of which usually occur within 1-2 days of vaccination and are self-limiting. Moderate problems include higher severity of mild problems and swelling of the entire arm in which the injection was given. Rarely, local injection site symptoms limit the ability to perform usual activities and require medical attention.

Problems that could happen after any injected vaccine:

- People sometimes faint after a medical procedure, including vaccination. Sitting or lying down for about 15 minutes can help prevent fainting, and injuries caused by a fall. Subjects should inform their doctor should they feel dizzy, or have vision changes or ringing in the ears.
- Some people get severe pain in the shoulder and have difficulty moving the arm where a shot was given. This happens very rarely.
- Any medication can cause a severe allergic reaction. Such reactions from a vaccine are very rare, estimated at about 1 in a million doses, and would happen within a few minutes to a few hours after the vaccination. As with any medicine, there is a very remote chance of a vaccine causing a serious injury or death.

There is a small risk of infection around the heel stick where the blood was collected. Maternal subjects may also experience dizziness or fainting. Each maternal subject will be asked to have up to 3 blood samplings with the total volume not to exceed 45mL. For infants participating in the 2-month study procedure, the volume of blood collected will be no more than 0.5 ml. There is no risk to the subject or their newborn for collection of cord blood, as the cord blood is drawn from the umbilical cord/placenta after the baby is not attached to it.

Randomized controlled trials of IIV during pregnancy have demonstrated efficacy in preventing maternal influenza infection as well as infant influenza infection for the first 6 months of life.[45,46] Further, these trials have not demonstrated any concerns for maternal-fetal-infant safety. Studies of Tdap during pregnancy have demonstrated an appropriate maternal immune response but have been too small to evaluate efficacy.[30] A recent large scale observational study of Tdap during pregnancy, demonstrated a reduction in neonatal/infant pertussis, which has been further supported by a recent retrospective case-control study. [47,48] Studies of Tdap in pregnancy have not demonstrated any concerns for maternal-fetal-infant safety. However, as with any vaccines we cannot assure or guarantee protection against influenza, tetanus, diphtheria, or pertussis given the potential for mismatch between circulating influenza vaccine strains and those included in the 2016-2017 and 2017-2018 vaccines and variation in individual immune responses.

The Advisory Committee on Immunization Practices (ACIP) does not make specific recommendations about simultaneous or sequential administration of Tdap and IIV for pregnant women. However, based on the ACIP Best Practice Guidelines for Immunization, Tdap and IIV (both inactivated vaccines) may be administered to pregnant women either simultaneously or sequentially (ACIP Best Practice Guidelines for Immunization accessed at https://www.cdc.gov/vaccines/hcp/acip-recs/general-recs/index.html)

1. Simultaneous administration of vaccines is defined as administering more than one vaccine on the same clinic day, at different anatomic sites, and not combined in the same syringe. Experimental evidence and extensive clinical experience provide the scientific basis for administering vaccines simultaneously. Simultaneously administering all vaccines for which a person is eligible at the time of a visit increases the probability that a child, adolescent, or adult will be vaccinated fully by the appropriate age.

Tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis (Tdap) and quadrivalent inactivated influenza vaccine can be administered simultaneously.

There is the potential risk of loss of confidentiality about information obtained as part of this study.

8.4 Adequacy of Protection Against Risks

8.4.1 Protections against Risk

Subjects will be counseled on possible side effects following vaccination and followed closely during the 7 days post-vaccinations for assessment of moderate to severe local or systemic reactogenicity. Subjects will be evaluated and cared for as described in the Unscheduled Visit section above. All subjects will be monitored in a sitting or lying position for 20 minutes following vaccinations to help prevent fainting, and injuries caused by a fall. Subjects with a prior history of any reaction following either IIV or Tdap will be excluded from study enrollment.

Every effort possible will be made to keep information about participants confidential. Computerized participant information will be kept in password protected files on secured servers. Paper case report forms will be kept in locked files belonging to the study personnel. Any publications resulting from this work will not contain any identifiable participant information.

8.4.2 *ClinicalTrials.gov Requirements*

The project is registered on ClinicalTrials.gov. (NCT02783170).

8.5 Human Subjects

In obtaining and documenting informed consent, the Investigator and study team will comply with the applicable regulatory requirements, Good Clinical Practices, and ethical principles. The written informed consent form must be signed and dated by the study participant prior to initiation of any study activities.

