Study Protocol

Feasibility study for the development of a sero-correlate of protection against invasive Group B Streptococcus disease (iGBS)

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Statement

The Chief Investigator (CI) and the Sponsor representative have discussed this protocol version. The investigators agree to perform the investigations and to abide by this protocol except where departures from it are mutually agreed in writing.

The Investigator agrees to conduct the trial in compliance with the protocol, Good Clinical Practice (GCP), the Data Protection Act (1998), the Trust Information Governance Policy (or other local equivalent), the UK Policy Framework for Health and Social Care Research, the Sponsor's Standard Operating Procedures (SOPs), and other regulatory requirements as appropriate.

This protocol has been written in accordance to the Sponsor's procedure identified as: JREOSOP0039 "Protocol Design" and is intended for use at UK sites only

Chief Investigator Paul Heath Professor St George's, University of London	Signature	Date

Acknowledgements and Protocol contributories

PTH and KLD conceived the study, PTH, KLD, TP, AK, JP and NA initiated the study design. PTH and KLD are grant holders, NA provided statistical expertise in clinical study design and is conducting primary statistical analysis. JP provided patient input. All authors contributed to refinement of the study protocol and approved the final manuscript

The funders played no role in the design, collection, management, analysis and interpretation of data, writing of any report, decision to submit a report. The Sponsor has the final decision about any of these aspects.

Contents

1 List of abbreviations

AE	Adverse Event
APR	Annual Progress Report
CI	Chief Investigator
CoP	Correlate of protection
CPS	Capsular Polysaccharide
CRF	Case Report Form
CTIMP	Clinical Trial of an Investigational Medicinal Product
EOD	Early Onset Disease
ELISA	Enzyme-linked Immunosorbent Assay
GBS	Group B Streptococcus
GCP	Good Clinical Practice
HRA	Health Research Authority
IAP	Intrapartum Antibiotic Prophylaxis
ID	Identification Number
iGBS	invasive Group B Streptococcal disease
IgG	Immunoglobulin G
IP	Intellectual Property
ISF	Investigator Site File
ITT	Intention to Treat
JREO	Joint Research & Enterprise Office
LOD	Late Onset Disease
NHS R&D	National Health Service Research & Development
PI	Principal Investigator
REC	Research Ethics Committee
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
TMF	Trial Master File

2 Roles and Responsibilities –

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3 Study synopsis

Brief title:	Sero-correlates of protection against Group B Streptococcus
Official title:	Feasibility study for the development of a sero-correlate of protection against invasive Group B Streptococcus disease (iGBS)
Sponsor reference number:	18.0089
Public database identifier	
Study type & Phase	Feasibility study
Study design	Prospective cohort study
Study Population/disease condition	Pregnant women and their infants.
Eligibility criteria:	*Inclusion criteria:* (i) pregnant, (ii) ≥ 18 years of age, (iii) delivering at one of the selected hospitals (iv) consented to participate during the study period
	Exclusion criteria: (i) any woman unable to meet the inclusion criteria above
Target number of participants	*Approximately 4000*
Criteria for evaluation	**Primary objective** To test the feasibility of collecting serum at delivery (either maternal or cord or both) from a large cohort of pregnant women. **Secondary objectives** To test the key operational aspects for a proposed large serocorrelates study: - enrolment rate (the rate (proportion) of eligible women who are willing to participate in the delivery blood collection study) - maternal and / or cord blood collection rate - key clinical exclusion data collection rate - infant iGBS surveillance consent rate In a sub-study of main study above, to assess: - rectovaginal swab study consent rate - rectovaginal swab collection rate - rectovaginal GBS colonisation rate - rectovaginal GBS CPS serotype-specific colonisation rates Within this sub-study of both mother/infant pairs with both swab and delivery bloods, to assess: - infant blood sample study consent rate - infant Guthrie card consent rate - infant Guthrie card collection rate

	Exploratory objectives To assess: - the impact of timing of processing of delivery blood sample and storage conditions on serotype-specific GBS anti-CPS IgG concentrations - the serotype-specific GBS anti-CPS IgG concentrations in maternal serum and cord blood in subjects colonised with GBS at delivery
Sources of funding	National Institute for Health Research HTA 17/153/01
Anticipated start date:	1st June 2018
Anticipated primary completion date:	1st December 2018
Sponsor/Co-Sponsor	St George's University Hospitals NHS Foundation Trust
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4 Background

Group B streptococcus (GBS) is the leading cause of neonatal sepsis and meningitis in most countries. GBS is also an important cause of disease in pregnant women, immunocompromised adults, and the elderly [1]. The highest incidence of invasive GBS (iGBS) is in the first 3 months of life and the condition is traditionally divided into early-onset disease (EOD, occurring in individuals aged <7 days) or late-onset disease (LOD, occurring in individuals aged from 7 - 89 days). GBS is an encapsulated bacterium and 10 serotypes are described; 5 serotypes (Ia, Ib, II, III, V) account for 97% of iGBS [2].

Overall, EOD accounts for 60–80% of iGBS disease in the first 3 months of life. Maternal colonization with GBS in the gastrointestinal tract or genital tract is a prerequisite for EOD with vertical transmission occurring during or just before birth. Around 20% of pregnant women are colonised with GBS [3] and 1-2% of neonates born to colonised women develop invasive disease in the absence of intrapartum antibiotic prophylaxis (IAP) [4]. EOD can occur rapidly, with signs evident at birth or within 12 hours in most cases and cases typically present with sepsis, pneumonia, and/or meningitis [5].

GBS is present in all regions of the world with an estimated 21.7 million pregnant women colonized [3]. In 2015 it was estimated that worldwide there were at least 319,000 infants <3 months of age with iGBS resulting in 90,000 infant deaths and at least 10,000 children with disability related to GBS meningitis. Additionally, 33,000 maternal cases and 57,000 stillbirths were attributed to GBS disease [6].

