



STUDY PROTOCOL

# Optimising the Timing of whooping cough Immunisation in MUMs: a randomised controlled trial investigating the timing of pertussis vaccination in pregnancy (OpTIMUM): a protocol paper [version 1; peer review: 1 approved, 1 approved with reservations]

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## Abstract

**Background:** Pertussis is a highly infectious respiratory illness caused by the bacteria *Bordetella pertussis*. A resurgence of pertussis, even in countries with good vaccine coverage, has led to an increase in infant deaths. In response to this, many countries have introduced pertussis vaccination in pregnancy. This strategy is effective at preventing infant disease, but there remains uncertainty about what gestational timing is best to ensure maximal protection of the infant. These uncertainties are the rationale for this randomised controlled trial and a sub-study investigating pertussis-specific antibody in breastmilk.

**Protocol:** We will recruit 354 pregnant women and will randomise them to receive their pertussis vaccination in one of three gestational age windows:  $\leq 23+6$ , 24-27+6 and 28-31+6 weeks of gestation. Vaccination will be with Boostrix-IPV® and participants will be asked to complete a symptom diary for seven days following vaccination. Blood sampling will be performed prior to vaccination, two weeks following vaccination and at the time of delivery. A cord blood sample will be collected at delivery and a blood sample collected from the infant 4-10

## Open Peer Review

Approval Status ? ✓

	1	2
<b>version 1</b> 14 Jun 2021	? <a href="#">view</a>	✓ <a href="#">view</a>

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weeks after completion of the primary immunisations. Individuals participating in the breastmilk sub-study will provide a sample of colostrum within 48 hours of delivery and samples of breastmilk at two weeks and around five-six months. Blood samples will be analysed using enzyme linked immunosorbent assay (ELISA) techniques for pertussis toxin, filamentous haemagglutinin and pertactin. A subset of serum samples will also be analysed using a functional assay. Colostrum and breastmilk samples will be analysed using functional assays.

**Discussion:** Although pertussis vaccination has been shown to be safe and effective in pregnancy there remains debate about the optimal timing for the administration during pregnancy. This study will investigate antibody responses in serum and breastmilk when vaccination is performed in three different time periods.

**Clinicaltrials.gov registration:** NCT03908164 (09/04/2019)

### Keywords

Pertussis, vaccination, pregnancy, antibody, breastmilk, gestational age

Any reports and responses or comments on the article can be found at the end of the article.

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## Introduction

Pertussis is a highly infectious respiratory illness caused by the bacteria *Bordetella pertussis*. Following the introduction of pertussis vaccination into infant schedules, the incidence of pertussis dropped significantly, however in recent years there has been a resurgence of pertussis disease, even in countries with good vaccination coverage, which led to an increase in infant deaths from pertussis<sup>1</sup>. In response to this, many countries have introduced pertussis vaccination in pregnancy including, in October 2012, in the United Kingdom (UK). This strategy increases pertussis vaccine antigen-specific immunoglobulin G (IgG) in pregnant women, resulting in more IgG available to cross the placenta, with a consequent increase in pertussis-specific IgG concentration in the infant at birth. This increased pertussis-specific IgG concentration protects the infant until they have completed their primary immunisations which, in the UK, is usually at around 16 weeks of age. Evaluation of pertussis vaccination in pregnancy has shown that this programme is effective in preventing severe pertussis disease in infants<sup>2-6</sup> and is safe for the mother and infant<sup>4,7,8</sup>. Whilst it is now clear that pertussis vaccination in pregnancy can safely reduce the burden of disease in young infants - prior to completion of their primary immunisations - it has not been established whether there is an optimal time to vaccinate in pregnancy to ensure maximal protection of the infant. This is reflected in the different guidelines currently in place in different countries: in the UK vaccination is offered between 16 and 32 weeks, in the US women are recommended to have the vaccination between 27 and 36 weeks, in Australia between 20 and 32 weeks, in Ireland from 16–36 weeks and in New Zealand from 16 weeks. When the programme was introduced in the UK, the recommendation was to give the vaccine at 28–32 weeks because of concerns that the speed of antibody decay would result in poor protection being provided to infants if vaccination were performed too early in pregnancy<sup>9</sup>. This position was challenged by a number of reports showing that vaccination earlier in the third trimester might provide higher antibody concentrations in the infant at birth compared with later in the third trimester<sup>10,11</sup> and a Swiss study showed that vaccination in the second trimester resulted in significantly increased antibody levels in cord blood compared to those vaccinated in the third trimester<sup>12</sup> and that vaccination in the second trimester can provide improved protection to preterm infants<sup>13</sup>. In April 2016 the guidance in the UK changed to recommend vaccination at any time from 16 weeks, after the detailed anomaly scan had been performed (Public Health England, 2018). There are clear logistical benefits to being able to offer the vaccination for the widest possible time period to maximise opportunities for pregnant women to receive the vaccine; however, there remains uncertainty about the best timing for the vaccination to be offered. A group in the US reported that vaccination within the recommended time window of 27–36 weeks was more protective than vaccination given during pregnancy but outside of this window, with a non-significant trend towards a greater benefit when given at 27–31 weeks of gestation<sup>14</sup>, although these findings have been challenged<sup>15</sup>.

The lack of agreement about the optimal timing of pertussis vaccination for the protection of the infant is the rationale for this randomised controlled trial investigating the impact of

administration of pertussis vaccination in three gestational age periods within the overall window currently recommended in the UK. As there is little information about functional immunity of anti-pertussis vaccine antigen IgG in serum and secretory(s) IgA in colostrum/breastmilk following vaccination in pregnancy we are also conducting exploratory sub-studies investigating these aspects.

## Protocol

The current protocol is version 3.0 dated 27.7.20. The key protocol contributors were Anna Calvert, Paul Heath, Christine Jones and Kirsty Le Doare. This is a phase IV randomised trial taking place at six sites in the UK to assess equivalence of vaccination given in three different time windows during pregnancy.