8.5.1 Vulnerable Subjects Research

Vulnerable subjects

This study proposes to include pregnant women and neonates.

Pregnant women

Tdap and IIV are FDA licensed vaccines and are recommended by the CDC and ACOG during pregnancy, however the risks for both vaccines are listed in section 8.3.

The specific procedures to be performed as part of this study are limited to minimal blood draw (<50mL over an 8-week period and not more than twice in a 7-day period), and other non-invasive procedures that are commonly performed during routine physical exams and are considered safe for pregnant women. These procedures do not pose greater than minimal risk to the fetus. Collection of the placenta for research purposes following delivery of the fetus will be conducted in accordance with 45 CFR 46.206.

This study will involve only those women who have given their free and informed consent in accordance with 45 CFR 46.116 and has the prospect of direct benefit to both the mother and infant. Randomization of vaccine administration provides minimal risk to the subject and fetus as these vaccines are routinely given either sequentially or simultaneously during pregnancy and do not go beyond the scope of standard of care.

The small amount of blood that will be drawn during the duration of pregnancy (up to 45 ml) are considered safe and are not expected to cause any harm to the baby. No inducement, monetary or otherwise, will be offered to terminate a pregnancy. Individuals engaged in the research will have no part in any decisions as to the timing, method or procedures used to terminate a pregnancy, or in determining the viability of a neonate.

Infants

Identifiable private information will be collected about the infants at the time of delivery. Significant infant complications identified during the delivery visit will be followed up to 60 days of life.

Mothers will be informed about the infant data collection at the time they consent for the study, and thus, the consent form for pregnant women will also serve as the parental permission for including the infant as a subject after delivery. A separate consent form will be used to obtain informed consent from pregnant or postpartum mothers or fathers for collection of an infant blood sample and documentation of any history of pertussis illness at 2-months of age. These procedures do not pose greater than minimal risk to the infant.

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Appendix A: Memory Aid Instructions

HOW TO COMPLETE THE MEMORY AID

Completing the Memory Aid

- Complete the Memory Aid every evening, starting the day of vaccination (Day 1) and continuing through Day 8. (Example: If you received your vaccination on Monday, you would start your memory aid Monday evening and finish your memory aid on the following Monday evening.)
- Assess symptoms and fill out the Memory Aid in the evening (after 4:00pm, but before midnight).
- For each symptom you experience, you must record the maximum severity and/or measurement.
- Fill out all the spaces each time. If you don't have any symptoms, write 0 in all the boxes.
- Record any other symptoms or medical problems that you have each day under the "other symptoms" portion of the Memory Aid.
- Record all new medications and any changes to previously reported medications that you take each day on page 3 of the Memory Aid.
    - You do not need to enter any regular medications you have already told us about. If you take a new medication, change a medication you were already on, or take something for comfort, please enter this information on the Memory Aid.
- Record any visits to the Emergency Room, Urgent Care, Labor and Delivery, or doctor's office (other than for a routine checkup or prenatal care) on the last page of the Memory Aid.
- Complete the Memory Aid for days 1-8 after vaccination.
- Be sure to return the paper Memory Aid to the study team at the next scheduled study visit.

HOW TO TAKE MEASUREMENTS

Temperature

- Measure your temperature at approximately the same time each day or when you feel feverish.
- Do not eat, drink, or smoke for 10 minutes prior to taking your temperature.
- To use the thermometer, place the skinny end of the thermometer under your tongue toward the back of your mouth and press the button to turn it on (it will beep).
- Keep your mouth closed and the thermometer still until the thermometer beeps rapidly, indicating completion.
- Record the temperature displayed on the screen.
- If your temperature is less than 97.0°F wait approximately 10 minutes and retake again. Record the highest temperature.
- If your temperature is at or above 100.0°F high, repeat approximately 20 minutes later to verify. Record the highest temperature.
- If multiple temperatures are measured during one day, record the highest temperature on the Memory Aid.

Measuring Swelling and Redness at the Site of the Vaccination

- Use the ruler that the study staff has given you and record the measurements in millimeters (mm).
- Follow these instructions to measure the size of the area with swelling or redness.
  - Determine the widest part (or diameter) of the area. Redness and swelling are often not in a perfect circle, so it is important to measure where it is the widest.
  - Measure this distance with the ruler and measure in **millimeters (mm)** by counting the small hash marks.
  - Measure the swelling and redness separately and record on the memory aid under the local vaccination site reactions.