The global burden of GBS is therefore high and represents an unmet public health need.

IAP can reduce the incidence of EOD, but have no impact on GBS-related stillbirth or LOD and only a limited impact on disease in pregnant women. Many high-income countries have established IAP policies. The incidence of EOD in the USA has declined significantly in the era of IAP and generally also in other countries adopting a swab-based screening policy [7,8]. However, in some countries, particularly those adopting a risk-based IAP strategy, such as the Netherlands [9] and the UK [10], recent increases in disease burden have been reported.

It is clear that even strict and universal implementation of guidelines does not eliminate EOD as it can occur despite administration of IAP, in infants of mothers who were negative on screening and where no risk factors are evident in labour [11]. Most significantly, IAP has no impact on GBS-related stillbirths or LOD infection, where the burden of disease is substantial. The majority of GBS meningitis occurs after the first week of life so this particular burden remains; in the USA and the UK GBS is now the most common cause of bacterial meningitis in children less than 5 years of age [12 13].

Given the very early onset of neonatal GBS disease, the shortcomings of IAP-based prevention strategies and evidence that suggests that maternal antibodies acquired after natural exposure, when transmitted transplacentally to the fetus, may protect the young infant from invasive infection [14], the prospect of protecting mothers and their infants through vaccination in pregnancy is an attractive one. Possible candidates for an effective vaccine include one or more of the conserved surface proteins or the capsular polysaccharide (CPS) [15].

Multiple studies of CPS - protein conjugate vaccines in non-pregnant and, more recently, in pregnant women have established the immunogenicity and safety of these candidates [15]. Recent estimates suggest that an effective GBS maternal vaccine (>80% efficacy) with high (90%) global coverage could prevent 231,000 infant and maternal GBS cases, 41,000 stillbirths and 66,000 infant deaths annually [6].

Several obstacles exist in moving the most advanced vaccines into phase III clinical trials. The first is that, given the relative rarity of GBS disease in Europe and the USA, large numbers of infants would need to be recruited to determine vaccine efficacy [16]. Second, obstacles exist in determining what concentration of antibody is required to protect the infant for the duration of the at-risk period (i.e., the first three months of life), as there are currently no internationally recognised standards with which to interpret individual study results [1]. Licensure and policy decisions would be significantly accelerated if an immune marker, measured in an analytically and clinically validated assay, was established as a correlate of protection. Licensure in such a scenario would come with a commitment to establish effectiveness post-licensure. This was the approach used for licensure of meningococcal C and meningococcal B vaccines [17].

Correlates of protection against disease.
The association between serotype-specific capsular antibody levels and invasive GBS disease in newborns was initially characterized in 1976 by Baker and Kasper [14]. In the majority of subsequent studies, low levels of CPS-specific antibodies were found in maternal delivery sera of women who had neonates with EOD and LOD caused by that type compared with sera from women delivering infants who remained healthy [1]. However, different "protective" levels have been defined in different studies as well as for the different CPS types.

In a recent meta-analysis undertaken to compare the proportions of cases and controls with antibody levels ≥2 µg/ml, the odds of iGBS disease was 6.6 (95% CI: 2.1–20.6) and 2.4 (95% CI: 1.2–4.7) times greater in infants whose mothers had antibody levels <2 µg/ml for types III and Ia, respectively [18]. A threshold of 1 µg/ml has also been proposed as a correlate for protection for types Ia and III [8]. Thresholds are much higher in other studies using different case-control designs and different Enzyme-linked immunosorbent assay (ELISA) methods [19,20], making direct comparisons difficult.

Interpretation of studies is confounded by the different assay methods used and the lack of standardized reference ranges for type-specific antibody levels. **Further studies using standardised methods are therefore warranted.**

Defining a correlate of protection against iGBS.
There is considerable evidence that serum immunoglobulin G (IgG) can protect infants against iGBS and that this IgG is maternally derived as a result of natural maternal infection (i.e. colonisation). We wish to know what level of serum IgG in women at delivery is protective so that we can mimic this through vaccination. We can do this by comparing IgG levels in mothers whose babies are exposed to GBS (through maternal colonisation) and go on to develop iGBS (**cases**), with IgG in women whose babies are exposed to GBS (through maternal colonisation) but **do not** develop iGBS (**controls**). In order to do this we need to have sufficient numbers of each of these (cases and controls) to be able to define the protective level of IgG (the correlate of protection: CoP) with sufficient precision. Although the level of IgG in women at delivery is most often proposed as the CoP there is a predictable decline in IgG level from the mother to the foetus (transplacental transfer ratio), and subsequently in the infant over the first 3 months of life (reflecting the half-life of maternal IgG). Measuring IgG in the cord blood and at different time points in the infant can allow these to be compared with maternal IgG to calculate the rate of antibody decline during the at risk period of the first three months of life. The level of IgG in the infant will be of particular relevance in cases of LOD where the median age at disease onset is around 21 days [21].

In order to generate a CoP, maternal delivery/cord sera from a cohort of mothers/babies must be collected prospectively. When an infant subsequently develops iGBS the relevant delivery samples can be retrieved for that infant and the antibody levels can be compared with those of suitable

controls. The antibody levels in the infant and the mother at the time of iGBS can also be obtained and may also be used to predict the levels present at the time of delivery as it is not expected that these will change significantly between birth and the onset of iGBS, certainly for EOD [22].

Study Rationale

From a global perspective the burden of iGBS in pregnant women and young infants has recently been quantified and shown to be a major problem [6]. From a UK perspective we have recently seen an increasing burden of disease, despite a national (risk-based) policy for IAP [10], and the UK National Screening Committee has recently recommended not to introduce an (arguably more effective) national screening program (https://www.gov.uk/government/news/screening-pregnant-women-for-gbs-not-recommended) - although the clinical and cost effectiveness of screening for GBS in pregnancy is the objective of another HTA application (17/86). Conversely, the UK population has widely accepted the concept of maternal vaccination, with high coverage of maternal pertussis vaccination (>70%) and the first (ever) demonstration of its effectiveness [23]. The UK is therefore in an excellent position to pursue the development, licensure and implementation of a maternal vaccine against GBS.