This version of the protocol includes several adaptations to facilitate the conduct of the study in the context of the coronavirus disease 2019 (COVID-19) pandemic: these include an extension to the period for completion of the infant visit, from 4–6 weeks after completion of the primary series to 4–10 weeks, and capacity for the infant visits to be done remotely.

## Objectives and outcome measures

Objectives and outcome measures are detailed below (Table 1).

## Study population and sample size

We will recruit pregnant women prior to them receiving their pertussis vaccination.

Based on previous studies of cord blood<sup>10,12,16</sup>, the log<sub>10</sub> Standard Deviation is about 0.5 for pertussis toxin (PT), 0.4 for filamentous haemagglutinin (FHA) and 0.55 for pertactin (PRN). To assess equivalence to within a 1.8 fold margin, and assuming the higher standard deviation of 0.55, a sample size of 100 per group is needed (two-sided 95% CI on the fold difference, 80% power) which, allowing for a drop-out rate of around 10% and a rate of prematurity of around 8% would require 354 women to be recruited.

## Methods

This is a parallel group randomised trial assessing equivalence which will be performed in six locations across England (St George's University Hospitals NHS Foundation Trust, Kingston Hospital NHS Foundation Trust, University Hospital Southampton NHS Foundation Trust, Oxford University Hospitals NHS Foundation Trust, University Hospitals Bristol NHS Foundation Trust, Manchester University NHS Foundation Trust). Two sites: St George's University Hospitals NHS Foundation Trust and University Hospital Southampton NHS Foundation Trust will take part in the sub-study investigating breastmilk. We will recruit 354 pregnant women. These women will be recruited in pregnancy and will be randomised into one of three groups to receive a pertussis containing vaccine: ≤23+6, 24–27+6 and 28–31+6 weeks of gestation.

## Recruitment

Women may be identified in a number of ways according to local arrangements. Some may be identified when they attend hospital or community appointments and approached about

**Table 1. Study objectives and outcome measures.**

	<b>Objectives</b>	<b>Outcome measures</b>
<b>Primary</b>	<ul style="list-style-type: none"> <li>To determine if pertussis vaccination at different time points in pregnancy results in equivalent concentrations of pertussis specific IgG in the term infant at birth</li> </ul>	<ul style="list-style-type: none"> <li>Anti-pertussis toxin (PT), filamentous haemagglutinin (FHA) and pertactin (PRN) specific IgG concentration in cord blood of term infants at delivery</li> </ul>
<b>Secondary</b>	<ul style="list-style-type: none"> <li>To determine if pertussis vaccination at different time points in pregnancy results in equivalent concentrations of pertussis specific IgG in the preterm infant at birth</li> <li>To investigate the rate of fever and local reactions in women receiving the vaccine in pregnancy comparing those who are receiving the vaccine for the first time and those who have previously received the vaccine in pregnancy</li> <li>To describe the kinetics of the IgG response to pertussis vaccination during pregnancy</li> <li>To describe the placental transfer of IgG following administration of vaccine at three discrete time points</li> <li>To evaluate the impact of timing of pertussis vaccination in pregnancy on pertussis vaccine antigen-specific IgG concentration in the infants following their primary immunisation schedule</li> <li>To explore the impact of repeated vaccination on the pertussis vaccine antigen-specific IgG response in women who have received a pertussis vaccination in a previous pregnancy</li> </ul>	<ul style="list-style-type: none"> <li>Anti-pertussis toxin (PT), filamentous haemagglutinin (FHA) and pertactin (PRN) specific IgG concentration in cord blood of preterm infants at delivery</li> <li>Rates of fever and local reaction following vaccination</li> <li>Anti-pertussis toxin (PT), filamentous haemagglutinin (FHA) and pertactin (PRN) specific IgG concentration in maternal blood prior to vaccination, at 14 days after vaccination and at delivery</li> <li>Anti-pertussis toxin (PT), filamentous haemagglutinin (FHA) and pertactin (PRN) specific IgG concentration in maternal blood and cord blood at delivery</li> <li>Anti-filamentous haemagglutinin (FHA) and Pertactin (PRN) specific IgG concentration in cord blood of term infants at delivery.</li> <li>Anti-pertussis toxin (PT), filamentous haemagglutinin (FHA) and pertactin (PRN) specific IgG concentration in infants one month after completion of their primary immunisations</li> </ul>
<b>Exploratory</b>	<ul style="list-style-type: none"> <li>To assess the function of anti-PT IgG in blood samples from women post vaccination, in the cord blood of infants and in the infant post vaccination.</li> <li>To investigate functional immunity of colostrum within 48 hours of delivery and of breastmilk at 14 days and 5 months following delivery in women vaccinated at three different time points.</li> </ul>	<ul style="list-style-type: none"> <li>Functional antibody assays on serum and breastmilk.</li> </ul>

the study, others may be identified from hospital lists of women due to attend for antenatal care and have information sent out to them. In the latter circumstance, this identification will be performed by members of the clinical team - the research team will not have access to identifiable information without consent. After a woman has received information about the study at any point from the time of the booking appointment, she may agree to being contacted by a member of the study team. This would require her to complete the information on the invitation letter and completion of this slip will be assumed to be consent to be contacted.

The following means of approaching women may be used at sites: information sent through the post/email about the study, information provided directly when women attend for a community or hospital appointment, women seeing a leaflet or poster about the study when attending a hospital appointment, women seeing information about the study on an institutional website (both hospital and university), on social media or in a press release.

We anticipate that recruitment will take place at different points according to the local clinical and research arrangements, but many women will be approached when they attend for their routine anomaly ultrasound scan at around 20 weeks of gestation.

