CLINICAL STUDY PROTOCOL

Title: Group-B streptococci: developing a correlate of protection for future vaccine trials with the help of pregnant Gambian women and their infants

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Protocol No:

SCC No:	**1350**
Brief Title	Group-B Streptococcal Antibody in Mothers and Infants (GAMI)
Protocol Version – Date	Version 1.0 17.1.14
Sponsor	Medical Research Council Unit, The Gambia Atlantic Road, Fajara PO Box 273 Banjul, The Gambia, West Africa
Principal Investigator	Dr Kirsty Le Doare BA (Hons.) MBBS MRCPCH

Protocol amendment(s)

Amendment #:

Principal Investigator:	**Signature:**	**Date:**
_____	_____	_____
Name, Title		

Sponsor's representative:	**Signature:**	**Date:**
_____	_____	_____
Name, Title		

Signature page

The study will be carried out in accordance with the protocol, the principles of good clinical practice as laid down in the ICH Harmonised Tripartite Guideline for Good Clinical Practice, and in accordance to local legal and regulatory requirements.

Principal Investigator:	**Signature:**	**Date:**
Dr Kirsty Le Doare		
		17.1.14
Name, Title		

Sponsor's representative:	**Signature:**	**Date:**
Prof Beate Kampmann		17.1.14
Name, Title		

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Key roles

For questions regarding this protocol, contact:

Dr Kirsty Le Doare,
Whittle Building
Department of Infant Immunology
Vaccinology theme
MRC Unit - The Gambia
Fajara
Tel: Switchboard (+220) 4495442/6 Ext.5010
Mobile: (+220) 7343780
Email: kdoare@mrc.gm, k.mehring-le-doare@imperial.ac.uk

Author:	Dr Kirsty Le Doare
Sponsor's representative:	Professor Beate Kampmann
Principal Investigator(s):	Dr Kirsty Le Doare
Investigator(s):	Professor Beate Kampmann Dr Ed Clarke
Trial Physician:	Dr Kirsty Le Doare
Statistician:	
Data Manager:	??
External Adviser:	Prof. Andrew Gorringe, Prof. Paul Heath
Clinical Laboratory:	
Other institutions:	Imperial College London Wellcome Trust UK
Ethics Committee	Gambia Government/MRC Joint Ethics Committee, c/o MRC Unit, The Gambia, PO Box 273, Banjul, The Gambia, West Africa

List of abbreviations

AE	Adverse Event
CRF	Case Report Form
DMC	Data Monitoring Committee
GCP	Good Clinical Practice
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
MRC	Medical Research Council; represents Medical Research Council Unit, The Gambia
PI	Principal Investigator

Protocol summary

Title:	**Group-B streptococci: developing a correlate of protection for future vaccine trials with the help of pregnant Gambian women and their infants**
Brief title:	GBS antibody: developing correlates of protection
Population:	Pregnant women and Newborn infants followed up until 3 months of age
Number of participants:	100
Number of Sites:	1
Location of Sites:	Faji Kunda Health Centre
Study Duration:	12 – 15 months
Duration for Participants:	3 months
Objectives:	To describe: 1) To describe the strain-specific prevalence of GBS carriage in women and their infants in a field setting in the Gambia 2) To develop functional tools which enable us to examine the relationship between maternal serotype-specific GBS antibody and carriage of GBS in neonates, which could serve as a surrogate marker for protection from disease in GBS vaccine trials.
Description of Study Design:	This longitudinal study will assess mothers and infants at three time points, birth, approx. 1 week and 2-3 months following the birth. A rectovaginal swab, breast milk (1-2mL) and umbilical cord blood sample (4-5mL) will be taken from women in labour and a nasopharyngeal and rectal swab will be taken from the infant at birth. At approx. 1 week a nasopharyngeal and rectal swab will be taken from the infants and breast milk (4-5mL) from the woman. At 2-3

months a (4-5mL) blood sample will be taken from the infant by venipuncture as well as a nasopharyngeal and rectal swab and (4-5mL) sample of breast milk from the mother. Recruitment will be from the maternity ward at the Faji Kunda health centre, and restricted to healthy singleton infants, birth weight >2.5kg, without congenital abnormalities. Informed consent will be obtained from mothers. 100 pregnant women and their newborn infants will be recruited over 6-months (allowing for a dropout rate of up to 20%).

1 Background information and rationale

1.1 Background information and rationale

GBS Epidemiology

In resource-rich countries GBS is the most common cause of serious bacterial infections in the first week of life (early onset, EO) and of meningitis in the first 3 months of life (late onset, LO), with long-term adverse neurodevelopmental outcomes in up to 30%(1,2). Limited data from three African countries reveal a significant burden of disease (1.21-2.06/1000 live births) including meningitis (4,5,6).

GBS-acquisition occurs through vertical transmission in 15%-50% of infants born to a vaginally/rectally-colonized mother (a prerequisite for EO and risk factor for LO disease)(7,8,9). EOGBS disease can be prevented by intrapartum antibiotics (IAP) and, in a number of resource-rich countries; EOGBS is significantly less common following the introduction of IAP strategies (10,11). However, IAP protection is incomplete and has no impact on LOGBS. In resource-poor countries IAP use poses additional logistical and financial problems and is poorly implemented (12). Maternal vaccination has the greatest potential for preventing invasive neonatal GBS-disease globally.

Although GBS-colonisation is common, few colonized infants subsequently develop invasive disease (estimated 1-2%)(13,14). Despite relatively high maternal carriage rates (22-23%)(15), 13-year old reports have previously shown low invasive GBS-disease rates in the Gambia (16). Possible explanations are: the prevalence of less virulent strains; differing GBS-serotypes, USA/European invasive GBS-serotypes are predominantly serotype-III (17-20) whilst Gambian GBS-serotype studies report serotype-V predominance(21). The difference in invasive disease prevalence could also be due to higher levels of transplacentally-acquired protective antibody; Suara et al. (1998) reported high mean maternal serotype-III antibody levels in women colonised with any GBS-serotype that might account for the rarity of GBS-disease in The Gambia and other resource-poor countries (21). Alternatively, the role of GBS might have been underestimated because of

inadequate culture techniques and microbiological methods (22). In the past six months we have confirmed the predominance of serotype V antibody in pregnant Gambian women and established GBS-invasive disease prevalence of 1.2/1000 live births in a small hospital-based cohort (unpublished data).

A major factor determining differences in GBS-disease incidence is likely due to unrecognized/undetermined causes of neonatal/premature deaths/stillbirths. The majority of deliveries in resource-poor settings occur outside hospital, increasing the probability that infants developing GBS-infection at birth die before infection is reported. Mulholland et al. (1999) excluded neonates dying before day 2 of life - a number of whom may have succumbed to GBS-disease, including meningitis (16). These fatalities would result in inaccurate measures of EO- and LO-disease incidence. The failure to recognize GBS as an important cause of neonatal sepsis in resource-poor countries may therefore reflect insufficient surveillance, true biological population differences or both.

The role of GBS Antibody

To foster effective vaccine development, data describing prevalent serotype differences by geographical region are urgently needed to ensure the inclusion of the most relevant components in a global GBS-vaccine. GBS serotype-specific multivalent CPS-conjugate vaccine candidates, including serotypes Ia, Ib, III and V have been evaluated for safety and immunogenicity in non-pregnant adults(23,24) and pregnant women (25). A major difficulty is the implementation of efficacy trials, which use the prevention of invasive neonatal GBS-disease as endpoint. Surrogate immunological markers, such as protective antibody levels, are required and have previously been used in vaccine licensure, e.g. meningococcal group-C conjugate vaccines.

A protective role for GBS-capsular polysaccharide (CPS) antibodies was predicted in the 1970s(26,27); Infants with invasive EOGBS disease were born to mothers with negligible type-III CPS-specific antibody in sera at delivery. The finding that human sera containing a sufficient concentration of CPS-specific antibody promoted efficient opsonisation and phagocytosis *in-vitro* affirmed the importance of capsular serotype specific antibodies in disease protection. This information suggested that active immunisation of pregnant women with purified GBSCPS could protect young infants (28-30).

GBS antibody in breast milk

Secretory immunoglobulin A (s-IgA), the predominant antibody class in breast milk, plays an important role in maternal-to-infant transfer of immunity in pneumococcal infection and immunization(31) and may also contribute to protection against neonatal GBS-disease(32, 33). Few small studies have assessed the transfer of GBS-antibody in breast milk, demonstrating persistence of serotype-III s-IgA-antibody up to two months of age, indicating their potential role in the prevention or amelioration of LOGBS(33, 34). In a population with a high breastfeeding rate this additional protection against GBS at the mucosal surface plays an important role in immunity from disease.