Licensure and policy decisions for a candidate GBS vaccine would be significantly accelerated if an immune marker was established as a correlate of protection. Regulatory bodies, including the European Medicines Agency and the Food and Drug Administration [24], have made it clear that they would now consider this approach to licensure if robust evidence can be developed. Additionally, the Joint Committee on Vaccination and Immunisation has indicated it would consider a recommendation for routine implementation of a vaccine licensed on the basis of correlates - as it has for other recent vaccines (meningococcal C [17] and meningococcal B).

The two critical gaps that have to be filled to make decisions on the use of such vaccines (both at the regulatory and recommending body level) are: (i) the development of a standardised immunoassay to measure antibody levels that act as the correlates of natural immunity, supported by measurement of functional antibody assays, and (ii) a large biobank of sera to establish the correlate using these new standardised assays. The first of these gaps is being addressed by a consortium of groups from academia, public health, and industry (lead by co-applicant KLD), and the second is the basis of this study.

Given the anticipated size and logistical complexities of a serocorrelates study that would be needed to address this, the aim of this initial feasibility study is to test key operational aspects of the study design.

5 Study objectives

5.1. Primary objective

To test the feasibility of collecting serum at delivery (either maternal or cord or both) from a large cohort of pregnant women.

5.2. Secondary objectives

To test the key operational aspects for a proposed large serocorrelates study:

- enrolment rate (the rate (proportion) of eligible women who are willing to participate in the delivery blood collection study)
- maternal and / or cord blood collection rate
- key clinical exclusion data (gestation at birth, receipt of IAP in labour (yes/no), type of IAP (list), time between administration of IAP and delivery (in hours)) collection rate
- infant iGBS surveillance consent rate

In a sub-study of the main study above to assess:

- rectovaginal swab study consent rate
- rectovaginal swab collection rate
- rectovaginal GBS colonisation rate
- rectovaginal GBS CPS serotype-specific colonisation rates
-

In the sub-study above where samples of maternal/cord blood and rectovaginal swabs are available to assess:

- infant blood sample study consent rate
- infant Guthrie card consent rate
- infant Guthrie card collection rate

5.3. Exploratory objectives

A sub-study to assess:

- the impact of timing of processing and blood sample storage conditions on serotype-specific GBS anti-CPS IgG concentrations
- the serotype-specific GBS anti-CPS IgG concentrations in maternal serum and cord blood in subjects colonised with GBS at delivery

6 Trial design

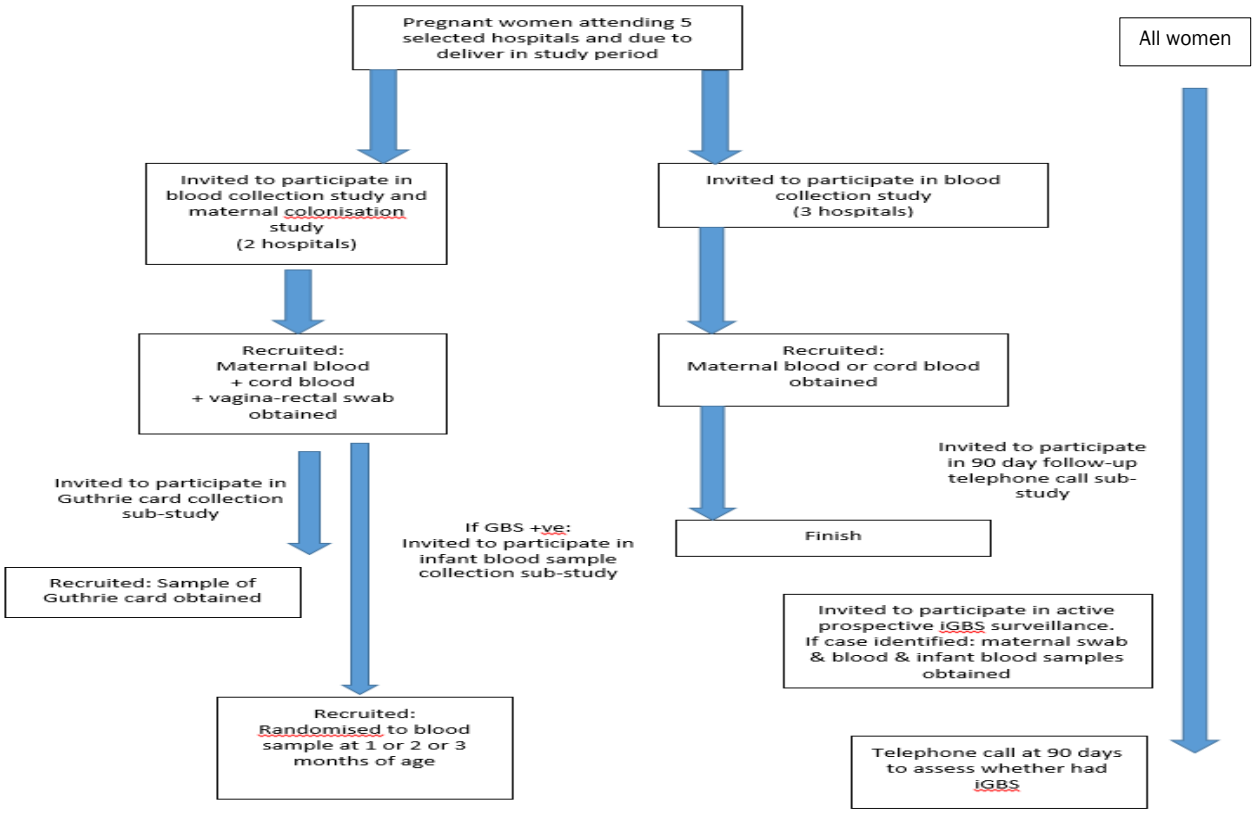
6.1. Overall design

This is a prospective cohort study of pregnant women and their infants.