### Eligibility

Women will be eligible to take part if they are pregnant and have not yet received pertussis vaccination, if they are willing and able to take part in the study and provide informed consent, if they have had a routine anomaly ultrasound scan with no evidence of life-limiting congenital abnormalities and if they are at or less than a gestation of 23+6 weeks of gestation at the time of screening. Women will be excluded from participation if they are aged less than 16 years, if they have had confirmed or suspected pertussis infection in the previous five years, if they have a known immune deficiency or have received immunosuppressive medication within six months of screening or if, in the opinion of the investigator, they are unlikely to complete follow up (Table 2)

### Consent

Informed consent can be taken by any appropriately trained member of the research team. Eligible women will be asked to

sign a consent form before any study procedures take place<sup>17</sup>. There is no minimum period between information being provided and consent being given providing that the individual has had sufficient time to consider the study and ask any questions. Informed consent will be taken for maternal and infant participation in the study at enrolment. Consent for infant participation in the study will be re-confirmed with parents verbally prior to any samples being taken from the infant. Participants will be asked if they agree to any remaining samples being retained for future related, ethically approved research. Consent to this will not be required for participation in the main study. Participants at St George's University Hospitals NHS Foundation Trust and University Hospital Southampton NHS Foundation Trust will be asked if they are willing to take part in an additional study investigating functional immunity in breastmilk following vaccination in pregnancy. If they are willing to participate in this aspect of the study, they will be asked to provide consent for this at the time of initial consent.

### Randomisation

Participants will be randomised on a 1:1:1 ratio to the three timing groups:  $\leq 23+6$ , 24–27+6 and 28–31+6 weeks of gestation. A computerised block randomisation list will be produced by the study statistician. Group allocations will be placed inside opaque envelopes bearing the corresponding participant number. Each centre will be provided with the necessary envelopes. On recruitment to the study, each participant will be allocated, in order of inclusion, the next available participant number. Neither the participants nor the researchers will be blinded as to group allocation.

### Study procedures

Participants will be involved in the study from the time of enrolment at around 20 weeks of gestation to the time of delivery (a period of around 20–22 weeks in total), and their infants will be involved in the study from birth until the age of 5–6 months (around 5–6 months in total) (Table 3)

**Screening visit.** This visit must take place at or before 23+6 weeks of gestation. Eligibility will be confirmed at this visit and eligible individuals will be asked to sign a consent form. Demographic details, past medical history, details of the current pregnancy, concomitant medication and vaccination history will be recorded. Participants will be randomised into one of the three study groups.

**Table 2. Eligibility criteria for the OpTIMUM study.**

Inclusion criteria	Exclusion criteria
<ul style="list-style-type: none"> <li>Pregnant and not yet having received pertussis vaccination</li> <li>Willing and able to comply with study procedures and provide informed consent</li> <li>Documentation of a routine anomaly ultrasound scan with no life limiting congenital anomalies identified</li> <li>Gestation <math>\leq 23+6</math> weeks</li> </ul>	<ul style="list-style-type: none"> <li>Age less than 16 years</li> <li>Confirmed or suspected pertussis in previous five years</li> <li>Known diagnosis of immune deficiency</li> <li>Receiving immunosuppressive medication within six months of enrolment in the study (this does not include inhaled or topical steroids)</li> <li>In the opinion of the investigator is unlikely to complete follow up</li> </ul>

**Table 3. Summary of study visits.**

Visit Number	Screening visit	Vaccination visit	Follow up visit	Delivery visit	Breastmilk sub-study visit 1*	Breastmilk sub-study visit 2*	Infant visit
<b>Timing</b>	At or before 23+6 weeks	According to study allocation	V+14 (+/- 2)	Delivery	<48 hours from delivery	D+14(+/- 2)	28–70 days following third pertussis vaccination
<b>Activity</b>	Screening & enrolment	Maternal blood sampling and vaccination. Diary card provided. Questionnaire.	Maternal blood sampling. Diary card collected.	Maternal blood and cord blood sampling	Colostrum sampling	Collection of breastmilk sample	Infant blood sampling. Collection of breastmilk sample if still breastfeeding

\*For those women participating in the breastmilk sub-study

**Vaccination visit.** This visit will take place at the time indicated by the study group allocation. For participants randomised to receive vaccination at  $\leq 23+6$  weeks of gestation, the vaccination visit may take place on the same day as the screening visit. Participants will have a blood sample taken (sample A) prior to receiving the pertussis containing vaccine. Participants will be asked to complete a short questionnaire regarding their views on how the whooping cough vaccine can best be administered in pregnancy<sup>17</sup>. Participants will receive a diary card following vaccination and will be given instructions about how this should be completed for the seven days following vaccination.

**Follow up visit.** This visit will take place 14 days following vaccination. At this visit all participants will have a blood sample collected (sample B), the diary card will be collected, and participants will be asked about any adverse events. Participants will be provided with blood sampling packs for blood sampling at the time of delivery.

**Delivery visit.** This visit will take place at the time of delivery. Following delivery, a blood sample will be taken from the cord (sample C) and the participant (sample D). If blood sampling is not performed at delivery, participants will be asked if a blood sample can be obtained from them within the first week following delivery and if a cord sample is not obtained parents will be asked if a blood sample can be obtained from the infant within the first week following delivery.

Participants who have agreed to take part in the breastmilk sub-study will be asked if they still plan to breastfeed and if they are happy to provide a sample of colostrum within the first 48 hours following delivery. If women are still willing to take part in the study, they will be provided with information about colostrum sampling and containers and instructions for the collection of the sample.

**Post-delivery visits for those women participating in the breastmilk sub-study.** For those participants in the breastmilk sub-study there will be a follow-up visit within 48 hours to collect a colostrum sample and a further visit at 14 days to collect a breastmilk sample.

**Infant visit.** This visit will take place 28–70 days following infant completion of the primary vaccination schedule. The primary vaccinations will have taken place outside the study in primary care (Table 4).