Data regarding the immunological correlates between maternal serotype, antibody transfer and invasive disease have focused on a limited range of serotypes. To date only one small study of vaccination in pregnant women has demonstrated persistence of serotype-III-antibody in infants up to two months of age (28).

The importance of functional assessments in GBS antibody

In addition to antibody levels, functionality is likely to play an important role. Several killing-based opsonophagocytosis assays (kOPA) that mimic the *in-vivo* process of GBS-killing by host effector cells, following opsonization by specific antibodies have been used for this purpose (35,36). However, bacterial growth, colony plating and counting are time- and resource-consuming steps and standardization presents challenges. Fluorescence-based OPAs (fOPA) can limit these obstacles and development is underway(37,38).In addition, our collaborators at HPA Porton have demonstrated for *Neisseria meningitidis* that antibody-mediated complement C3 deposition correlates strongly with opsonophagocytosis. This assay has advantages of requiring small serum volumes and a large number of sera can be processed in a single assay.

Preliminary data from the Gambia

Using microbiological data from the MRC hospital at Fajara, it is possible to give crude estimates of GBS invasive disease over an eight-year period in a hospital with 1,500 births annually. A crude estimate based solely on blood and CSF cultures equates to a burden of invasive disease in neonates under 3 months of age was 1.4/1,000 live births, comparable to larger cohorts in South Africa, Kenya and Malawi. A clinical trial testing the utility of single-dose Azithromycin in the prevention of neonatal sepsis in the Gambia is currently enrolling pregnant women at the time of delivery and has also confirmed that carriage rates identified in earlier studies are similar today (24%, Roca, unpublished data).

In collaboration with MRC Infant Nutrition Group in Gambia, we have been able to estimate serotype-specific antibody prevalence in pregnant mothers at 30 weeks gestation and birth and in infants at 12, 24 and 56 weeks of life. Interestingly, infant antibody levels rise between 24 and 56 weeks, which could indicate, that colonisation plays a role in antibody development in otherwise healthy children (Figure 1 and 2). The Reverse cumulative distribution curve confirms the prevalence of serotype V antibody in Gambian women, followed by serotype Ia, II, III and Ib. This work now needs to be extended and functional assays added in order to answer questions concerning the correlation between antibody concentration and infant carriage in this group and assess the suitability of this approach for vaccine testing.

Figure 1 – Reverse cumulative distribution curve for GBS serotypes in cord blood in Gambian women

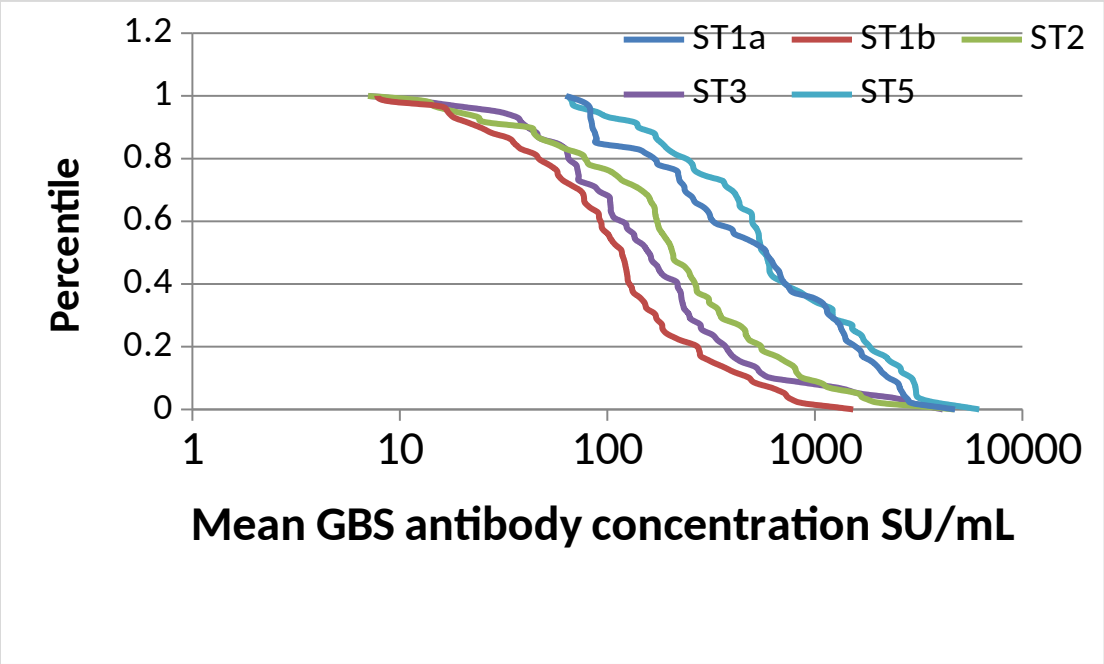
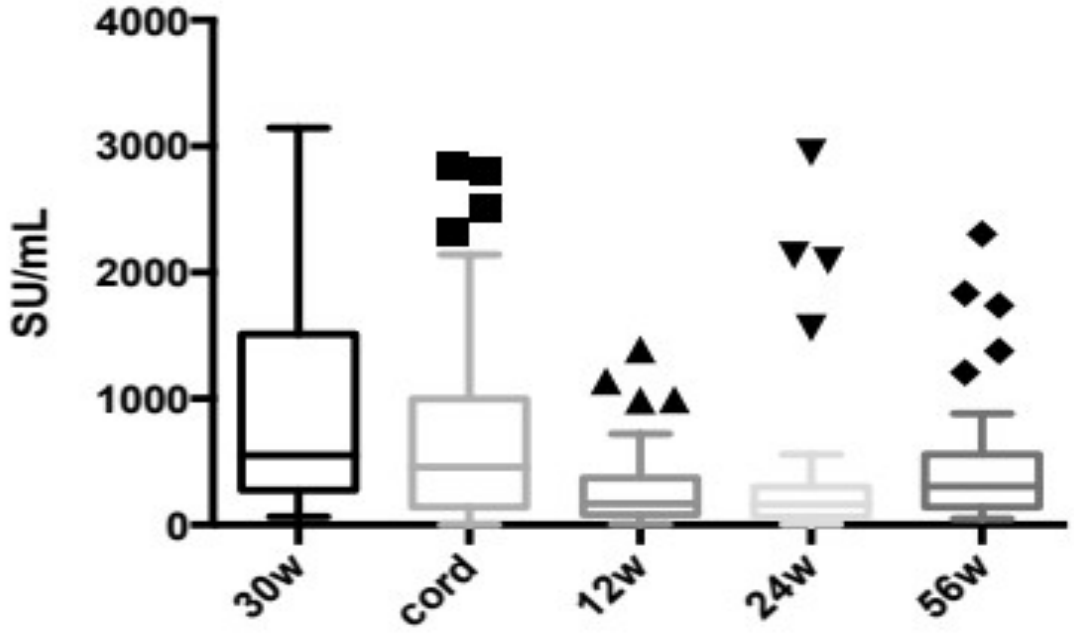


Figure 2 Median concentration of GBS serotype specific antibody during the first year of life in Gambian infants



References of literature and data are listed in Section 11.

1.2 Potential risks and benefits

The potential risks to human subjects and known benefits, if any, are summarised in Section "Human Subject Protection".

Improving the health of people of The Gambia

This study will provide much-needed data on prevalence of colonization in mothers and carriage in infants in the Gambia. We wish to provide evidence that any potential vaccine candidates are effective in Gambian women and their infants and will examine the link between maternal carriage, infant colonization and antibody-responses by studying both trans-placental antibody and antibody contained in breast milk. Prospective cohort studies measuring the development of disease in infants born to colonized mothers as an endpoint would have to be extremely large and are beyond the scope of a single-site, resource-limited study. Instead, we will investigate serotype-specific antibody transfer, neonatal antibody persistence and its function in-vitro in order to gain insight into the potential for assays measuring functionally active antibody to be used as a surrogate marker of protection from disease. A functional antibody assay could serve as a surrogate marker for disease for use in vaccine licensure and limit the need for extensive and expensive phase III trials. The main important aspect is that ultimately infants in The Gambia and other resource-poor as well as resource-rich settings can benefit. This represents a long-term aim of course, as we are not conducting a clinical trial but wish to gain deeper insights into mechanisms, which can be exploited for future interventions. We hope that this study will help health professionals to understand more about how maternal immunity can protect infants from GBS disease. We hope that what we find out in this study will help to stop infants in The Gambia and other parts of the world from becoming sick in the future.