6.2. Treatment/intervention plan and rationale

No treatment / intervention planned as part of this feasibility study

6.3. Schematic of Study design



7 Participation selection criteria

There will be no exceptions (waivers) to eligibility criteria prior to participant inclusion into the study. Any questions raised about eligibility should be addressed prior to entering the participant.
The eligibility criteria have been carefully considered and are standards used to ensure the trial results can be appropriately used to make future decisions for other people with similar disease or medical condition. It is therefore vital exceptions are not made to the following detailed selection criteria.

All participants that are screened for inclusion into the study must be entered onto the Sponsor screening log JREOLOG0001 and will be assigned a sequential number. Participants will be considered eligible for enrolment into this trial if they fulfil all of the inclusion criteria and none of the exclusion criteria as defined below.

Eligible participants will be entered onto the Sponsors Subject Identification Number (ID) log JREOLOG0002 and assigned a Trial specific Identification number in a pre-agreed format in accordance with Site identifier and next sequential numerical value e.g. SG001

7.1. Inclusion criteria

(i) pregnant,
(ii) ≥ 18 years of age,
(iii) delivering at one of the selected hospitals
(iv) consented to participate during the study period

7.2. Exclusion criteria

(i) As this is a feasibility study there are no exclusion criteria other than inability to fulfil the inclusion criteria above.

8 Participant Recruitment process

Patient recruitment at a site will only commence once evidence of the following approval/essential documents are in place:

1. Research Ethics Committee (REC) approval, if applicable, Health Research Authority (HRA) approval
2. Final sponsorship and host site permissions (confirmation of capacity and capability),

All subjects who wish to enter the study will be fully screened and consented by the Chief Investigator, or an appropriate delegate. As detailed below:

All women will be made aware of the study through information on social media, posters on notice boards, institutional websites, letters, text messages or through direct approach in antenatal clinics. Women will also have the opportunity to ask questions of the research team by telephone or email. Women who are interested in participating will have this indicated on hand-held or hospital notes (as appropriate) to ensure that delivery staff are aware.

Collection of delivery bloods: Following confirmation of consent, blood samples will then be obtained from the mother at any time during labour (or within 48 hours of delivery) and / or from the cord once the placenta has been delivered. At Kingston and St George's Hospitals, both maternal blood and cord blood will be obtained. At other sites, maternal blood will only be obtained if it is not possible to obtain a sample of cord blood after delivery.

Collection of swabs: At Kingston and St George's Hospitals only, pregnant women will also be consented to take part in the colonisation study.

Collection of infant blood: At Kingston and St George's Hospitals, women who are participating in both the delivery blood collection study and the colonisation study and who are shown to be colonised with GBS will also be invited to participate in the infant antibody kinetics sub-study. Women will be asked to attend a hospital appointment when their infant is 4, 8 or 12 weeks old for an additional blood sample.

Collection of Guthrie cards: At Kingston and St George's Hospitals, women who are participating in both the delivery blood collection study and the colonisation study will also be invited to participate in the infant Guthrie card collection sub-study. Women will be asked for consent to access their baby's routine newborn blood spot screening card from the National Screening Laboratory and to obtain a samples from this card.

iGBS surveillance: When a case of iGBS occurs, the relevant paediatrician will be contacted by the study team and asked to recruit the mother and baby to the iGBS sub-study. Women will be approached in the hospital and asked to consent to providing a rectovaginal swab and an infant blood sample.

Follow-up of babies: A follow-up telephone call will be made to all consenting parents 90 days after their babies' birth to confirm whether their infants developed iGBS disease during this time period.

9 Study procedures

9.1. Informed consent

Informed consent from the participant, legally authorised representative or the parents/guardians/person with legal responsibility for children must be obtained following explanation of the aims, methods, benefits and potential hazards of the trial and before any trial specific procedures are performed. The only procedures that may be performed in advance of written informed consent being taken are those that would have been performed on all participants in the same situation as routine clinical practice.

Consent will be re-sought for children where the legal guardian may change.

Women will be given information about the study by one of the research midwives/clinical team and will be given the opportunity to ask questions. There will be no minimum period between receiving information and providing consent, but the study teams will ensure that women have had sufficient opportunity to consider the information and ask any questions. Ideally formal written consent will be taken for participation in the study at enrolment and then re-confirmed with the mother verbally at / during / following delivery. Consenting pregnant women will be encouraged to attach a study sticker to their hand-held notes to indicate their potential interest in the study. Participants can withdraw at any time.

The Investigator or designee will explain that the patients are under no obligation to enter the trial and that they can withdraw at any time during the trial, without having to give a reason.
A copy of the signed Informed Consent Form along with a copy of the most recent approved Patient Information Sheet will be given to the study participant. An original signed & dated consent form will be retained in the Investigator Site File (ISF) and a copy will be placed in the medical notes.

9.2. Randomisation procedure

N/A

9.3. Emergency unblinding

N/A

9.4. Discontinuation/withdrawal of participants and stopping rules

In consenting to the trial, participants are consenting to trial involvement, trial follow up and data collection. However, an individual participant may withdraw early or the study may be stopped early for any one of the following reasons:

- Unacceptable adverse event
- Intercurrent illness that prevents further protocol participation
- Any change in participant's condition that in the investigator's opinion justifies the discontinuation of the participation
- Withdrawal of consent from the participant

As participation in the trial is entirely voluntary, the participant may choose to withdraw at any time without penalties or loss of benefits to which they may be entitled. Although not obliged to give a reason for discontinuing their protocol inclusion a reasonable effort should be made to establish this reason, whilst remaining fully respectful of the participant's rights. Participants who discontinue protocol involvement, for any of the above reasons, should remain in the trial for the purpose of follow up and data analysis.