CONTACT THE RESEARCH TEAM AND/OR DOCTOR if…

- If you have **any severe symptoms** (grade 3). These are any symptoms that **prevents daily activity and/or requires medical care and/or missing work/school**.
- If you have any other symptoms or health complaints of concern, even if they are not the events listed on the Memory Aid.
- Please note that if you feel you are experiencing a life threatening reaction or emergency, please call 911 or go to your nearest emergency room to seek treatment. Once everything is safe, you can then contact the research team about your reaction or emergency.
- If you have any concerns or questions about completing the Memory Aid or about symptoms you are experiencing, please contact the study team at the number below:

Contact name:_____ **Phone #:**_____

Appendix B: Memory Aid for Simultaneous Group – Visit 1

CISA TDAP/IIV Data Collection Form
Memory Aid Simultaneous Group
TI07A

Subject ID: _ _ _ _ _

Visit 1

Please assess symptoms and fill out memory aid in the evening (after 4pm)

Post-Vaccination Day	**Day 1(Day of vaccination)**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**
Date (dd/MMM/yyyy)	/ /	/ /	/ /	/ /	/ /	/ /	/ /	/ /
Oral Temperature °F	.	.	.	.	.	.	.	.
General Systemic Symptoms: Grade 0 = **None** Grade 1 = **Mild** (noticeable but does not interfere with activity) Grade 2 = **Moderate** (interferes with activity but did not need a medical visit or absenteeism [i.e. missing work or school]) Grade 3 = **Severe** (prevents daily activity and/or resulted in medical visit and/or absenteeism [i.e. missing work or school])								
Chills/Shivering								
Fatigue								
Body Aches								
Headache								
Joint pain								
Nausea								
Vomiting								
Diarrhea								
Abdominal Pain								
Body rash								

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CISA TDAP/IIV Data Collection Form
Memory Aid Simultaneous Group
TI07A

Subject ID: _ _ _ _ _ _

Flu Vaccine Local Vaccination Site Reactions: ☐ **RIGHT ARM** ☐ **LEFT ARM** Grade 0 = **None** Grade 1 = **Mild** (noticeable but does not interfere with activity) Grade 2 = **Moderate** (interferes with activity but did not need a medical visit or absenteeism [i.e. missing work or school]) Grade 3 = **Severe (**prevents daily activity and/or resulted in medical visit and/or absenteeism [i.e. missing work or school])								
Pain								
Tenderness								
Swelling– size (mm)	____mm	____mm	____mm	____mm	____mm	____mm	____mm	____mm
Redness- size (mm)	____mm	____mm	____mm	____mm	____mm	____mm	____mm	____mm
Tdap Vaccine Local Site Reactions: ☐ **RIGHT ARM** ☐ **LEFT ARM** Grade 0 = **None** Grade 1 = **Mild** (noticeable but does not interfere with activity) Grade 2 = **Moderate** (interferes with activity but did not need a medical visit or absenteeism [i.e. missing work or school]) Grade 3 = **Severe (**prevents daily activity and/or resulted in medical visit and/or absenteeism [i.e. missing work or school])								
Post-Vaccination Day	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**
Pain								
Tenderness								
Swelling- size (mm)	____mm	____mm	____mm	____mm	____mm	____mm	____mm	____mm
Redness- size (mm)	____mm	____mm	____mm	____mm	____mm	____mm	____mm	____mm

CISA TDAP/IIV Data Collection Form
Memory Aid Simultaneous Group
TI07A

Subject ID: __ __ __ __ __

Other symptoms
1 = Mild (noticeable but does not interfere with activity or measurement)
Grade 2 = **Moderate** (interferes with activity but did not need a medical visit or absenteeism [i.e. missing work or school])
Grade 3 = **Severe (**prevents daily activity and/or resulted in medical visit and/or absenteeism [i.e. missing work or school])
Other symptoms to report: ☐ **None**

Symptom	Intensity: Mild, Moderate or Severe	Start date	Stop date

Medications
New medications used following vaccination or changes to previously reported medications: ☐ No ☐ Yes (complete chart below)