Potential benefits to wider communities

This research will contribute to developing methods that require very small blood samples and yet yield a large amount of information through use of flowcytomeric technologies. This will be of benefit not just to my project but also to the wider academic community. I aim to identify serotype

specific antibody to GBS and develop techniques to test the ability of antibody to kill GBS, which could be amenable to industry as a surrogate marker of protection from disease in subsequent phase IV clinical trials. Such correlate markers of protection have already been used in the licensing of meningococcal group C vaccine and have the huge advantage of limiting phase III trial size that often limits vaccine launch.

To examine a possible connection between carriage of organisms and subsequent GBS-antibody concentration and killing ability is a new and evolving area of research, which has become possible through cutting edge flowcytometric techniques allowing the assessment of high sample numbers in a small specimen. This technology is ideally suited to infant serology work. In addition to simply measuring antibody concentration at each time point, we wish to link infant colonisation to functional antibody responses in neonates, which is a novel approach and additionally strengthened by the longitudinal approach within the study, which will swab a number of infants repeatedly.

2 Study objectives

To describe:

1) to describe the strain-specific prevalence of GBS carriage in women and their infants in a field setting in the Gambia
2) to develop functional tools which enable us to examine the relationship between maternal serotype-specific GBS antibody and carriage of GBS in neonates, which could serve as a surrogate marker for protection from disease in GBS vaccine trials.

2.1 Study endpoints

1. **<u>Serotype-specific GBS colonisation in mothers and infants</u>**

Prevalence of serotype-specific GBS carriage in mothers and their infants and changes in infant carriage over time will be determined by culturing rectovaginal swabs and infant nasopharyngeal and rectal swabs in selective microbiological media for GBS.

2. **<u>Serotype-specific GBS antibody concentration</u>**

Serotype-specific GBS antibody-concentration (Ia, Ib, II, III, V) in blood and breast milk will be determined at each of the three time points using flow cytometry.

3. **GBS-antibody function**

Serotype-specific GBS antibody function (Ia, Ib, II, III, V) will be determined in blood and breast milk using complement C3b/C5 deposition and flow cytometric methods.

3 Study design

3.1 Type of study and design

Study design

Study Design (See Figure 3 for study design).

This is a prospective cohort study, based at Faji Kunda Health Centre of pregnant women recruited at 37 weeks gestation from the antenatal clinic at Faji Kunda and their infants from birth to 3 months of life.

Interventions

a. Samples in labour: combined rectovaginal swabs will be taken from mothers in labour and nasopharyngeal and rectal swabs their infants at birth. 1mL of colostrum taken within 72 hours of birth, 4-5 mL of cord blood.
b. Samples at day 6: nasopharyngeal and rectal swabs from infants, 1-2 mL breast milk
c. Samples at day 56-90: nasopharyngeal and rectal swabs from infants, 4-5 mL breast milk, 2-3mL infant serum

Data Collection

A case report form (CRF) will capture information on:

Epidemiological data:

a) maternal: Site of delivery, mode of delivery, antenatal risk factors for infection (Premature rupture of membranes/chorioamnionitis/maternal fever/raised white cell count), number of live births, information on maternal vaccination status, information of any infections leading up to delivery requiring antibiotics, time in hours of rupture of membranes prior to delivery.

b) infant: gestational age, birth weight, stillbirths/neonatal deaths/perinatal complications

(birth asphyxia/meconium staining/signs of severe infection in the first three months of life (fever >38.5 degrees, raised white cell count, meningism). Gestational age calculated by last menstrual period or midwife palpation or ultrasonography if available.

Sample Collection

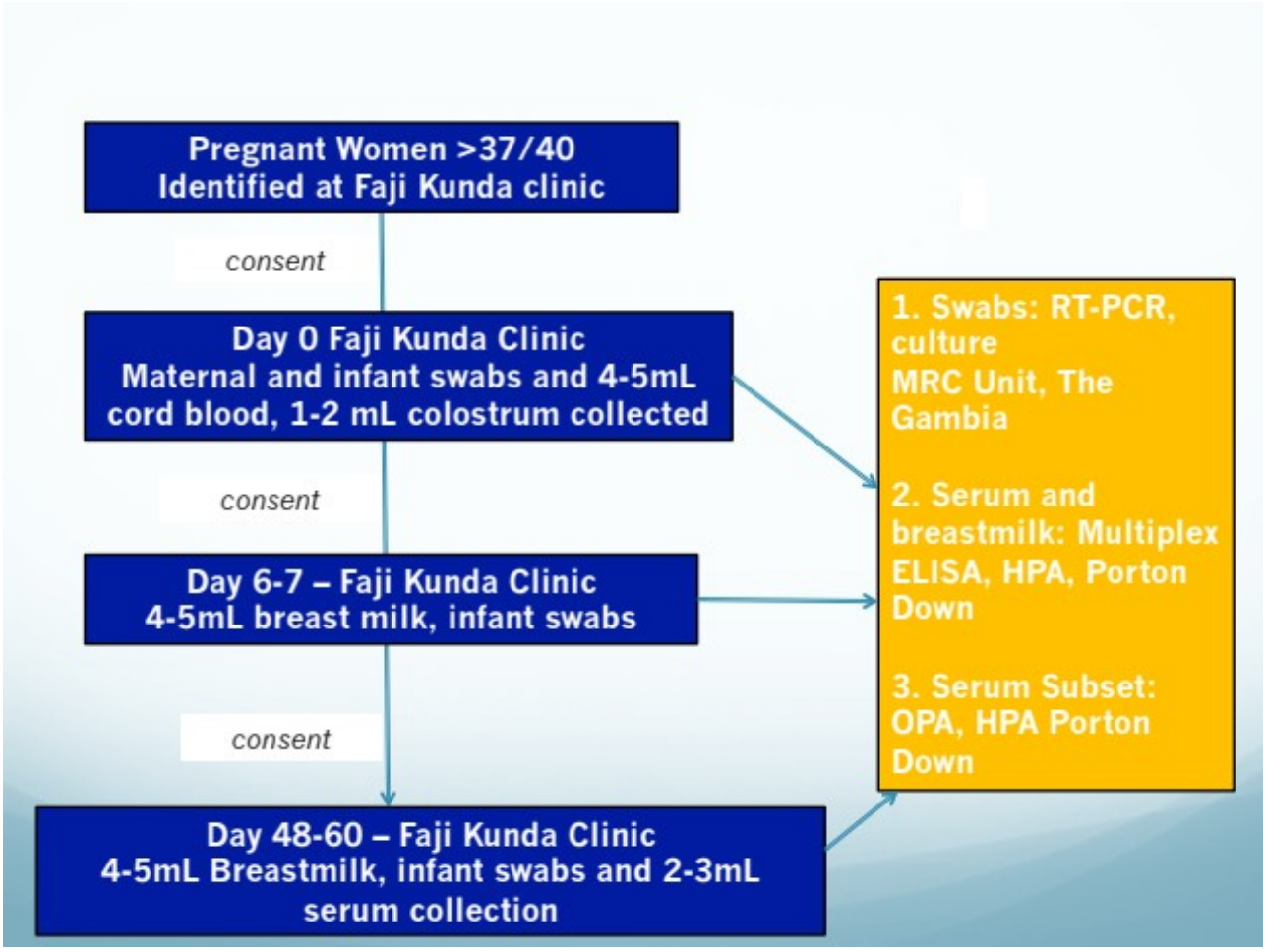
1) *Maternal/infant carriage:*
   a) Maternal swabs: Combined-vaginal-rectal swabs in labor from all mothers who have been consented in the antenatal clinic.
   b) Infant swabs: nasopharyngeal and rectal swabs in the first 24 hours after birth of all infants whose mothers have been enrolled into the study, second infant swabs on day 6 and final infant swabs collected at day 48

2) *Antibody Carriage Serum:*
   a) Maternal serum: Cord blood or maternal serum sample at delivery to determine antibody levels.
   b) Infant serum: at two months using venipuncture.

3) *Antibody Carriage Breast milk:*

Breast milk will be collected at day 0, 6 days and 2-3 months post-partum. The women will be given non-sterile surgical gloves and asked to manually express several drops of milk, which were discarded before collecting the specimen directly into the test tube.

Figure 3 – Study Design



All infants will be followed-up from birth to 3 months of age and will receive their EPI schedule normally as shown in Table 1. The flow chart of the study is presented in Figure 7.