Women may withdraw at any time during the study without giving a reason without detriment to their or their infants' care. If women withdraw for any reason, any data collected will be analysed up to the point of withdrawal as long as the women has not withdrawn consent for her sample and/or data to be used in this study. Any woman who discontinues the study will not be replaced.

If a participant chooses to discontinue they should continue to be followed up as closely as possible to the follow-up schedule defined in the protocol, providing they are willing. However if the participant confirms they do not wish to participate in the scheduled follow up phone call then data that has already been collected should be kept and analysed according to the intention to treat principle for all participants who stop follow up early.

9.5 Participant transfers

If a participant moves from the area making continued follow up at their consenting centre inappropriate, every effort should be made for them to be followed up at another sponsor approved trial centre. Written consent should be taken at the new centre and then a copy of the participant's Case Report Form (CRF) should be provided to the new centre. Responsibility for the participant remains with the original consenting centre until the new consent process is complete.

9.6 Lost to Follow up

For studies conducted in the UK we will utilise the National Health Service (NHS) number to trace participants whom may have changed their General Practitioner if consent is provided for us to inform the general practitioner about the study. For the sub-study of infants followed up to 12 weeks of age, we will make two phone calls, one week apart, to the number given by the woman and one attempt to contact the family general practitioner. If following these calls there is no response, we will deem the infant lost to follow up.

9.7 Definition of the End of Trial

The study will have ended at the Last Patient Last Visit. We will notify the REC of the end of trial within 90 days of its planned completion or within 15 days if the study is terminated early.

10 Study Procedures

10.1 Screening assessments

All women who indicate an interest in the study will be assessed to ensure they meet the inclusion criteria before enrolment.

10.2. Baseline assessments

As above.

10.3. Study procedures

Delivery blood samples: Following oral confirmation of consent, blood will be obtained from the mother at any time during labour (or within 48 hours of delivery) and / or from the cord once the placenta has been delivered and when the obstetric/midwifery team deem it is safe to do so. 5mL of blood will be collected into a BD® serum separator tube. Blood samples will be transported to the laboratories to be processed and the serum collected into eppendorf tubes and frozen at -20°C to -80°C for later processing.

Relevant clinical information will be extracted from the mothers hand-held or hospital notes onto the CRF. This will include: baby/ies' gestation at birth (wks), receipt of IAP (yes/no), type of IAP including dose and quantity (list), time between administration of first IAP and delivery (hours), recent blood transfusion (in last 1 month), elective c/s (yes/no).

Vaginal and rectal swabs: *At Kingston and St George's Hospitals only*, and for those who consent to participate in the colonisation study, a single rectovaginal swab (Copan swab) will be obtained at any time from 35 weeks gestation up to (and including) delivery, either by the study midwife or by the mother herself if she prefers (with appropriate written guidance). The swab is inserted into the lower half of the vagina and turned slowly clockwise once before removing and inserting past the anal sphincter into the low rectum. If there is any resistance to insertion the procedure should be abandoned. The swab is then inserted into a sterile Amiens media tube and transported to the local clinical laboratory for processing according to standard methods. The result (positive / negative) will then be entered into the maternal notes and the mother informed by phone (if GBS positive) and by letter (if positive or negative). It is anticipated that women will subsequently be managed according to the relevant Royal College of Obstetrics and Gynaecology Greentop Guideline on GBS with regard to the swab result – if positive the mother would be offered IAP (https://www.rcog.org.uk/en/guidelines-research-services/guidelines/gtg36/).

Collection of infant blood: *At Kingston and St George's Hospitals only*, and only in those women who are participating in both the delivery blood collection study and the colonisation study and who are shown to be colonised, they will also be invited to participate in the infant antibody kinetics sub-study. In this sub-study babies born to colonised mothers will be randomized to have one blood sample obtained, at 4, 8 or 12 weeks of age. This will be undertaken by suitably trained research nurses or doctors using venipuncture or capillary sampling (2 ml) and then blood will be processed and stored as described above.

Collection of Guthrie cards: *At Kingston and St George's Hospitals only*, and only in those women who are participating in both the delivery blood collection study and the colonisation study, they will also be invited to participate in the infant Guthrie card collection sub-study. In this sub-study mothers will be asked for permission to obtain a sample of the Guthrie card, which is obtained routinely in all babies at around 5 days of age. This will be obtained from the National Screening Laboratory and stored for antibody analysis.

iGBS surveillance: Microbiologists and paediatricians at the 5 participating hospitals will be engaged at the start of the study and information provided about the study and its rationale. They will be asked to make contact with study staff should a case of iGBS occur (only up to 5 cases would be expected with the cohort of this feasibility study). Study staff will additionally make contact with each laboratory on a weekly basis to ensure that cases have not been missed. When a case of iGBS occurs, the relevant paediatrician will be contacted by the study team and asked to recruit the mother and baby to the iGBS sub-study. Following consent from the mother, a sample of blood will be obtained from her and from her baby and processed as above. She will also be asked to provide a single rectovaginal swab to be processed as above.

Follow-up of babies: A follow-up telephone call will be made to all parents 90 days after their babies birth to establish whether the infants developed iGBS disease during this time period. This phone call will be made by a member of the study team.

Subsequent assessments

As indicated, a follow-up telephone call will be made to parents 90 days after their baby/babies were born to confirm whether the infants developed iGBS disease during this time period. In the event of a case of iGBS occurring, the relevant paediatrician will be contacted by the study team and asked to recruit the mother and baby to the iGBS sub-study. Following consent from the mother a sample of blood will be obtained from her and from her baby by suitably trained research nurses or doctors and processed as above. She will also be asked to provide a single rectovaginal swab, either taken by the midwife or mother herself, to be processed as above.