Medication name	Dose	Frequency	Start date	Stop date	Reason

Any visit to the Emergency department (ED), Urgent Care, Labor and Delivery, or doctor's office other than routine check-up or prenatal visit? ☐ No ☐ Yes (please explain below and add dates of the visit to ED, Urgent Care, Labor and Delivery, or doctor's office)

Appendix C: Memory Aid for Sequential Group – Visit 1

CISA TDAP/IIV Data Collection Form
Memory Aid Sequential Group
TI07B

Subject ID: _ _ _ _ _ _

Visit 1

Please assess symptoms and fill out memory aid in the evening (after 4pm)

Post-Vaccination Day	**Day 1(Day of vaccination)**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**
Date (dd/MMM/yyyy)	/ /	/ /	/ /	/ /	/ /	/ /	/ /	/ /
Oral Temperature °F	.	.	.	.	.	.	.	.
General Systemic Symptoms: Grade 0 = **None** Grade 1 = **Mild** (noticeable but does not interfere with activity) Grade 2 = **Moderate** (interferes with activity but did not need a medical visit or absenteeism [i.e. missing work or school]) Grade 3 = **Severe** (prevents daily activity and/or resulted in medical visit and/or absenteeism [i.e. missing work or school])								
Chills/Shivering								
Fatigue								
Body Aches								
Headache								
Joint pain								
Nausea								
Vomiting								
Diarrhea								
Abdominal Pain								
Body rash								
Flu Vaccine Local Vaccination Site Reactions: ☐ **RIGHT ARM** ☐ **LEFT ARM** Grade 0 = **None** Grade 1 = **Mild** (noticeable but does not interfere with activity) Grade 2 = **Moderate** (interferes with activity but did not need a medical visit or absenteeism [i.e. missing work or school]) Grade 3 = **Severe** (prevents daily activity and/or resulted in medical visit and/or absenteeism [i.e. missing work or school])								
Pain								
Tenderness								
Swelling– size (mm)								
Redness- size (mm)	mm	mm	mm	mm	mm	mm	mm	mm

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CISA TDAP/IIV Data Collection Form
Memory Aid Sequential Group
TI07B

Subject ID: _ _ _ _ _ _

Other symptoms
1 = Mild (noticeable but does not interfere with activity or measurement)
Grade 2 = **Moderate** (interferes with activity but did not need a medical visit or absenteeism [i.e. missing work or school])
Grade 3 = **Severe (**prevents daily activity and/or resulted in medical visit and/or absenteeism [i.e. missing work or school]) **Other symptoms to report:** ☐ **None**

Symptom	Intensity: Mild, Moderate or Severe	Start date	Stop date

Medications
New medications used following vaccination or changes to previously reported medications: ☐ No ☐ Yes (complete chart below)

Medication name	Dose	Frequency	Start date	Stop date	Reason

Any visit to the Emergency department (ED), Urgent Care, Labor and Delivery, or doctor's office other than routine check-up or prenatal visit? ☐ No ☐ Yes (please explain below and add dates of the visit to ED, Urgent Care, Labor and Delivery, or doctor's office)

Appendix D: Memory Aid for Simultaneous Group – Visit 4

CISA TDAP/IIV Data Collection Form
Memory Aid Simultaneous Group
TI09A

Subject ID: _ _ _ _ _ _

Visit 4

Please assess symptoms and fill out memory aid in the evening (after 4pm)

Post-Visit Day	**Day 1 (Day of visit)**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**
Date (dd/MMM/yyyy)	/ /	/ /	/ /	/ /	/ /	/ /	/ /	/ /
Oral Temperature °F								
General Systemic Symptoms: Grade 0 = **None** Grade 1 = **Mild** (noticeable but does not interfere with activity) Grade 2 = **Moderate** (interferes with activity but did not need a medical visit or absenteeism [i.e. missing work or school]) Grade 3 = **Severe** (prevents daily activity and/or resulted in medical visit and/or absenteeism [i.e. missing work or school])								
Chills/Shivering								
Fatigue								
Body Aches								
Headache								
Joint pain								
Nausea								
Vomiting								
Diarrhea								
Abdominal Pain								
Body rash								

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CISA TDAP/IIV Data Collection Form
Memory Aid Simultaneous Group
TI09A