At the infant visit a blood sample will be obtained from the infant (sample E). This will be a venous sample where possible, although a capillary sample can be used if a venous sample cannot be obtained. At this visit details will be taken about the infant's past medical history, including a detailed history of any respiratory illnesses or contact with individuals with a diagnosis or clinical symptoms of pertussis, and a full vaccination history will be recorded.

Those participating in the breastmilk sub-study who are still breastfeeding at this visit will be asked for a sample of breastmilk.

#### Contact with participants

Participants will be phoned at 36 weeks of gestation to remind them about the importance of the delivery samples. Parents will be contacted at 8 weeks to remind them about the infant vaccinations and again at 14–16 weeks to check that vaccinations have been scheduled and to arrange the infant visit. We hope that this contact will help to encourage retention of participants in the study.

#### Intervention

All participants will receive a pertussis containing vaccine licensed for use in pregnancy - Boostrix-IPV<sup>®</sup> manufactured by GlaxoSmithKline, unless the specific brand of pertussis containing vaccine routinely used in the UK changes during the course of the trial. Boostrix-IPV<sup>®</sup> contains PT (8 micrograms), FHA (8 micrograms) and PRN (2.5 micrograms) as well as diphtheria toxoid (not less than 2 international units), tetanus toxoids (not less than 20 international units) and inactivated polio virus types 1–3 (type 1 40 D-antigen unit, type 2 8 D-antigen unit, type 3 32 D-antigen unit). There is no placebo. All participants will receive one dose of the vaccine by intramuscular injection into the non-dominant arm according to the information in the summary of product characteristics (SmPC). All vaccines will be requested in the usual way by individual

**Table 4. Routine infant vaccinations included in the primary schedule.**

Vaccine	Product names	Age (months)		
		2	3	4
DTaP/IPV/Hib/Hep B	Infanrix Hexa®	x	x	x
Rotavirus	Rotarix®	x	x	
MenB	Bexsero®	x		x
PCV13*	Prevenar13®	x		x

\*It is possible that during this study the routine infant vaccine schedule may change to include a single pneumococcal vaccine in the primary schedule followed by a booster at 12 months. Because the infant would be vaccinated according to the routine vaccine schedule this would not necessitate a change to the protocol.

sites and their handling and management will be subject to standard procedures of the pharmacy.

This vaccine will be given at the time period assigned by randomisation. All possible time periods are within that recommended in the UK as part of routine practice. The time period for the first group is defined as  $\leq 23+6$  weeks of gestation. This is to allow for recruitment following the routine anomaly ultrasound scan of the small number of women who attend just before they are 20 weeks of gestation. No pertussis vaccine will be given as part of the study at less than 16 weeks of gestation.

## Laboratory processing and analysis

### Serum

#### • Sample collection

Blood samples will be obtained by venepuncture by appropriately trained members of the research team. If it is not possible to obtain a venous sample from the infants, capillary blood sampling is acceptable, although venous blood samples are preferable. The required volume of maternal blood is 7.5mls, cord blood 7.5mls and infant blood 3–5mls (no more than 1% of circulating blood volume). Samples will be labelled with the participant number (prefixed with OPT), date of sampling and study visit information using stickers provided checking that the sample letter corresponds with the correct visit number.

For multiple births there will be an amended system for labelling samples. The first infant will have the same number as the mother with the addition of the suffix T1, the second infant will have the same number as the mother with the addition of the suffix T2 and in the case of triplets the third infant will have the same number as the mother with the addition of the suffix T3 (e.g. OPT005T2 for infant two born to participant 005). NB: Only one cord sample needs to be obtained in the case of multiple births, but if a cord sample is not obtained an infant sample should be collected from each infant.

#### • Sample transport

The preference will be for samples to be sent on the same day they are obtained, however if this is not possible, they will be stored in the fridge at 2–8°C. Samples will be packaged appropriately in accordance with regulations for posting of biological specimens through public post. Samples will be sent to the Public Health England (PHE) laboratory at Porton Down. They will be transported at ambient temperature and will not need monitoring.

#### • Sample processing and analysis

On arrival at the PHE laboratory the sample will be centrifuged for 10 minutes at 2600rpm in Sorval Legend RT centrifuge and the serum will be aliquoted and stored frozen at -70°C. An aliquot of 200µl will be saved from all the cord and infant samples for functional analysis. All samples will have IgG antibody against PT, FHA and PRN measured, relative to the 1st World Health Organisation (WHO) International Pertussis Standard Serum 06/140, using in house, validated, enzyme linked immunosorbent assay (ELISA) techniques<sup>18</sup> following controlled standard operating procedures (SOPS) in a good practice (GxP) facility. Briefly, this involves coating 96 well microplates with antigen, then after washing off unbound material serial dilutions of test serum samples, standard serum and IQC serum prepared in a separate low binding plate are added and incubated to facilitate antibody binding. Plates are washed to remove unbound antibody and incubated with goat anti-human IgG antibody conjugated to alkaline phosphatase to detect the bound antibody. After a further wash AP yellow substrate (p-nitrophenyl phosphate) is added and the reaction is stopped after 1hr by the addition of 3M sodium hydroxide. The optical density of plates is measured on a VERSAmax plate reader. Data analysis is done using **SOFTmax<sup>®</sup> PRO** (Enterprise) software using validated templates for each assay which fit an unweighted four parameter logistic model curve to the standard serum dose response data on each plate. OD values from duplicate serial dilutions for each test and QC serum are used to interpolate antibody concentrations from the standard dose response curve on each plate and reportable values assigned following defined rules detailed assay SOPs to ensure that all assay and test sample acceptance criteria are met.

For the sub-study investigating functional aspects of anti-pertussis vaccine antigen immunity a smaller set of samples will be used. Details will be provided to the laboratory team and the aliquots from the cord and infant samples of these participants will be transferred to the pathogen immunology group to be analysed using a serum bactericidal antibody assay<sup>19</sup>.