10.4. Summary flow chart of study assessments

See previous flow chart

10.5. Methods

10.5.1. Laboratory procedures

Swab microbiological testing: The swab will be sent to the microbiology laboratory for processing according to United Kingdom Accreditation Services accredited methods for the testing of Group B Streptococcus from rectovaginal swabs.

Antibody testing: in a sub-study of samples from the swab / delivery blood sub-study, the impact of timing of processing of delivery blood sample storage conditions on serotype-specific GBS anti-CPS IgG concentrations will be assessed. 100 blood samples (samples where there is sufficient blood) will be divided further in order to assess the effect on anti-GBS concentrations of: 1. Different times between sample collection and sample processing (6 hours / 10 hours / 18 hours / 24 hours / 48 hours / 72 hours / 5 days / 7 days); 2. Different times between serum processing and serum freezing (24 hours / 48 hours / 72 hours / 5 days / 7 days).

Antibodies to GBS will be quantified using a multiplex ELISA and a opsonophagocytosis killing assay at St George's University of London laboratories using the standardized assays that are currently being established as part of a Gates Foundation funded collaboration led by a co-applicant (KLD). It is expected that these will be in place before the end of 2018.

10.5.2. Radiology or any other procedure(s)

N/A

11. Safety Reporting

11.1. Definitions

Adverse Event (AE)— any untoward medical occurrence in a participant whether it is considered to be related to the intervention or not, that includes a clinical sign, symptom, or condition and /or an observation of a near incident such as incorrect dosing of antibiotics. (This does not include pre-existing conditions recorded as such at baseline).

Serious Adverse Event (SAE)— any Adverse Event or untoward medical occurrence in a trial participant that can be wholly or partly to the intervention that resulted in any of the following:

- Results in death; or
- Is life-threatening (places the subject, in the view of the Investigator, at immediate risk of death)
- Requires hospitalisation or prolongation of existing hospitalisation (hospitalisation is defined as an inpatient admission, regardless of length of stay; even if it is a precautionary measure for observation; including hospitalisation for an elective procedure, for a pre-existing condition)
- Results in persistent or significant disability or incapacity (substantial disruption of one's ability to conduct normal life functions)
- Consists of a congenital anomaly or birth defect (in offspring of subjects or their parents receiving the trial intervention regardless of time of diagnosis).
- Or is another important medical condition

Important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the outcomes listed in the definition of serious will also be considered serious.

11.2. Investigator responsibilities relating to safety reporting

Collection, recording and reporting of AEs (including serious and non-serious events and reactions) to the Sponsor will be done according to the Sponsor's Safety reporting for clinical studies Standard Operating Procedure (SOP) JREOSOP0033.

All AEs whether serious or not will be recorded in the hospital notes in the first instance. A record must also be kept in the participant's CRF and the Sponsor's AE Log JREOLOG0007. The AE log will be sent to the Sponsor on request and every 2 months.

All SAEs will be reported both to the Sponsor via the JREO & REC using the SAE report form for research other than CTIMPs (non-CTIMPs) published on the HRA website The Chief or Principal Investigator at any participating site will complete the SAE form which will be faxed both to the JREO on 020 8725 0794 or E-mailed to adverseevents@sgul.ac.uk, within 48hrs of the Investigator becoming aware of the event, and via email to the relevant REC.

The CI or PI will respond to any SAE queries raised by the Sponsor as soon as possible. Follow up reports must continually be completed within acceptable time-frames and sent as detailed above until the reportable event is considered resolved.

11.3. Notification of deaths

Only deaths that are assessed to be caused by the trial intervention will be reported to the Sponsor. This report will be immediate or within 24 hours of first knowledge.

11.4. Annual Progress Reports (APRs)

The CI will prepare the APR in accordance with JREOSOP0043. Following review by the sponsor the report will be sent to the REC. The APR is due for submission annually within 30 days of the

anniversary date on which the Ethics committee gave the favourable opinion, until the trial is declared ended.

12. Data management and quality assurance

12.1. Confidentiality

All data will be handled in accordance with the Data Protection Act 1998.

CRFs will not bear the participant's name or other directly identifiable data. The participant's trial ID only, will be used for identification. The sponsor Subject ID log JREOLOG0002 can be used to cross reference participant's identifiable information.

12.2. Data collection tool

The CI will design CRFs, either on paper or online on a secure RedCap database. For paper CRFs, all data will be entered legibly in black ink with a ball-point pen. If the Investigator makes an error, it will be crossed through with a single line in such a way to ensure that the original entry can still be read. The correct entry will then be clearly inserted. The amendment will be initialled and dated by the person making the correction immediately. Overwriting or use of correction fluid will not be permitted.

It is the Investigator's responsibility to ensure the accuracy of all data entered and recorded in the CRFs. The Staff Delegation of Responsibilities Log JREOLOG0004 will identify all trial personnel responsible for data collection, entry, handling and managing the database.

Following receipt of informed consent, maternal demographics and medical history will be collected at delivery. CRFs will be designed for the collection of relevant information. Priority will be given to the collection of key exclusion data: gestation at birth, receipt of IAP (yes/no), type of IAP (list), time between first administration of IAP and delivery (in hours), recent blood transfusion (in last 1 month), in addition to collection of other routine data.

12.3. Incidental Findings

N/A

12.4. Data handling and analysis

A secure password-protected database will be designed and set up using REDCap and will be used by the study sites for entering data. Data from CRFs will be reviewed and corrected in the database by the site study team referring to the original documentation.

12.5. Personal information data flow

Women who agree to participate in this trial will be identified and consented as per the procedures above. Once recruited, a CRF form will be created as above and stored in a locked cabinet. Samples taken will be identified with a unique number that is linked to a participant's CRF. Samples sent to the laboratory at each site will therefore not contain any patient identifiable data. The samples will be stored in a -20°C to -80°C freezer in a locked research laboratory with access restricted to research staff.