Subject ID: _ _ _ _ _ _

Other symptoms
1 = Mild (noticeable but does not interfere with activity or measurement)
Grade 2 = **Moderate** (interferes with activity but did not need a medical visit or absenteeism [i.e. missing work or school])
Grade 3 = **Severe (**prevents daily activity and/or resulted in medical visit and/or absenteeism [i.e. missing work or school])
Other symptoms to report: ☐ **None**

Symptom	Intensity: Mild, Moderate or Severe	Start date	Stop date

Medications
New medications used following vaccination or changes to previously reported medications: ☐ No ☐ Yes (complete chart below)

Medication name	Dose	Frequency	Start date	Stop date	Reason

Any visit to the Emergency department (ED), Urgent Care, Labor and Delivery, or doctor's office other than routine check-up or prenatal visit? ☐ No ☐ Yes (please explain below and add dates of the visit to ED, Urgent Care, Labor and Delivery, or doctor's office)

Appendix E: Memory Aid for Sequential Group – Visit 4

CISA TDAP/IIV Data Collection Form
Memory Aid Sequential Group
TI09B

Subject ID: _ _ _ _ _ _

Visit 4

Please assess symptoms and fill out memory aid in the evening (after 4pm)

Post-Vaccination Day	Day 1(Day of vaccination)	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Date (dd/MM/yyyy)	/ /	/ /	/ /	/ /	/ /	/ /	/ /	/ /
Oral Temperature °F	.	.	.	.	.	.	.	.

General Systemic Symptoms:
Grade 0 = **None**
Grade 1 = **Mild** (noticeable but does not interfere with activity)
Grade 2 = **Moderate** (interferes with activity but did not need a medical visit or absenteeism [i.e. missing work or school])
Grade 3 = **Severe** (prevents daily activity and/or resulted in medical visit and/or absenteeism [i.e. missing work or school])

Chills/Shivering								
Fatigue								
Body Aches								
Headache								
Joint pain								
Nausea								
Vomiting								
Diarrhea								
Abdominal Pain								
Body rash								

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CISA TDAP/IIV Data Collection Form
Memory Aid Sequential Group
TI09B

Subject ID: _ _ _ _ _ _

Tdap Vaccine Local Site Reactions: ☐ **RIGHT ARM** ☐ **LEFT ARM** Grade 0 = **None** Grade 1 = **Mild** (noticeable but does not interfere with activity) Grade 2 = **Moderate** (interferes with activity but did not need a medical visit or absenteeism [i.e. missing work or school]) Grade 3 = **Severe (**prevents daily activity and/or resulted in medical visit and/or absenteeism [i.e. missing work or school])								
Pain								
Tenderness								
Swelling- size (mm)	_ _ _ mm	_ _ _ mm	_ _ _ mm	_ _ _ mm	_ _ _ mm	_ _ _ mm	_ _ _ mm	_ _ _ mm
Redness- size (mm)	_ _ _ mm	_ _ _ mm	_ _ _ mm	_ _ _ mm	_ _ _ mm	_ _ _ mm	_ _ _ mm	_ _ _ mm

Other symptoms **1 = Mild** (noticeable but does not interfere with activity or measurement) Grade 2 = **Moderate** (interferes with activity but did not need a medical visit or absenteeism [i.e. missing work or school]) Grade 3 = **Severe (**prevents daily activity and/or resulted in medical visit and/or absenteeism [i.e. missing work or school]) **Other symptoms to report:** ☐ **None**			
Symptom	**Intensity: Mild, Moderate or Severe**	**Start date**	**Stop date**

Medications New medications used following vaccination or changes to previously reported medications: ☐ No ☐ Yes (complete chart below)					
Medication name	**Dose**	**Frequency**	**Start date**	**Stop date**	**Reason**

CISA TDAP/IIV Data Collection Form
Memory Aid Sequential Group
TI09B

Subject ID: _ _ _ _ _ _

Any visit to the Emergency department (ED), Urgent Care, Labor and Delivery, or doctor's office other than routine check-up or prenatal visit? ☐ No ☐ Yes (please explain below and add dates of the visit to ED, Urgent Care, Labor and Delivery, or doctor's office)

Appendix F: Phone Script

CISA TDAP/IIV Memory Aid Phone Script

To be used for visits: 2, 3, 5, 6, & 7

Hello, my name is _____ calling from the Tdap and Flu vaccine study at _____. I would like to review your memory aid information since your most recent study vaccination(s). This call should only take a few minutes. Is now a good time to review this information with you? Do you have your memory aid available to review with me?