### Colostrum/breastmilk

#### • Sample collection

Colostrum and breastmilk samples will be collected by hand expression into a sterile container after the breast has been cleaned. The samples will be taken immediately following a feed and will include colostrum/breastmilk from both breasts.

Samples will be labelled with the participant number, date of sample collection and study visit information using stickers provided.

### Sample processing and analysis

The colostrum/breastmilk will be centrifuged for 30 minutes at 3000 rpm in Heraeus Function Line 400e centrifuge and the lipid layer removed at the local participating site. The aqueous fraction will then be transferred to cryovials and frozen at -70°C. When sample collection is completed, samples from University Hospitals Southampton NHS Foundation Trust will be couriered to St George's, University of London where functional assays will be performed. No colostrum or breastmilk samples will be transferred to other labs. Breast milk and colostrum samples will be analysed for anti- PT, FHA and PRN specific IgG antibodies using a multiplex immunoassay (MIA) according to good laboratory practice. Magplex microspheres will be coupled to PT, FHA and PRN followed by incubation with the samples. The 1<sup>st</sup> WHO International Pertussis Standard Serum 06/140 will be used as a reference standard and for interpolation of IgG levels.

### Follow up duration

Participants will be followed up until delivery of their infant (with a further maternal visit within 48 hours, at two weeks and five months for those participating in the breastfeeding sub-study). Infants of participants will be seen following completion of their primary vaccinations.

### Data collection and management

Participants will be given a unique participant identification number at the time of enrolment which will be used to identify all study documents. Relevant data for the study will be recorded using REDCap electronic data capture tools hosted at St George's, University of London.

### Case report forms

For this study, data will be entered directly into REDCap using the unique participant identification number and this electronic case report form (eCRF) will be the source document. These eCRFs will be accessible to the local site and the central site throughout the study. The central team will check the data being entered by sites and will communicate with local sites about missing data. Paper copies of CRFs will be available for use in case of technical issues.

### Patient questionnaire

The patient questionnaire will also be completed directly into REDCap by participants with a member of the research team present to answer any questions.

### Study diaries

Participants will be asked to complete a paper diary for seven days after vaccination. These will be collected at visit three and the information entered into REDCap. The original will be retained in the participant study folder.

### Other documents

Local sites will have paper copies of the participant contact details, consent forms and a copy of the participant information sheet<sup>17</sup>. These will be retained by the local site and will not be shared with the central team.

### Data protection and patient confidentiality

All investigators and trial site staff must comply with the requirements of the Data Protection Act 2018 with regards to the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles. Local sites will collect contact details from participants and will retain consent forms which contain identifiable information. The paper copies of this information will be stored in a secure location in a private office; the digital records of this information will be stored on password protected files on secure university or hospital computers. This information will be used by the local team only and no identifiable information will be shared with the central team. We will be collecting information about the participants' demographics including ethnicity and age- past medical history, obstetric history, details of their current pregnancy and a detailed vaccination history. At each visit we will collect information about whether any adverse events have been experienced. None of this information will be stored alongside any identifiable information. This information will be retained in the study database for five years after the end of the study. No data will leave the UK.

All members of the trial management group will have access to the full dataset on request. This will include the principal investigators at all sites.

### Safety

This study uses a licensed vaccine given according to national guidelines. There is no increased risk to participation in the study compared with receiving the vaccine in routine care.

### Post-trial care

Women will receive a single pertussis containing vaccine in their pregnancy according to national guidelines in the UK. There is no need for any post trial care to be arranged.

### Clinical oversight

The Chief Investigator will provide clinical oversight of the safety of participants in the trial, including an ongoing review of the risk / benefit. He will immediately review all reports of suspected unexpected adverse reactions (SUSARS) and will review all serious adverse event (SAE) reports as these become available throughout the study. He will review a line listing of adverse events (AEs) provided by the Sponsor on a three-monthly basis throughout the study.

### Vaccine administration

All vaccinations will be given by trained members of staff who are able to deal with an allergic reaction to the vaccine. All participants will be observed for 20 minutes following



vaccine administration to ensure that they remain well. All participants will have access to contact details for the study team which they will be able to use if they have any concerns about adverse events following vaccine administration. This is a licensed vaccine so significant adverse reactions are extremely unlikely.

#### Adverse events/serious adverse events

AEs and SAEs will be asked about at every study visit. AEs which could reasonably be expected to occur after vaccination (those which are 'very common' or 'common' according to the summary of product characteristics) do not need to be recorded on the AE log although a note should be made of these on the eCRF. These include:

-Headache

-GI disorders such as vomiting, abdominal pain and nausea

-Injection site reactions such as redness and/or swelling, haematoma at injection site, pruritis, induration or injection site pain

-Fatigue

-Pyrexia (fever  $\geq 37.5^{\circ}\text{C}$ )

Other adverse events which occur within 28 days of the vaccine administration will be recorded on the AE log as will medically attended adverse events which occur at any time in the study period. Medical attendance which is a routine part of antenatal care will not be considered an AE.

We will not require SAE reporting for women being admitted to hospital for delivery, including those women who are admitted for induction of labour and caesarean section. Neither instrumental delivery nor caesarean section would be reported as an SAE although the reasons for this may be, for example, if a caesarean section took place because of a significant antepartum haemorrhage the latter would be reported as an SAE although the caesarean section would not. Whether to report complications of delivery as an SAE should be decided at the discretion of the local PI following discussion with the Sponsor and CI as necessary.

#### Serious adverse reactions and suspected unexpected serious adverse reactions

Any SUSAR will need to be reported to the Sponsor irrespective of how long after vaccine administration the reaction has occurred until resolved. Where a participant withdraws consent for further processing of data, this does not preclude the reporting of SARs and SUSARs which are required to continue being reported according to the protocol for regulatory purposes.

#### Specific issues for studies in pregnancy

The collection of safety data in studies involving vaccination in pregnancy raises specific issues and it is important that the information collected about some outcomes of special interest is collected as consistently as possible<sup>19</sup>.