Table 1: EPI Schedule in Faji Kunda

Age (week)	Vaccine
0 (within 48 hours of birth)	BCG, HBV, OPV
8	DTP, Hib, HBV, OPV, PCV-13* RV
12	DTP, Hib, HBV, OPV, PCV-13* RV

**PCV-13 replaces PCV-7 in April 2011.*

Key: **BCG**: Bacillus Calmette-Guerin, **HBV**: Hepatitis B vaccine, **OPV**: oral Polio vaccine, **DTP**: Diptheria, Tetanus, Pertussis, **Hib**: *Haemophilus Influenza* Type b, **PCV-13**: Pneumococcal Conjugate Vaccine-13. **RV:** Rotavirus (Rotateq)

4 Selection and withdrawal of participants

4.1 Selection of participants

Recruitment

Mothers and their newborns will be recruited by MRC Infant Immunology group's field site at Faji Kunda health centre, located in a peri-urban area in The Gambia. Field workers and nurses known to the local community will do sensitization of mothers to the study. Government staff working in the antenatal clinic and on the labour ward at the health centre may provide additional information. When possible, field workers/nurses will talk to potential participants and their families in advance of the delivery to sensitize them to the study and to provide them with a copy of the informed consent document. If a pregnant woman is interested in the study, a sticker will be placed in her yellow antenatal record card. She will also be given a card with contact numbers for the study staff. The study team will be contacted when women present in labour either by the women themselves, or the midwives on duty. Mothers presenting to the health centre in labour

may additionally be sensitized to the study if this has not occurred in advance and when adequate time is available.

Oral consent will be gained from the mothers at the time of delivery in order to collect rectovaginal swabs before delivery and cord blood (which has to be processed within 6 hours of collection). The midwives will collect cord blood samples on duty or by a member of the study team. Written informed consent will then be obtained by a fieldworker/nurse shortly after birth, when they have recovered sufficiently and will include consent to collect a nasopharyngeal and rectal swab from the newborn and to collect breast milk. Final enrolment will occur after informed consent has been obtained and only if the neonate meets the inclusion criteria and does not meet any of the exclusion criteria (see below).

They will be asked to provide further informed consent to a nasopharyngeal and rectal swab of the newborn and collection of breast milk coinciding with the first postnatal check at approximately 6 days post-partum. A Final breast milk sample, infant venipuncture sample and nasopharyngeal and rectal swab will be taken at 2 months at the infant's first immunisation clinic visit.

4.2 Eligibility of participants

Newborns must meet all of the inclusion criteria and none of the exclusion criteria to be eligible to participate in the study.

4.2.1 Inclusion criteria

Maternal:

1. Written informed consent obtained from the infant's mother
2. Maternal age over 18 years
3. >37/40 gestation
4. Born to known HIV negative mothers (tested and documented on the yellow antenatal card in the current pregnancy

4. Ability to comply with the study procedures as judged by a member of the research team
5. Remaining in the Faji Kunda area for at least the first 12 weeks following delivery
6. Planning to breastfeed

Infant:

1. Singleton
2. Birth weight >=2.5kg
3. Healthy infants without congenital birth defects requiring prolonged hospital stay (>48 hours)

4.2.2 Exclusion criteria

1. Significant congenital abnormalities
2. Symptoms or signs of significant illness or infection
3. Planning to move outside the study area (preventing follow-up visits) during the 3 months of study participation
4. Enrollment in other studies requiring blood/breast milk sampling or swabs
5. Any other condition or situation meaning that participation would not be in the best interests of the infants
6. Any reason that would prevent the study endpoints being assessed in the infant effectively as judged by the investigator
7. Multiple Pregnancies
8. Complications of delivery (preeclampsia, antepartum hemorrhage, caesarian section)
9. Premature infants (born prior to 37 weeks gestation)
10. Bottle-fed infants
11. Known HIV infection
12. Birth weight <2.5kg.

4.3 Withdrawal of participants

A study participant will be discontinued from participation in the study if:

- Any medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant.
- Development of any exclusion criteria.

For further details on participant's premature termination see corresponding section below.

Participants are free to withdraw from the study at any time without giving a reason.

5 Study procedures and evaluations

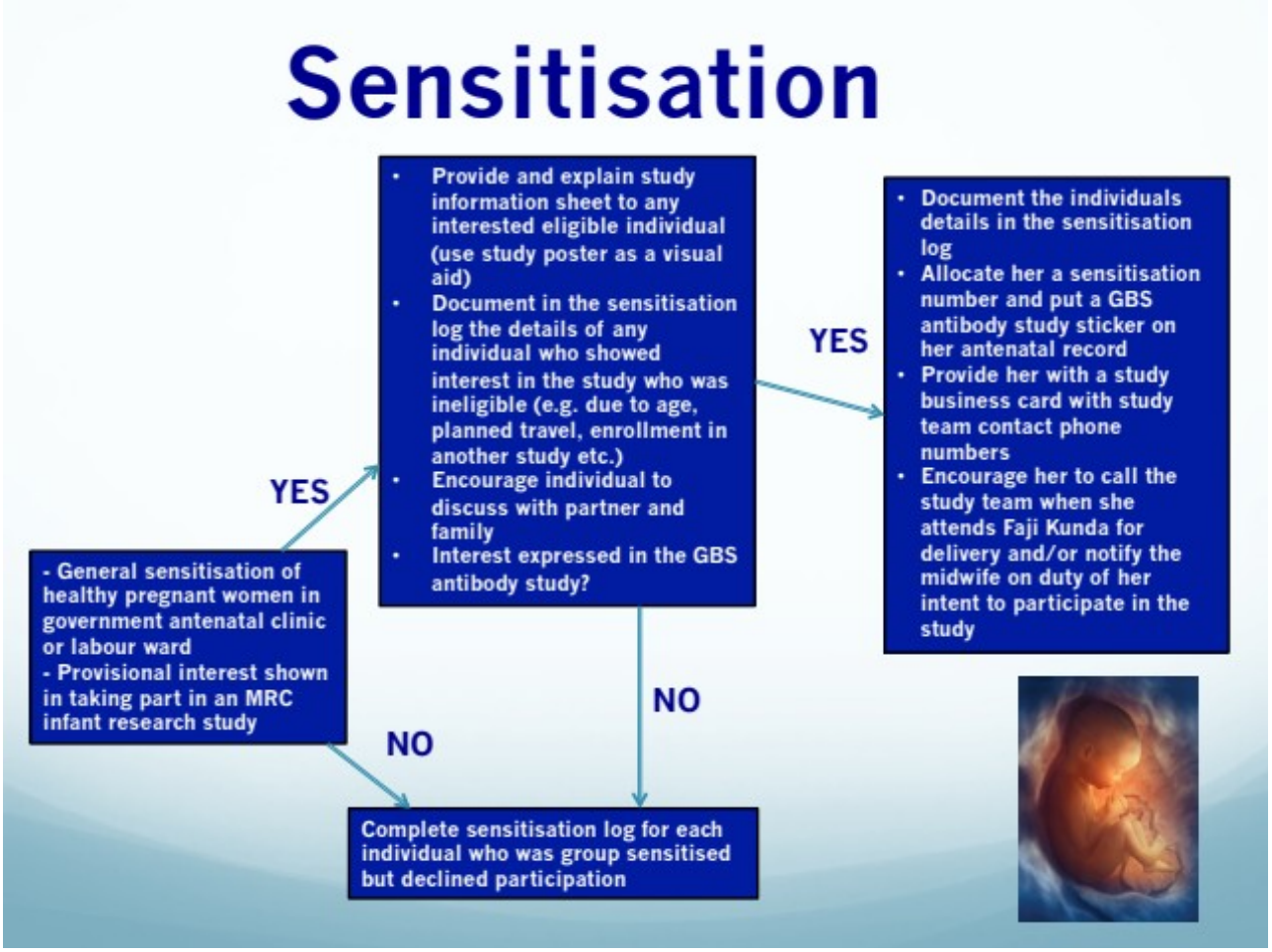
For an overview see annex "Schedule of Events".

5.1 Study schedule

All infants will be followed-up at 3 time-points between births to age 3 months and will receive their EPI schedule normally as shown in Table 1.