13. Archiving arrangements

The trial essential documents along with the trial database will be archived in accordance with the sponsor SOP JREOSOP0016. The agreed archiving period for this trial will be 5 years.
Each PI at any participating site will archive the trial essential documents generated at the site for the agreed archiving period in accordance with the signed Clinical Trial Site agreement.

14. Statistical design

14.1. Statistical input in trial design

Statistical design has been discussed with Prof. Nick Andrews, Senior Statistician at Public Health England.

14.2. Endpoints

14.2.1. Primary endpoints

As this is a feasibility study in order to understand how the study can be done in a large cohort of pregnant women, a range of parameters (as detailed) will contribute to the feasibility objective.

14.2.2. Secondary endpoints

N/A

14.3. Sample size and recruitment

14.3.1. Sample size calculation

There is no formal sample size calculation as this is a pragmatic study conducted among a representative network of hospitals that is intended to assess the feasibility of approaching a much larger network of hospitals that is needed to achieve the main objective of this research. The proportions achieved against the various study endpoints will allow sample size calculations to be made for the main study.

In the feasibility study, the total number of livebirths represented at the 5 hospitals is approximately 7000 over the 4-month study period. We hope to recruit around 80% of eligible pregnant women during the study period, to collect cord blood in around 80% of women recruited and to capture key clinical data in 80% of recruited women. We would expect that approximately 15% of births will not be recruited leaving around 6600 eligible women. With this sample size we will be able to estimate a rate of 80% recruitment to within a 95% confidence interval of +/-1%, with similar confidence intervals for other estimates and for each individual hospital also (to assess relevance of hospital).

With regards to the swab study it is proposed to approach pregnant women in 2 hospitals only (St George's & Kingston Hospitals). Over the 4-month period there will be 3600 eligible women and thus a target of 1000 women implies a 28% recruitment rate that we believe is achievable. If 1000 participants are recruited we will continue to recruit until the end of the study period. Again the estimates of confidence intervals around these recruitment rates etc. will enable sample size calculations to be made for the main study.

14.3.2. Planned recruitment rate

As this is a feasibility study, there are no planned recruitment rates, however, see above for estimates.

14.4. Statistical analysis plan

14.4.1. Summary of baseline data and flow of patients

A descriptive analysis will be undertaken. For example, the proportion of women approached who agree to participate in the blood collection study, the proportion of women who agree to participate from whom a blood sample is obtained, etc.

14.4.2. Primary endpoint analysis

As this is a feasibility study in order to understand how the study can be done in a large cohort of pregnant women, a range of parameters (as detailed) will contribute to the feasibility objective.

14.5. Secondary endpoint analysis

N/A

14.6. Sensitivity and other planned analyses (if applicable)

N/A

14.7. Interim analysis

N/A

14.8. Other statistical considerations

N/A

15. Committees in involved in the trial

15.1. Trial Steering Committee – To be assigned by the National Institute for Health Research.

16. Direct access to source data

The Investigator(s)/institution(s) will permit trial-related monitoring, audits, REC review, and regulatory inspection(s), providing direct access to source data/documents. Trial participants are informed of this during the informed consent discussion. The process will include participants being asked to consent to provide access to their medical notes.

17. Ethics and regulatory requirements

The site must conduct the trial in compliance with the protocol as agreed by the Sponsor and, which was given favourable opinion by the REC where applicable and the HRA approval.

The Chief Investigator will be provided (via the Sponsor) with file indexes E.G. JREODOC0003 Trial Master File (TMF) index and JREODOC0004 ISF index for use with SOP JREOSOP0019 'Preparation and Maintenance of the TMF' The CI will be responsible for the maintenance of the TMF and may delegate the responsibility of ISF maintenance to the PI at each participating site.

It is the responsibility of the PI at each site to ensure that all subsequent amendments gain the necessary approval. Refer to JREOSOP0011 'Management of Amendments'.

Within 90 days after the end of the trial, the CI and Sponsor will ensure that the REC is notified that the trial has finished. If the trial is terminated prematurely, those reports will be made within 15 days after the end of the trial. Refer to JREOSOP0015 'End of study declaration'

The CI will supply an End of Study report of the clinical trial to the REC within one year after the end of the trial. The sponsor can provide JREODOC0059 End of study Report template.

18. Finance

This study is funded by the National Institute for Health Research HTA 17/153/01.

19. Insurance and indemnity

St George's University Hospitals NHS Foundation Trust holds insurance to cover participants for injury caused by their participation in the clinical trial. Participants may be able to claim compensation if they can prove that St George's University Hospitals NHS Foundation Trust has been negligent. This includes negligence in the writing of the protocol, or selection of trial resources.

Where the Trial is conducted in a hospital, the local hospital has a duty of care to participants. St George's University Hospitals NHS Foundation Trust will not accept liability for any breach in the hospital's duty of care, or any negligence on the part of hospital employees. Hospitals selected to participate in this clinical trial shall provide clinical negligence insurance cover for harm caused by their employees and a copy of the relevant insurance policy or summary shall be provided to St George's University Hospitals NHS Foundation Trust, upon request.

Participants may be able to claim compensation for injury caused by participation in this Trial without the need to prove negligence on the part of St George's University Hospitals NHS Foundation Trust or another party.

If a participant indicates that they wish to make a claim for compensation, this needs to be brought to the attention of St George's University Hospitals NHS Foundation Trust immediately.

Failure to alert St George's University Hospitals NHS Foundation Trust without delay and to comply with requests for information by the sponsor or any designated Agents may lead to a lack of insurance cover for the incident.

NHS bodies are liable for clinical negligence and other negligent harm to individuals covered by their duty of care. NHS Institutions employing researchers are liable for negligent harm caused by the design of studies they initiate.

20. Intellectual and development policy

Unless otherwise specified in agreements, the following guidelines shall apply:

All Intellectual Property Rights and Know How (IP) related to the Protocol and the Trial are and shall remain the property of the Sponsor excluding:
1) Pre-existing IP related to clinical procedures of any Hospital.
2) Pre-existing IP related to analytical procedures of any external laboratory.