The Global Alignment of Immunization safety Assessment in pregnancy (GAIA) consortium have developed case definitions for a range of outcomes of interest. These should be used for the following events.

- Congenital microcephaly
- Failure to thrive
- Low birth weight
- Neonatal encephalopathy
- Respiratory distress in the newborn
- Small for gestational age
- Antenatal bleeding
- Dysfunctional labour
- Fetal growth restriction
- Gestational diabetes mellitus
- Congenital anomalies
- Neonatal death
- Neonatal infections
- Preterm birth
- Stillbirth
- Hypertensive disorders of pregnancy
- Maternal death
- Non-reassuring fetal status
- Post-partum haemorrhage

More details about these can be found [here](#).

#### Study monitoring

As this study uses a licensed vaccine currently used in pregnancy there is no need for a data monitoring committee and interim analysis is not required. The study will be monitored remotely and via site self-assessment. If required, participating sites will permit trial-related on-site monitoring, audits, and regulatory inspection, providing direct access to source data/documents. Trial participants are informed of this during the informed consent discussion. Participants will consent to provide access to their medical notes.

#### Protocol amendments

For any amendment to the study, the Chief Investigator or designee, in agreement with the Sponsor will submit information to the appropriate body in order for them to issue approval for the amendment. The Chief Investigator or designee will work with sites (R&D departments at NHS sites as well as the study delivery team) so they can put the necessary arrangements in place to implement the amendment to confirm their support for the study as amended.

#### Statistical plan

**Summary of baseline data and flow of patients.** At the end of the study, a flowchart will be used to summarise the number of women approached, consented, assigned to the different study

arms, receiving vaccinations at the planned times, completing the study protocol and analysed for the primary outcome, as recommended by the [CONSORT](#) statement. Baseline data comparing the three trial groups will be summarised in a table format and will compare median age, number of previous pregnancies, recruiting study site, history of previous pertussis containing vaccination and underlying medical conditions. Statistical analyses will be performed on [STATA](#) version 15.

#### Primary outcome analysis

##### **To investigate if pertussis vaccination at different time points in pregnancy results in equivalent concentrations of pertussis specific antibodies in the term infant at birth.**

Geometric mean concentrations will be calculated for each group with 95% confidence intervals (CIs). To assess equivalence the geometric mean fold ratio with 95% CI will be calculated for group 3/group 1 and group 3/group 2 (i.e. late vs. early) and equivalence achieved if the upper end is below 1.8 and the lower end above 1/1.8 (0.55) (the equivalence margin). In addition, groups 1 and 2 will be compared for equivalence and all groups will be compared with one-another for differences using ANOVA on log-concentrations, or Kruskal-Wallis test in the event of non-normal log-concentration distributions. Finally, normal errors regression will be used to compare groups 1 and 2 to group 3 with adjustment for covariates including maternal age, gestation and prior vaccination history.

#### Secondary outcome analysis

##### **To investigate if pertussis vaccination at different time points in pregnancy results in equivalent concentrations of pertussis specific antibodies in the preterm infant at birth.**

The exact number of infants born preterm is not known, but local data suggests a rate of prematurity of about 8%. If this is the case, there will be around 30 preterm infants in this study. Geometric mean concentrations will be calculated for each group with 95% confidence intervals. The groups will be compared according to timing of maternal vaccination as is done with the term infants.

##### **To investigate the rate of fever and local reactions in women receiving the vaccine in pregnancy comparing those who are receiving the vaccine for the first time and those who have previously received the vaccine in pregnancy**

Within each group the rates of fever ( $>38^{\circ}\text{C}$ ) and local reactions (redness, swelling and tenderness) will be calculated (with 95% exact CIs) and compared using Fisher's exact test.

##### **To describe the kinetics of the antibody response to pertussis vaccination in pregnancy**

Within each group geometric mean concentrations at each time point will be calculated with 95% CIs as well as the geometric mean fold change between each successive time points. These will be compared between groups using ANOVA or Kruskal-Wallis test in the event of non-normal log-concentrations distributions. Normal errors regression will be used to compare groups 1 and 2 to group 3 with adjustment

for covariates including maternal age and prior vaccination history. Multivariable analysis will be performed allowing for various factors and modelling will be performed for the antibody kinetics in the mother following vaccination based on a relationship such as log-titre vs log-time. This will include random effects mixed models to allow for individual declines and to take into account the different timing between the 14-day sample and birth sample.

##### **To describe the placental transfer of antibody following administration of vaccine at three discrete time points**

The transfer ratio will be calculated as the ratio of cord to maternal blood and the geometric mean ratio calculated with 95%CI. This ratio (on a log-scale) will be compared between groups using ANOVA or Kruskal-Wallis test in the event of non-normal log-ratios. Finally, normal errors regression will be used to compare log-ratios for groups 1 and 2 to group 3 with adjustment for covariates including maternal age.

##### **To explore the impact of repeated vaccination on the antibody response in women who have received a pertussis vaccination in a previous pregnancy**

This will be done as described above by inclusion as a covariate in the normal errors regression models. We expect that around 40% will have received a prior pertussis vaccine in pregnancy. Assuming 40%, when combining groups, we will have approximately 120 with prior history of vaccination and 180 without; with a log-10 SD of 0.55 this will allow 1.5-fold differences to be detected (80% power, 5% significance). In addition, the interaction term between prior vaccination and group on antibody response will be assessed to see if the effect of prior vaccination depends on timing.

##### **To evaluate the impact of timing of pertussis vaccination in pregnancy on antibody concentration in the infants following completion of their primary immunisation schedule.**

Geometric mean concentrations will be calculated for each group with 95% confidence intervals. Groups will all be compared with one-another for differences using ANOVA on log-concentrations, or Kruskal-Wallis test in the event of non-normal log-concentrations distributions. Finally, normal errors regression will be used to compare groups 1 and 2 to group 3 with adjustment for covariates including time of blood sample.