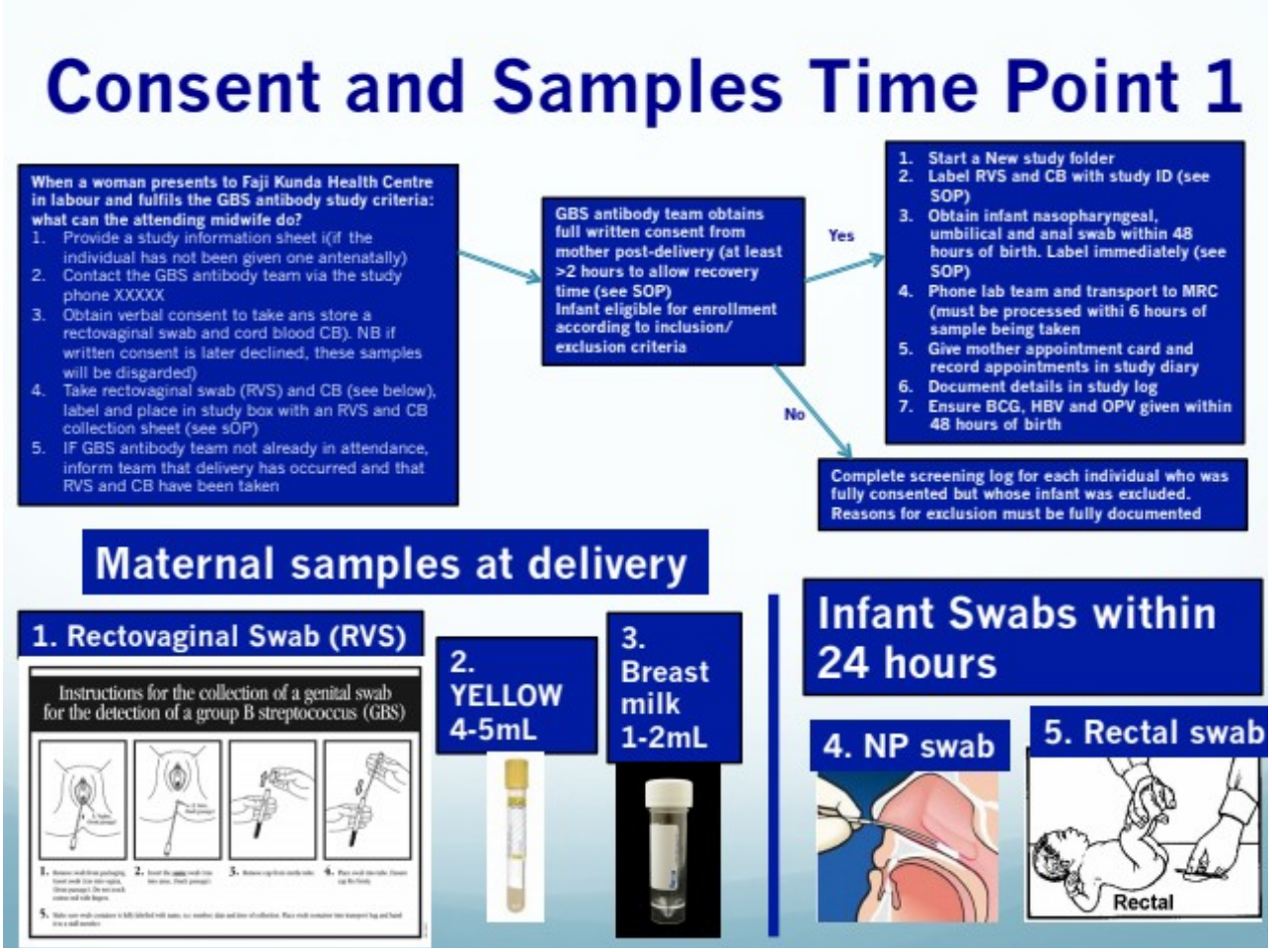
5.1.1 Screening

Figure 4 - Sensitisation



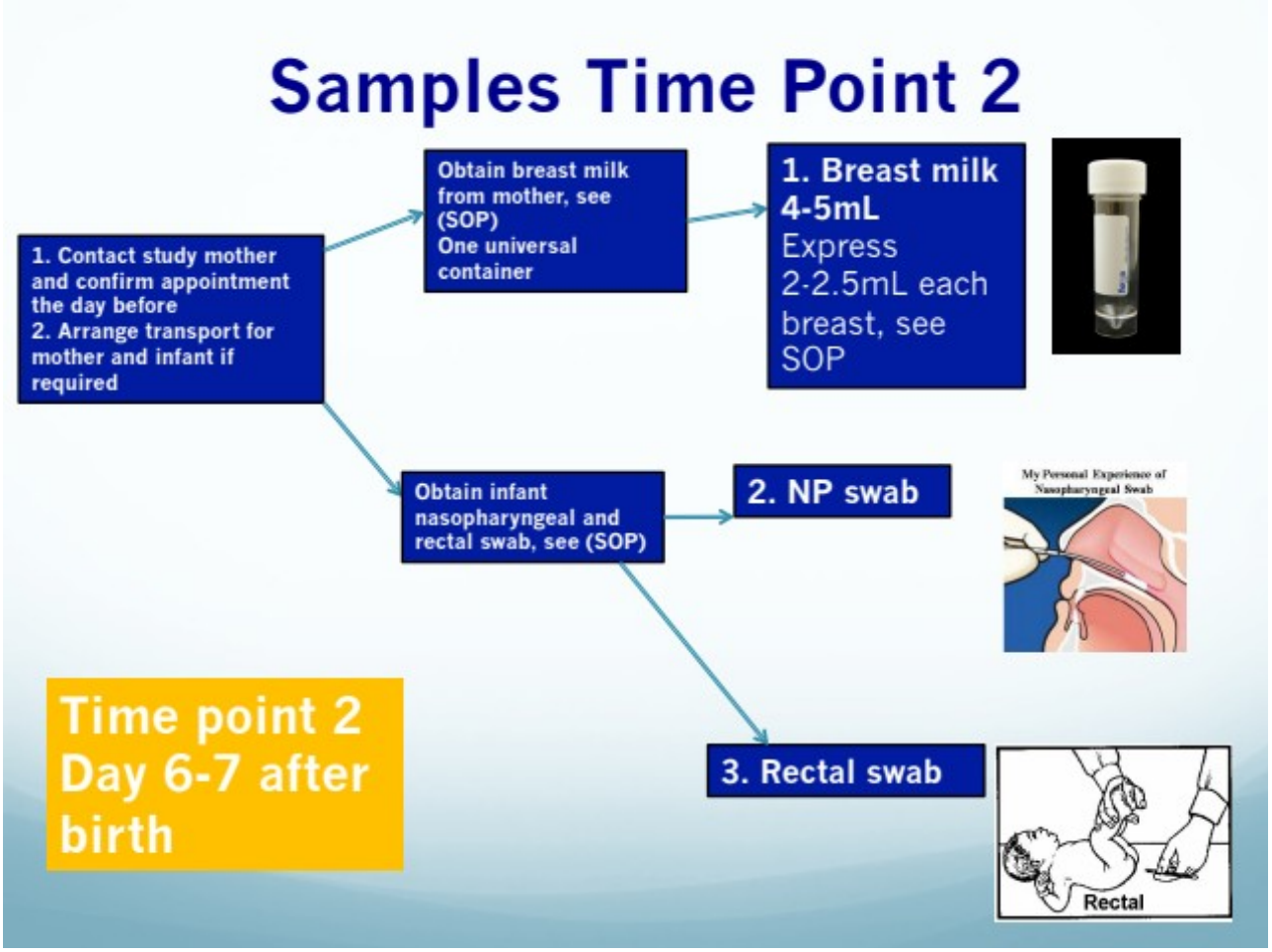
5.1.2 Enrollment (Baseline)