Starting from day ___, please provide me with the temperatures that you recorded so far.

Starting with day ___, please tell me if you have recorded any reactions other than 0 (none). If so, in what category/ies? What number(s) did you record for the reactions? Did any of your symptoms prevent you from doing your daily activities or going to work/school?

Next on day ___, please tell me if you have recorded any reactions other than 0 (none). If so, in what category/ies? What number(s) did you record for the reactions? *(continue to ask for each day until all completed days have been reviewed with the subject).* Did any of your symptoms prevent you from doing your daily activities or going to work/school?

Did you report any other symptoms on your memory aid? Please provide me with the symptom, what you rated it, as well as the start and stop dates. *(continue to ask for any additional other symptoms*)

Did you take any new medications or change any of your current medications since you were vaccinated for the study? Please provide me with the medication name, dose, how often you took it, start and stop dates, and why you took the medicine.

Did you have any visits to the emergency room, urgent care, labor and delivery, or unplanned visits to the doctor's office, other than routine check-ups? If yes, please explain.

That concludes our study follow up call. Your next study visit is planned for: _____. Please remember to bring your memory aid with you to that visit so that we can place it in your study file.

Do you have any questions at this time? Please do not hesitate to contact the study team if you have any questions or concerns. We can be reached at _____.

Thank you for your continued study participation. Have a wonderful day.

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Appendix G: Recruitment Letter

Letter from Physician and PI to Potential Participant

[Physician Letterhead]

Date

Dear Potential Participant's Name,

We at [OB/GYN PRACTICE] are committed to improving the care of our mothers and babies through knowledge obtained from research studies. Our practice is participating in a vaccine study titled "A Prospective, Randomized, Open-label Clinical Trial to Assess the Safety and Immunogenicity of Simultaneous versus Sequential Administration of Tetanus Toxoid, Reduced Diphtheria Toxoid, and Acellular Pertussis (Tdap) Vaccine and Inactivated Influenza (Flu) Vaccine in Pregnant Women – Pilot". I think you may be eligible to take part in this study. Participating in this study is voluntary and whether or not you choose to participate, your access to healthcare at [OB/GYN PRACTICE] or Duke will not be affected.

The purpose of this study is to determine if there is a difference in how the immune system responds when giving pregnant women the Tdap and Flu vaccines on the same day versus on different days. Both vaccines are currently licensed in the United States and recommended by the Advisory Committee on Immunization Practices (ACIP) and the American College of Obstetrics and Gynecology (ACOG) for use in pregnancy. Previous studies have shown that both vaccines provide protection to both the pregnant mother and her baby from the Flu, as well as Tetanus, Diphtheria, and Pertussis (Whooping Cough).

If you decide to participate you will be randomized (like flipping a coin) to one of two groups. In one group, you will receive injections of the Tdap vaccine and the Flu vaccine on the same day. In the other group you will receive an injection of the Flu vaccine on one day and approximately 3 weeks later you would receive an injection of the Tdap vaccine. You will be followed for the remainder of your pregnancy through six weeks after delivery.

A member of the research team will speak with you either on the phone or at one of your next clinic visits to tell you more about the study and answer any questions you may have. If you do not want to be approached about the study, please contact the research team by phone at 919-613-9630 or by email at obresearch@duke.edu and indicate that you don't want to be approached. However, if you would like more information, please contact the research team by phone at 919-613-9630 or by email at obresearch@duke.edu.

Thank you in advance for your research consideration!

Sincerely,

Patient's OB/GYN Practice, Lead Provider [OB/GYN PRACTICE]	Geeta Swamy, MD Principal Investigator

Appendix H: Recruitment Email

Recruitment Email (sent from obresearch@duke.edu)

Dear [**woman's name**],

I hope this email finds you well. Please see the letter attached from your prenatal care provider at [**clinic's name**] about a research study evaluating the difference between giving pregnant women the Flu vaccine and Tetanus, Diphtheria, and Pertussis (Tdap) vaccine on the same day versus on different days. A staff member from the study team will be contacting you soon to talk about the study and to see if you may be interested in participating.

We look forward to speaking with you.

Sincerely,

Dr. Geeta Swamy

[Attach recruitment letter from appropriate clinic to email]

Version 102214

Appendix I: Informed Consent Form