#### Ethical approval

The study has been approved by the NHS Health Research Authority and York and Humber Research Ethics Committee (19/YH/0050).

#### Patient and public involvement

This study has been discussed during its development with a patient and public involvement (PPI) group based at St George's, University of London which is specifically interested in studies relating to maternal and infant infections. This group is made up of pregnant women and those who have

recently had a baby and their partners, and parents of young children including those who have previously participated in research studies. The study design, management of the study and participant facing documents have previously been discussed at meetings of this group and we will continue to discuss the study as it progresses.

### Trial registration

This trial was registered with Clinicaltrials.gov ([NCT03908164](https://clinicaltrials.gov/ct2/show/study/NCT03908164)) on 9<sup>th</sup> April 2019.

### Dissemination of information

We plan to present the data from this study at relevant national and international conferences and to report the results in a peer reviewed journal. Authorship will be determined by the Chief investigator and senior author and no professional writers will be sought. The results from the study will also be shared with participants of the study in a summary form and with a link to any related publications. We will discuss the results of the study with members of our PPI group as part of our cycle of meetings. The data will be registered in an open access repository.

### Limitations

This study has been significantly affected by the COVID-19 pandemic which has necessitated several protocol amendments. The main change has been to extend the period during which infant follow up can take place to try to maximise the number of infants who could be followed up during the periods between lock downs and the related restrictions on research activities.

### Current status

We have completed recruitment and follow up for this study and are currently performing the laboratory analysis.

### Role of sponsor and funders

The sponsor and funders had no input into the study design and will have no input into data collection or data analysis.

### Study organisation

The OpTIMUM study is sponsored by St George's, University of London and the study activities are coordinated by the research team at St George's, University of London. Details of the contact details for the Sponsor and coordinating team can be provided by the corresponding author.

### Conclusions

Pertussis is a highly infectious respiratory disease which can cause significant morbidity and mortality, particularly in young infants. The introduction of childhood pertussis vaccination programmes has significantly reduced the number of cases and associated morbidity and mortality; however, there has been a recent resurgence of disease even in countries with high vaccine coverage. In response to this, many countries have introduced pertussis vaccination in pregnancy in order to increase the maternal antibody and thus the amount of antibody available for transplacental transfer leading to higher

concentrations of pertussis specific antibody in the infant at birth. The pertussis vaccination programme has been shown to be safe<sup>4,8</sup> and effective<sup>2-6</sup> but there has been debate about the best timing at which to offer the vaccination, which is reflected in the variation of recommendations in different countries.

In this randomised controlled prospective clinical trial, we will compare the antibody responses at delivery following vaccination in three time periods in pregnancy and thus provide evidence for subsequent guidelines.

### Data availability

#### Underlying data

No data are associated with this article.

#### Extended data

Figshare: Participant information for the OpTIMUM study. <https://doi.org/10.24376/rd.sgul.1450175117>.

This project contains the following extended data:

- OpTIMUM ICF v1.1\_non-breastmilk site\_14.03.19\_TC.docx (informed consent form)
- OpTIMUM ICF v1.2\_breastmilk site\_31.10.19\_clean.docx (informed consent form)
- OpTIMUM PIS v3.0\_breastmilk site\_28.10.19\_clean.docx (participant information sheet)
- OpTIMUM PIS v3.0\_non-breastmilk sites\_28.10.19\_clean.docx (participant information sheet)
- Questionnaire for participants\_v1.0\_30.12.18.docx

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/) (CC-BY 4.0).

### Acknowledgements

We gratefully acknowledge the input of the OpTIMUM study group for their contribution to the development of the protocol and the reviewing and editing of this manuscript: **Sneha Basude** (University Hospital Bristol and Weston NHS Foundation trust, Bristol), **Hannah Cuthbertson** (Immunoassay group, National Infection Service, Public Health England, Porton Down), **Olwenn Daniel** (Paediatric Infectious Diseases Research Group and Vaccine Institute, St George's, University of London, London, UK), **Jessica Fretwell** (Immunoassay group, National Infection Service, Public Health England, Porton Down), **Andrew Gorrington** (Public Health England, Porton Down), **Vanessa Greening** (Paediatric Infectious Diseases Research Group and Vaccine Institute, St George's, University of London, London, UK; St George's University Hospitals NHS Foundation Trust, London, UK), **Tom Hall** (Paediatric Infectious Diseases Research Group and Vaccine Institute, St George's, University of London, London, UK), **Edward Johnstone** (Manchester Academic Health Science Centre, St Mary's Hospital, Manchester University NHS Foundation Trust; Maternal and Fetal Health

Research Centre, School of Medical Sciences, University of Manchester), **Uzma Khan** (Paediatric Infectious Diseases Research Group and Vaccine Institute, St George's, University of London, London, UK), **Suzy Lim** (Paediatric Infectious Diseases Research Group and Vaccine Institute, St George's, University of London, London, UK), **Elisabeth Peregrine** (Kingston Hospital NHS Foundation Trust), **Deborah Powell** (Immunoassay group, National Infection Service, Public Health England, Porton Down), **Laxmee Ramkhelawon** (Paediatric

Infectious Diseases Research Group and Vaccine Institute, St George's, University of London, London, UK), **Lorraine Stapley** (Immunoassay group, National Infection Service, Public Health England, Porton Down), **Stephen Taylor** (Immunoassay group, National Infection Service, Public Health England, Porton Down), **Manu Vatish** (Nuffield Department of Women's and Reproductive Health, University of Oxford), **Rosie Watts** (Immunoassay group, National Infection Service, Public Health England, Porton Down).

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# Open Peer Review

Current Peer Review Status: ? ✓

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## Version 1

Reviewer Report 09 November 2022

<https://doi.org/10.21956/wellcomeopenres.18492.r52782>

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✓ **Annette K. Regan** 

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Pertussis immunization guidelines vary by country and as the authors mention, the optimal time to vaccinate remains under debate. Guidelines for immunizing earlier in the second trimester are partly driven by the increased opportunities to vaccine - in the absence of any evidence for lower antibody transfer. However, robust clinical trial data are really needed to appropriately guide effective national immunization guidelines.

This protocol proposes a nicely designed parallel three-arm randomized controlled trial to identify the optimal time to vaccinate during pregnancy. The primary outcome of the trial is antibody concentration measured in cord blood. Secondary endpoints will address potential adverse effects (including AEFI and blunting) and maternal responses to vaccination. Exploratory endpoints include functional assessment of antibodies found in sera and breastmilk. The results of the trial are likely to provide essential information for optimizing maternal vaccine policies to prevent pertussis in neonates.

I recommend approval; however, I have some comments where the protocol could provide further clarity:

1. Eligibility criteria: Because vaccine would need to be withheld until 28-31 weeks of gestation for one arm of participants, it is possible that this would mean withholding vaccine for some participants with preterm birth prior to 32 weeks. For ethical reasons, it may be reasonable to exclude pregnant individuals who are at risk of preterm birth (i.e., previous preterm birth).
2. The exclusion criteria "In the opinion of the investigator is unlikely to complete follow up" is not well described. This seems subjective and details should be provided.
3. Clearly blinding is not possible, but can the statistician be blinded to participant allocation?
4. For infant sera collection after primary immunization, will repeat attempts to follow up be

made for those who have not yet scheduled immunization appointments?

5. Please clarify the definition of the trials' exploratory outcome (i.e., maternal sera?).

**Is the rationale for, and objectives of, the study clearly described?**

Yes

**Is the study design appropriate for the research question?**

Yes

**Are sufficient details of the methods provided to allow replication by others?**

Partly

**Are the datasets clearly presented in a useable and accessible format?**

Not applicable

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Vaccine epidemiologist with primary experience in real world evidence generation for the evaluation maternal vaccine programs (i.e., evaluation of uptake, safety and effectiveness)

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Reviewer Report 23 June 2022

<https://doi.org/10.21956/wellcomeopenres.18492.r50841>

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This article describes in detail the study protocol from a randomised controlled trial investigating the impact of timing of pertussis vaccination in pregnancy. Besides the main objective to determine if pertussis vaccination at different time points in pregnancy results in equivalent concentrations of pertussis specific IgG in term infants at birth, several secondary objectives are also investigated in this study.

Despite the fact that the study protocol is explained in a very detailed and complete way, I still have several small comments/suggestions listed below.

### **Introduction**

"It has not been established whether there is an optimal time to vaccinate in pregnancy to ensure maximal protection to the infant" Is providing optimal protection to the infant the only factor that needs to be considered when thinking about the timing of vaccination during pregnancy? Which factors also need to be taken into account?

### **Protocol**

"To assess equivalence to within a 1.8 fold margin..." Please remove to.

### **Methods**

Women were recruited in three cohorts: is there a lower boundary for vaccination in the first cohort? Or are all women vaccinated before 23+6 weeks gestational age eligible for participation in the first cohort?

Eligibility: How will you monitor "confirmed or suspected pertussis infection in the past 5 years". What is your case definition?

Vaccination visit: "Participants will be asked to complete a short questionnaire regarding their views on how the whooping cough can be best administered during pregnancy" What do you mean by how the vaccine can be administered? Please give some clarification.

Vaccination visit: "diary card must be completed for seven days following vaccination". Are serious adverse events monitored during the complete study? Did you monitor pregnancy outcomes?

Follow-up visit: please change "blood sampling packs" into "blood collection kits" or something comparable.

Delivery visit: "Following delivery, a blood sample will be taken from the cord (sample C) and the participant (sample D)". Please change participant into mother.

Delivery visit: What is the range at which maternal blood samples were taken at delivery? If samples were not taken at delivery, did you correct for this in the analysis? Did you use infant samples taken in the first week postpartum to calculate transplacental transport ratio?

Infant visit: what was the rationale for the broad time interval to take blood samples in the infant post-primary vaccination? Were these blood samples only taken from infants that completed the complete primary vaccination schedule? What if parent decided for an alternative for example prolonged vaccination schedule? Were the infant blood samples still taken then?

Sample transport: What is the maximum timeframe in which samples need to be transported?

Colostrum/breast milk: Sample collection: Did you monitor from which breast the milk was taken, what the time interval was with the previous feeding, breast previous feeding... these are determinants that can have an influence on the breast milk composition. What was the required volume needed?

Secondary outcome analysis: preterm infants: Is a sample size of approximately 30 preterm infants enough to draw conclusions on the best timing to vaccinate during pregnancy in this subgroup? Maybe add that the outcomes will not be reliable or that this will be an exploratory analysis from which no firm conclusions can be drawn?

### **Table 1**

In the objectives, you talk about vaccination at different time points in pregnancy. Should this not be time intervals since you did not vaccinate the pregnant women in the different groups at fixed time points but somewhere in a predefined interval.

Just out of curiosity, what was the rationale to take blood samples in women 14 days after vaccination while immune responses are in most cases measured 28-35 days after vaccination?

What are the outcome measures to explore the impact of repeated vaccination on the pertussis vaccine antigen-specific IgG responses in women who received a pertussis vaccination in a previous pregnancy?

### **Table 2**

Since one of the secondary objectives is to explore the impact of repeated vaccination, were there also exclusion criteria regarding the interval since previous pertussis vaccination?

### **Table 3**

Timing of infant visit is very broad. Was this originally planned like this or adapted due to COVID?

**Is the rationale for, and objectives of, the study clearly described?**

Yes

**Is the study design appropriate for the research question?**

Yes

**Are sufficient details of the methods provided to allow replication by others?**

Yes

**Are the datasets clearly presented in a useable and accessible format?**

Not applicable

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Vaccination during pregnancy



**We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.**

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