Figure 5 – Visit 1



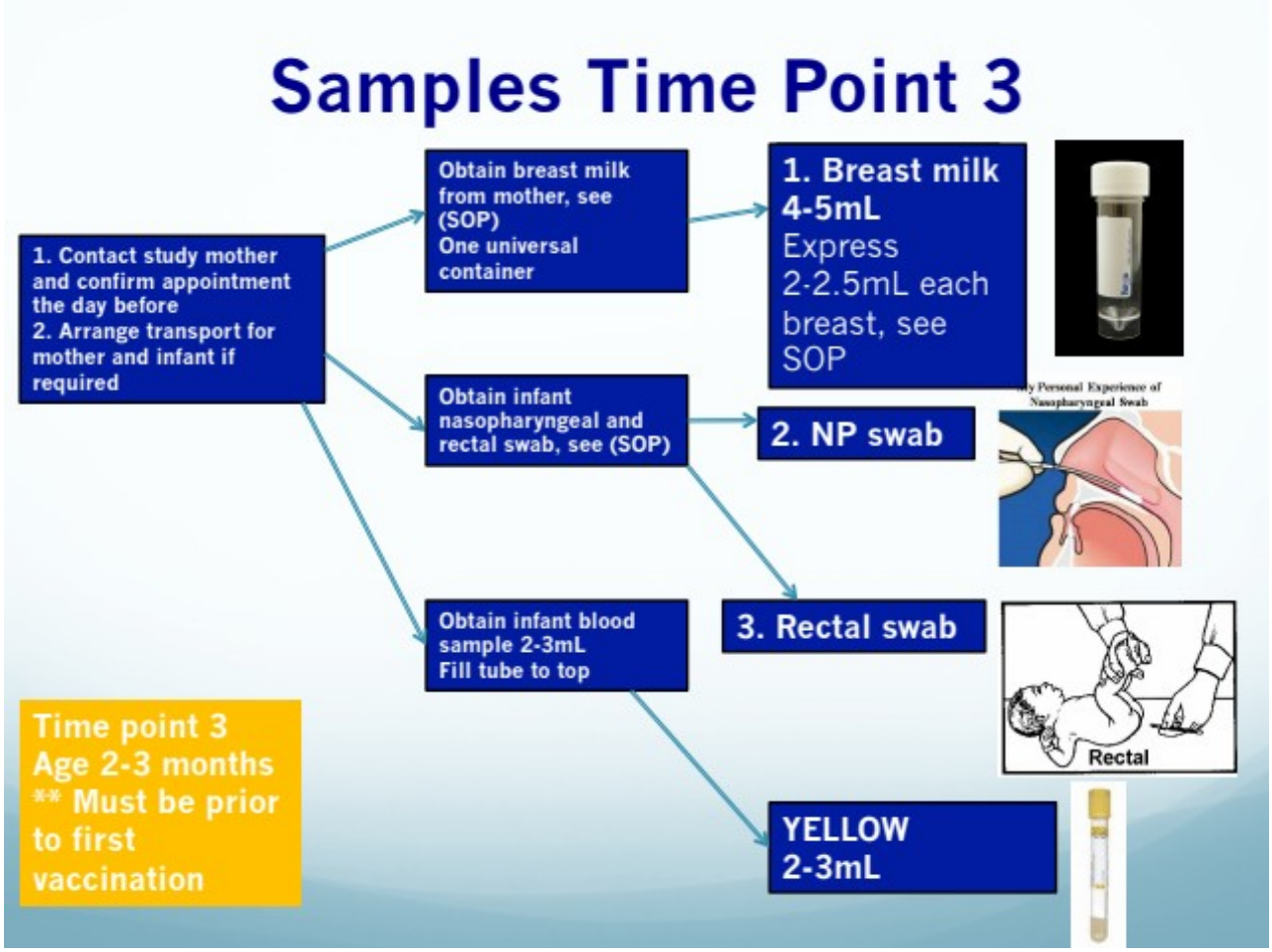
5.1.3 Follow-up – Visit 2

Figure 6 – Visit 2



5.1.4 Final study visit

Figure 7 – Visit 3



5.2 Study evaluations

A case report form will be completed at each time point. At the time of screening for study eligibility, the following information will be recorded on the appropriate case report form (CRF):

- Study mother demographic information
- Study mother medical information to include: Gestational age calculated by last menstrual period or midwife palpation or ultrasonography if available, gravida, parity, living children, HIV status, time of rupture of membranes prior to delivery, mode of delivery, antenatal risk factors for infection (Premature rupture of membranes/chorioamnionitis/maternal fever), information on maternal vaccination status, information of any infections leading up to delivery requiring antibiotics, current medication.
- Study infant demographic information

- Study infant medical information to include: gestational age, birth weight, perinatal complications (birth asphyxia/meconium staining/fever >38.5 degrees, hypothermia, poor feeding, floppiness, hypoglycaemia.

At each subsequent time point the following information will be recorded on the appropriate case report form (CRF):

- Feeding type
- Any overnight hospital admissions.
- Any episodes of malaria, septicaemia, pneumonia, meningitis, worms, otitis media, tonsillitis, upper respiratory tract infection (URTI)
- Any courses of intravenous or oral antibiotic therapy.
- Any current medications

5.2.1 Clinical evaluations

At recruitment a limited newborn examination will be performed by the study clinician or nurse as detailed in the screening record.

At each time-point infant anthropometry will be recorded including:

- Weight (grams)
- Head circumference/Occipitofrontal circumference (OFC) (cm)
- Length (cm)

At each time-point infant vital signs will be recorded including:

- Respiratory rate (breaths per minute)
- Oxygen saturations (%)
- Heart rate (beats per minute)
- Temperature (°C)

In addition to the study time-points the infants will be given their routine EPI vaccines by the study staff. The vaccine details will be recorded on their infant welfare card as well as in the study paperwork.

5.2.2 Laboratory evaluations

Swab culture

Rectovaginal swabs will be taken from all mothers at presentation in labour and sent same day to the MRC laboratory. Nasopharyngeal and rectal swabs will be taken from all infants and sent same day to the MRC laboratory. Maternal and infant swabs will be pre-cultured overnight in Todd Hewitt Broth and cultured by plating on selective blood agar plates for 48 hours. If there is any bacterial growth this will be identified as GBS by latex agglutination test and serotyping by RT-PCR.

Cord blood and at 2 to 3-months of age

Additional experiments will be done from the cord blood samples and from the infants at 3-months of age in order to complete the antibody aspect of this study. The blood samples will need to be transported to MRC laboratories in EDTA tubes within 6 hours of collection, spun down and frozen at -70 degrees. The samples will be batched and sent to Porton Down, UK for serotype-specific antibody analysis and complement deposition studies.

Breast milk

Additional experiments will be done from breast milk samples collected at birth, day 6 and day 60. Women will be asked to hand express 1-2mL of colostrum at the first visit and 4-5mL of breast milk on day 6 and day 60. Breast milk should be expressed from each breast and collected in two sterile universal containers. The samples need to go immediately into cold storage and transferred same day to the laboratory. The samples then need to be aliquoted into 100uL samples and frozen at -70 degrees. Batches of breast milk will then be sent to Porton Down for antibody analysis and complement deposition studies.

Sample handling

Cord blood and 3 months

Spin sample, take off serum into two aliquots and PLACE SAMPLE STRAIGHT IN -70°C & RECORD LOCATION IN WORKSHEETS

Table 2 – Laboratory experiments

Experiment	Time course	Volume required	Method	Sample storage
1. Microbiological swab culture	48 hours (12-56 hours)	1 combined rectovaginal swab (mother) 1 nasopharyngeal swab, 1 rectal swab (infant)	See SOP	Store at 4 degrees until culture. Culture at 37 degrees
2. Antibody surface labeling studies	4 hrs (3.5-4.5 hrs)	>2mL	See SOP	Serum in YELLOW tubes, spin down and aliquot into 2 aliquots and store at -70°C

3. Complement deposition studies	4 hrs (3.5-4.5 hrs)	>2mL	See SOP	Serum in YELLOW tubes, spin down and aliquot into 2 aliquots and store at -70°C

NB/ For full details of the full laboratory procedures please see the corresponding SOPs

6 Safety considerations

Morbidity will be closely monitored as a safety net for participants, and free access to basic health care will be provided to the mothers and their child. This includes basic medical treatment for non-severe infections (urinary, respiratory, malaria, skin infections *etc.*) provided at Faji Kunda clinic during opening hours (open Monday to Thursday 8am until 4pm, and Friday 8am to 12pm). If the infection is severe then the mother/child will need to be referred either to a government hospital or to the MRC site in Fajara for further assessment and/or hospital admission. In most cases, if the family incurs costs at referring facilities or out of hours centres, these costs will be reimbursed by the study team. The free health care will be provided to our participants until the child is 3 months of age, (including participants subsequently excluded) as well as 24/7 access to study staff via mobile phones.

6.1 Methods and timing for assessing, recording, and analysing safety parameters

Adverse events (AEs) or serious adverse events (SAEs) will be monitored during the study

6.1.1 Adverse event reporting procedure

If any adverse events occur which are directly related to the participation in the study, study staff will document these. Records will be in accordance with the MRC Gambia SAE reporting protocol

and include the following information: nature/description of the AE, start and stop date/time, severity of the AE and action taken. Staff will then monitor the events until their resolution.