All contributors:

- shall assign their its rights in relation to all IP Rights and in all Know How, not excluded above to the Sponsor and at the request and expense of the Sponsor, shall execute all such documents and do all such other acts as the Sponsor may reasonably require in order to vest fully and effectively all such IP Rights and Know How in the Sponsor or its nominee.

- shall promptly disclose to the Sponsor any Know How generated pursuant to this Protocol and not excluded above and undertake to treat such Know How as confidential information jointly owned between it and the Sponsor.

Nothing in this section shall be construed so as to prevent or hinder any medical professional from using Know How gained during the performance of the Trial in the furtherance of its normal business activities, to the extent such use does not result in the disclosure or misuse of Confidential Information or the infringement of any IP Right of the Sponsor.

21. Publication policy

Publication: "Any activity that discloses, outside of the circle of trial investigators, any final or interim data or results of the Trial, or any details of the Trial methodology that have not been made public by the Sponsor including, for example, presentations at symposia, national or regional professional meetings, publications in journals, theses or dissertations."

All scientific contributors to the Trial have a responsibility to ensure that results of scientific interest arising from Trial are appropriately published and disseminated. The Sponsor has a firm commitment to publish the results of the Trial in a transparent and unbiased manner without consideration for commercial objectives.

To maximise the impact and scientific validity of the Trial, data shall be consolidated over the duration of the trial, reviewed internally among all investigators and not be submitted for publication prematurely. Lead in any publications arising from the Trial shall lie with the Sponsor in the first instance. The study will be registered on www.clinicaltrials.gov.

21.1. Before the official completion of the Trial,

All publications during this period are subject to permission by the Sponsor. If an investigator wishes to publish a sub-set of data without permission by the Sponsor during this period, the Steering Committee shall have the final say.

Exempt from this requirement are student theses that can be submitted for confidential evaluation but are subject to embargo for a period not shorter than the anticipated remaining duration of the trial.

21.2. Up to 180 days after the official completion of the Trial

During this period the Chief Investigator shall liaise with all investigators and strive to consolidate data and results and submit a manuscript for peer-review with a view to publication in a reputable academic journal or similar outlet as the Main Publication.

- The Chief Investigator shall be senior and corresponding author of the Main Publication.
- Insofar as compatible with the policies of the publication outlet and good academic practice, the other Investigators shall be listed in alphabetic order.
- Providers of analytical or technical services shall be acknowledged, but will only be listed as co-authors if their services were provided in a non-routine manner as part of a scientific collaboration.
- Members of the Steering Group shall only be acknowledged as co-authors if they contributed in other capacities as well.
- If there are disagreements about the substance, content, style, conclusions, or author list of the Main Publication, the Chief Investigator shall ask the Steering Group to arbitrate.

21.3. Beyond 180 days after the official completion of the Trial

After the Main Publication or after 180 days from Trial end date any Investigator or group of investigators may prepare further publications. In order to ensure that the Sponsor will be able to make comments and suggestions where pertinent, material for public dissemination will be submitted to the Sponsor for review at least sixty (60) days prior to submission for publication, public dissemination, or review by a publication committee. Sponsor's reasonable comments shall be reflected. All publications related to the Trial shall credit the Chief and Co-Investigators as co-authors where this would be in accordance with normal academic practice and shall acknowledge the Sponsor and the Funders.

22. Statement of Compliance

The trial will be conducted in compliance with the protocol, Sponsor's SOPs, GCP and the applicable regulatory requirement(s).

The study conduct shall comply with all relevant laws of the EU if directly applicable or of direct effect and all relevant laws and statutes of the UK country in which the study site is located including but not limited to, the Human Rights Act 1998, the Data Protection Act 1998, the Human Medicines Regulations 2012, the Medicines for Human Use (Clinical Trial) Regulations 2004, and with all relevant guidance relating to medicines and clinical studies from time to time in force including, but not limited to, the International Conference on the Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use, the World Medical Association Declaration of Helsinki entitled 'Ethical Principles for Medical Research Involving Human Subjects' (2008 Version), the UK policy framework for Health and Social Care Research (Version 3.2, October 2017).

This study will be conducted in compliance with the protocol approved by the REC and according to GCP standards and UK Clinical Trials Regulation. No deviation from the protocol will be implemented without the prior review and approval of the Sponsor and REC except where it may be necessary to eliminate an immediate hazard to a research subject. In such a case, the deviation will be reported to the Sponsor and REC as soon as possible.

23. List of Protocol appendices

Appendix 1 Summary of Protocol Revision History

Appendix 2 Summary chart of Study assessments

24. References

1. Heath PT, Culley FJ, Jones CE, et al. Group B streptococcus and respiratory syncytial virus immunisation during pregnancy: a landscape analysis. *The Lancet Infectious diseases.* 2017.
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prevention of early- and late-onset invasive disease in young-infants. *Vaccine.* 2013;31 Suppl 4:D52-57.
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Appendix 1

Protocol amendment /Revision History

Protocol Version and Date Amendment type (Substantial/non-substantial) and Number	Justification/explanation	Protocol section

Appendix 2. Template summary chart of study assessments

Please note that the summary chart below should only be used as guidance and ensure that the list of procedures in the chart as well as treatment and follow up timelines relate to procedures in you study.

Study Procedures	swab sub-study (SGH & KH only) @ 35 weeks to delivery	Delivery study (all participants)	Guthrie card collection sub-study (swab sub study participants who are GBS colonised only)	iGBS surveillance (all participants)iGBS surveillance	Follow up at 90 days (all participants in swab sub-study)*3months*)
Informed consent	x	x			
Inclusion criteria	x	x			
Medical history	x	x			
Demographics	x	x			
Blood sample (maternal blood and /or cord blood)		x			
Guthrie card collection			x		
iGBS surveillance				x	
Telephone call					x