6.2 Participant's premature termination

A study participant will be discontinued from participation in the study if:

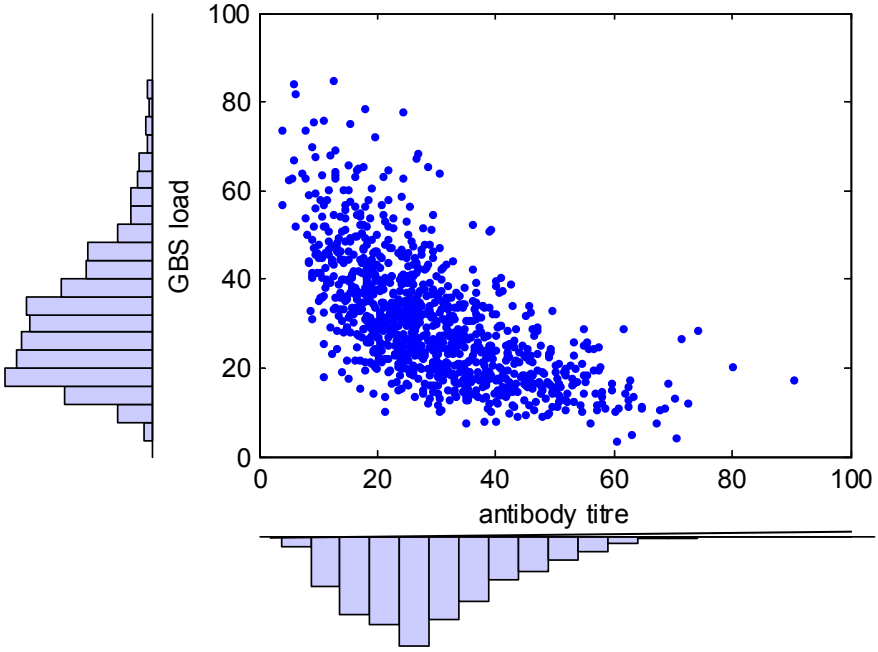
- Intercurrent illness, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject.
- Development of any exclusion criteria.

Subjects are free to withdraw from participating in the study at any time without giving a reason. This withdrawal will not affect their standard medical care.

7 Statistical considerations

Sample Size:
Assume negatively correlated skewed distributions for maternal antibody and maternal (or infant) GBS load, with Figure 2 showing a typical simulated bi-variate distribution (note scale factor not modelled – correlation is unaffected). A sample size of 150 gives 90% power to detect a correlation of -0.45 as significantly different from zero at the 1% level (adjusted down to allow for serotype multiplicity).

Figure 2 Bi-variate distribution of antibody and bacterial load.



With the absence of further variability parameters it is not possible to conduct a classical sample size calculation. Although the study is powered on measuring correlation (i.e. linear trend), the analysis plan focuses on measuring the actual trend rather than relying on linear assumptions.

Analysis Plan

Initial analyses will be based on standard summary statistics comparing maternal and infant responses. Graphical methods with spline smoothers will be used to examine trends and if appropriate (linear trends), correlations will be used to summarize the relationships. Cross-tabulations will compare means (with 95% confidence intervals), medians (with inter quartile ranges), with histograms to compare distributions.

Formal analysis will be based on linear regression for each serotype and will test whether maternal antibodies predict colonization in newborns. Antibody responses will be suitably transformed to satisfy the normality and constant variance assumptions for the regression based analyses. As the relationships are likely to be non-linear fractional polynomials will be used to model non-linearity. In addition to analyses based on continuous data, responses will be categorized and contrasts used to make appropriate comparisons. Where possible regression will be adjusted for potential confounders including mothers with high levels of carriage who have not yet mounted an antibody response, infants without antibody and maternal and infant infections. With both maternal and infant longitudinal sampling, separate analyses will be repeated at each time point. Discrete analyses

based on carriage will be analyzed using a survival analysis approach. With multiple time points and multiple serotypes from the same subjects, false discovery rates will be used to control for false positives from multiple hypothesis tests.

8 Data handling and record keeping

8.1 Data management and processing

Data storage and handling

Paper clinical record forms will be transcribed to a dedicated database, which will be stored on the MRC-Unit the Gambia's secure SQL server. A database will be designed with the help of an MRC data manager. This will reflect the content of the study clinical record forms CRFs. All the scientific data will be maintained within a pre-designed excel database, which will be backed up on 2 external hard drives. The data management department at the MRC will provide guidance on the data quality control systems and tracking systems for patients and laboratory samples. The database will adhere to good clinical practice (GCP) where possible.-

Data sharing

This work contributes to the advancement of scientific knowledge and the broadening of understanding of human physiology using state-of-the art laboratory methods. Our research aims to identify key antibody-mediated parameters as potential targets of future vaccine trials to prevent and treat infection in newborns.

8.2 Source documents and access to source data

The Principal Investigators will maintain appropriate medical and research records for this study in compliance with the principles of good clinical practice and regulatory and institutional

requirements for the protection of confidentiality of participants. The study team members will have access to records.

The authorised representatives of the sponsor and the ethics committee—may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) for the participants in this study. The clinical study site will permit access to such records.

9 Ethical considerations

This study is conducted in accordance with the principles set forth in the ICH Harmonised Tripartite Guideline for Good Clinical Practice and the Declaration of Helsinki in its current version (see appendix), whichever affords the greater protection to the participants.

9.1 General considerations on human subject protection

9.1.1 Evaluation of risks and benefits

Risks

There are few risks to the individual mothers and babies in the study. Collecting blood, and taking a rectovaginal, nose and rectal swabs are safe procedures, which will not cause any harm. However, all swabs require the patient to remain quite still, and may cause a little temporary discomfort. At the study visits parents will have the opportunity to discuss any concerns they may have about their baby's health. If the investigator discovers a study baby is sick and decides that he/she cannot participate in the study because of that, he/she will receive immediate care at the study site and then be referred to the MRC hospital ward/clinic or other health facility as appropriate. Parents will get either transport by MRC or get the costs for the transport reimbursed.

Benefits to participants

The research does not directly benefit the participants, but may benefit babies in the future. The mothers of the babies involved in the study will have the opportunity to meet with and ask questions of myself, a qualified paediatrician. They will also have access to health care at the MRC hospital facility, which is available to any participants of an MRC sponsored study.

9.2 Informed consent

See study specific SOP. Parents or guardians of all participants will be fully informed of the aims and practical implications of my study during a rigorous process of informed consent, which will be done in their first language (or English) both verbally and in writing.

9.3 Participant confidentiality

Guarantees of confidentiality and anonymity given to the research participants will be honoured, unless there are clear and overriding reasons to do otherwise. Researchers will practice in accordance with the 'duty of confidentiality' and not pass on identifiable data to third parties without participants' consent. Paper clinical record forms will be transcribed to a dedicated database, which will be stored on the MRC-Unit the Gambia's secure server. The data management department at the MRC will provide guidance on the data quality control systems and tracking systems for patients and laboratory samples. (All lab samples will have a unique identifier). All aspects of the database will adhere to good clinical practice (GCP).

9.4 Future use of stored specimen

Maternal and infant swabs may be used to test for further organisms not specified in this proposal. Although no excess blood or breast milk is expected to be obtained during the study, any leftovers would be stored to test for additional antibody responses that turn out to be important as a result of ongoing research. Additional permission will be sought from the SCC/EC for further tests.

10 Financing and insurance

The study is financed by a Wellcome Trust Clinical Research Training Fellow grant. Study specific funding has been transferred to the MRC The Gambia for all study costs.

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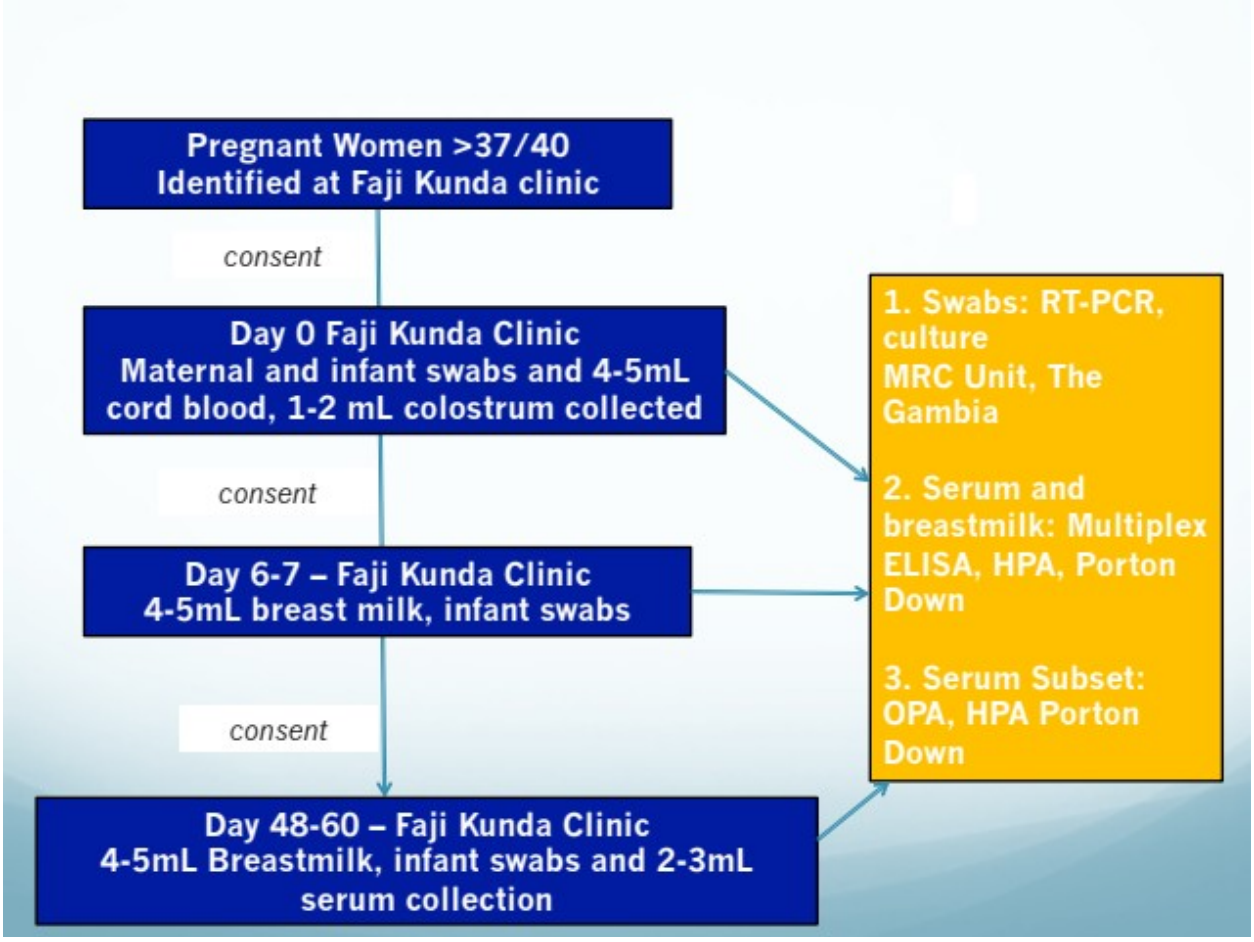
Supplements, appendices and other documents

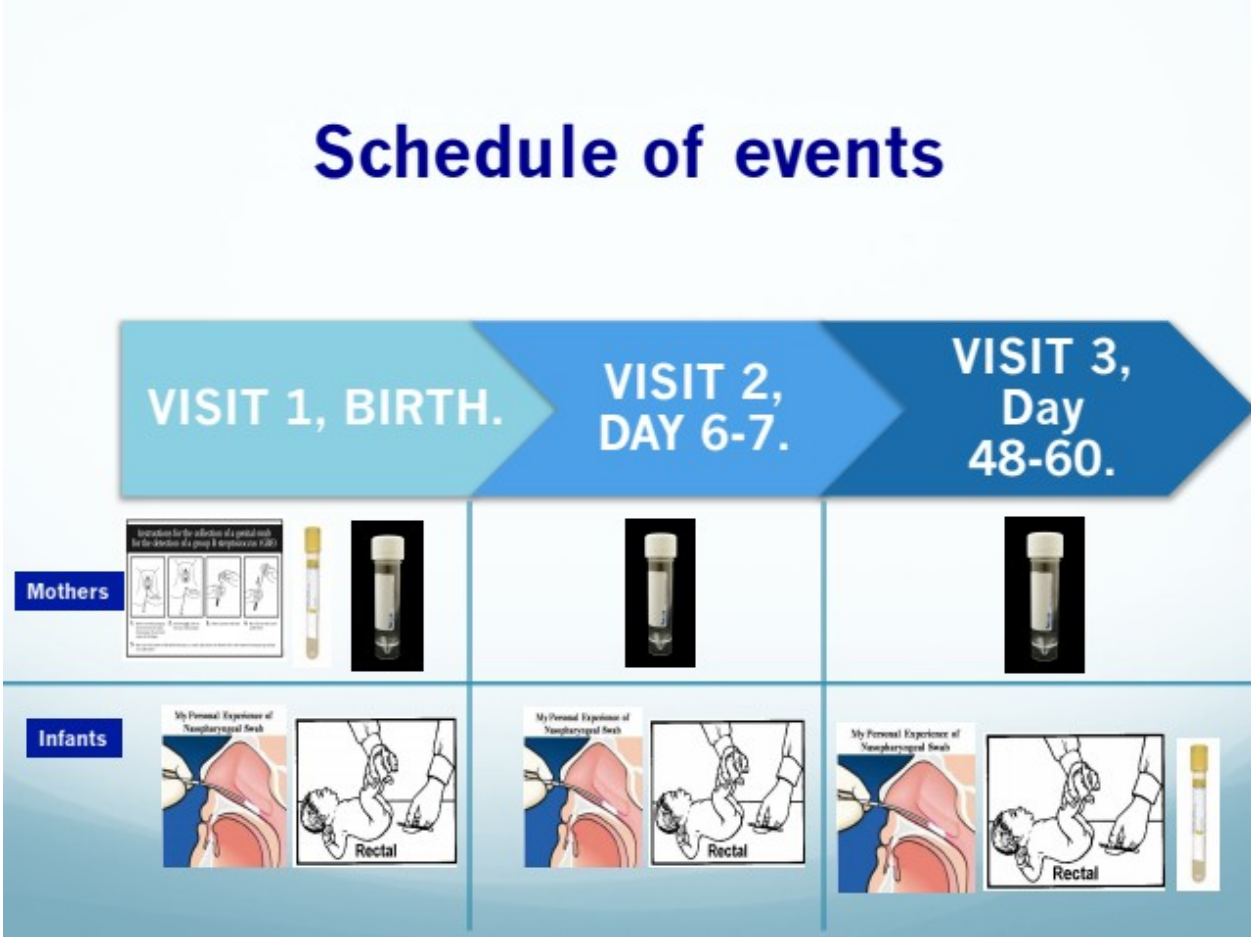
Schematic of Study Design:

Figure 8 – schematic of study design



Appendix: schedule of events

Figure 9 – schedule of events


Schedule of events
VISIT 1, BIRTH.
VISIT 2, DAY 6-7.
VISIT 3, Day 48-60.
Mothers
Infants
Rectal
Rectal
Rectal

Expected timelines:

Figure 10 – Gantt chart of time line.

	2014				2015												2016												2017		
	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
Approvals Process																															
Research and Development approval MRC Gambia																															
Research and Development approval Imperial College																															
Ethical Approval MRC Gambia																															
Ethical Approval Imperial College REC																															
Conversion MPhil to PhD																															
Research Time																															
Laboratory Training																															

and analysis HPA Porton Down																													
Laboratory Training MRC Unit, The Gambia																													
Statistical Training																													
Staff Recruitment and Training, laboratory preparation and pilot - Gambia																													
Recruitment of mothers at 37 weeks gestation																													
Sampling of mothers and infants(rectovaginal swabs, cord blood, maternal breast																													

milk in mothers, nasopharyngeal and rectal swabs and serum in infants); 3-4 days per week																													
Laboratory analysis of swabs using real-time PCR and conventional culture techniques in Gambia																													
Surface labelling and complement deposition work serum																													
Optimisation of assays for breast milk																													
Breast milk																													

antibody assays																														
Laboratory time at Novartis Vaccines																														
write up thesis																														
final papers																														
Review of current literature throughout study																														

Appendix:

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI
Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964 and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington, DC, USA, October 2002
(Note of Clarification on paragraph 29 added)
55th WMA General Assembly, Tokyo, Japan, October 2004
(Note of Clarification on Paragraph 30 added)
59th WMA General Assembly, Seoul, Korea, October 2008

A. INTRODUCTION

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data. The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.
2. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.
3. It is the duty of the physician to promote and safeguard the health of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.
4. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."
5. Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.
6. In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.
7. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best current interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.
8. In medical practice and in medical research, most interventions involve risks and burdens.

9. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.
10. Physicians should consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

B. PRINCIPLES FOR ALL MEDICAL RESEARCH

11. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.
12. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.
13. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.
14. The design and performance of each research study involving human subjects must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.
15. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No change to the protocol may be made without consideration and approval by the committee.
16. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician

or other health care professional. The responsibility for the protection of research subjects must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.

17. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.
18. Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.
19. Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.
20. Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.
21. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.
22. Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.
23. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.
24. In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.
25. For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would

pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.

26. When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed