Accuracy of a rapid intrapartum test for maternal group B streptococcal colonisation and its potential to reduce antibiotic usage in mothers with risk factors

ISRCTN74746075

PROTOCOL

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Full Title	*Accuracy of a rapid intrapartum test for maternal group B streptococcal colonisation and its potential to reduce antibiotic usage in mothers with risk factors*
Short Title/Acronym	GBS2
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Setting:	A minimum of sixteen NHS maternity units

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1. GLOSSARY of Terms and Abbreviations

BCTU	Birmingham Clinical Trials Unit at the University of Birmingham
CI	Chief Investigator
CRT	Cluster Randomised Controlled Trial
DMEC	Data Monitoring and Ethics Committee
EOGBS	Early onset Group B s*treptococcus* disease
GBS	Group B s*treptococcus*
GCP	Good Clinical Practice
ICC	Intraclass Correlation Coefficient
IAP	Intrapartum Antibiotic Prophylaxis
IMP	Investigational Medicinal Product
ISRCTN	International Standard Randomised Controlled Trial Number
MREC	Multicentre Research Ethics Committee
NICE	National Institute for Health and Clinical Excellence
NICU	Neonatal Intensive Care Unit
NSC	National Screening Committee
PCR	Polymerase chain reaction
PI	Principal Investigator – the local lead investigator for the GBS2 Trial
POC	Point of care
PROM	Prolonged rupture of membranes
RCOG	Royal College of Obstetricians and Gynaecologists
RR	Relative Risk
RRR	Relative Risk Ratio
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SCBU	Special Care Baby Unit
TMG	Trial Management Group
TSC	Trial Steering Committee

1. SIGNATURE PAGE

Chief Investigator Agreement

The clinical study as detailed within this research protocol **(Version 1.0, dated 22nd December 2015)**, or any subsequent amendments will be conducted in accordance with the Research Governance Framework for Health & Social Care (2005), the World Medical Association Declaration of Helsinki (1996) and the current applicable regulatory requirements and any subsequent amendments of the appropriate regulations.

Chief Investigator Name: *Prof. Khalid Khan*

Chief Investigator Site: *QMUL, Blizard Institute, Barts and The London School of Medicine and Dentistry, Yvonne Carter Building, 58 Turner Street, London, E1 2AB*

Signature and Date:

Principal Investigator Agreement *(if different from Chief investigator)*

The clinical study as detailed within this research protocol **(Version 1.0, dated 22nd December 2015)**, or any subsequent amendments will be conducted in accordance with the Research Governance Framework for Health & Social Care (2005), the World Medical Association Declaration of Helsinki (1996) and the current applicable regulatory requirements and any subsequent amendments of the appropriate regulations.

Principal Investigator Name: *Mr Matthew Hogg*

Principal Investigator Site: *Barts Health NHS Trust, Royal London Hospital,* Whitechapel, London E1 1BB

Signature and Date:

2. SUMMARY/SYNOPSIS

Short Title	*GBS2*
Methodology	*Prospective test accuracy study embedded within a cluster randomised controlled trial (CRT), and economic evaluation*
Research Sites	*A minimum of sixteen, consultant lead NHS Hospital maternity units*
Objectives/Aims	*To establish the real time accuracy of the GeneXpert rapid test for GBS colonisation among women presenting to a labour ward with risk factors associated with GBS transmission, comparing against the reference standard of selective enrichment culture, in a prospective cohort study.* *To evaluate if rapid GBS testing reduces maternal and neonatal antibiotic usage, compared with usual care where administration of Intrapartum Antibiotic Prophylaxis (IAP) is directed based on maternal risk factors alone, in a cluster randomised trial*
Number of Participants/Patients	*A total of 1340 women with risk factors associated with GBS colonisation*
Main Inclusion Criteria	*Women with defined GBS risk factors presenting to a labour ward will be eligible and included.*
Statistical Methodology and Analysis (if applicable)	*The accuracy of the rapid test for detection of GBS will be expressed as sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios, and their 95% confidence intervals.* *The mother's baseline characteristics will be summarised as means and standard deviations, or medians and inter-quartile ranges, and grouped by unit allocation (risk factor based screening or rapid test screening).* *Analyses of outcomes will be by intention to treat. As randomisation will be at the maternity unit level, appropriate statistical methods to account for the clustering within units will be used in the analysis*
Proposed Start Date	29th January 2016
Proposed End Date	31st July 2018
Study Duration	*30 months*

3. INTRODUCTION

Background

Group B streptococcus

Group B *streptococcus* (GBS) is a ubiquitous bacterium and forms part of the normal bacterial flora of the gut and genital tract. In adults, GBS is an occasional cause of serious systemic infections in immunocompromised patients, but is more commonly seen as an opportunistic pathogen of the female urogenital tract. However if a neonate, whose immune system is immature, is exposed to GBS it can lead to sepsis and death. Most systemic GBS infections usually present within 24 hours of delivery as rapidly progressing septicaemia, although early onset disease is defined by NICE[1] as occurring within the first 72 hours of life. The RCOG define early onset disease as that which occurs within the first seven days of life[2]. Exposure to GBS present in the gut and genital tract of the mother during birth is thought to be the most common route for early onset colonisation in the neonate.

Any infection with GBS in children between eight days and 3 months of age is deemed late-onset and is more often associated with localised infections (especially meningitis and pneumonia). Colonisation from environmental sources is thought to be the most common cause of late-onset GBS and is beyond the remit of GBS2.

Incidence

GBS is the leading cause of serious early-onset neonatal sepsis in developed countries. The incidence of early-onset GBS sepsis in the newborn in England, Wales, and Northern Ireland has changed little between 2003 and 2010 at 0.37-0.41 per 1,000 livebirths.[3]

Maternal GBS colonisation

The gastrointestinal tract is the natural reservoir of GBS in humans, and is the likely source of vaginal colonization. Asymptomatic colonisation of the genital and lower gastrointestinal tracts with GBS has been reported at 10-30% in pregnant women,[4] although this figure can vary with age, sexual activity, race and the method of laboratory culture used for its detection.[5] A single vaginal or rectal swab during pregnancy has been shown to have a poorer predictive value for neonatal sepsis than either multiple site swabbing or repeated culture from a single site.[6] Boyer *et al* noted that whilst 35% of their sample of pregnant women were colonised with GBS at some point in pregnancy, only 17% were persistent carriers and some lost or gained GBS strains during the pregnancy.[7] Nine UK studies of untreated women in labour have suggested rates of maternal colonisation of between 5-15% [8-10] based only on vaginal culture and 15-21% if both vaginal and rectal swabs are cultured.[11-14] The mean colonisation rate for all studies is 13.6% (95%CI: 9.6 - 18.3%).[15]

There is ample evidence to suggest that the lower gastrointestinal tract often acts as the primary site for genesis of new GBS strains. Gastrointestinal colonisation is thought to be more persistent than vaginal colonisation.[16-18] Urinary tract infections due to GBS are associated with perinatal infection and late spontaneous abortion.[19]

Transmission

Neonates with early onset infection show initial colonisation mainly in the mucous membranes of their respiratory tract, and the major route of vertical transmission at the time of delivery is thought to be through aspiration of vaginal, rectal, and amniotic fluid aerosols during birth. Vertical transmission *in utero* is thought to occur as a consequence of prolonged rupture of membrane and is regarded as one of the causes of stillbirth.[20] Colonisation of the mother is less predictive for late onset GBS infection, with prematurity being the major risk factor.[21]

The association between the rates of maternal colonisation, transmission, and infection has been established. A meta-analysis of six studies of the maternal and baby colonisation rates in an untreated general population showed a transmission rate of 36.4% (95% CI 27-41%). A further meta-analysis, in the same report, of early onset GBS disease in colonised babies of untreated mothers gave an average incidence of 3.0% (95% CI 1.6-4.7%).[15]

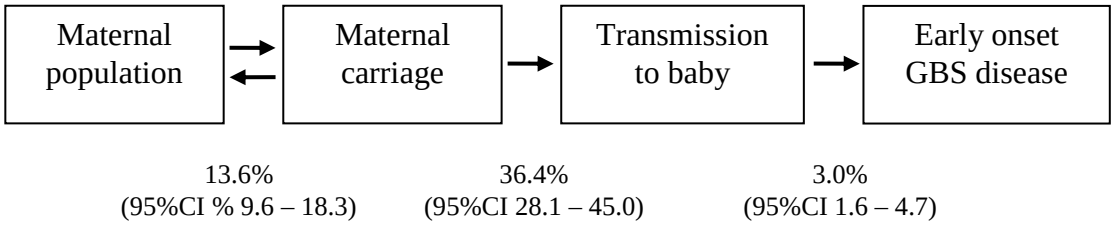


Figure 1: Model of colonisation, transmission and early onset GBS disease

Epidemiology of Early onset Group B s*treptococcus* disease (EOGBS)

The incidence of Early onset Group B s*treptococcus* disease (EOGBS) can be decreased if women with risk factors associated with GBS colonisation are given Intrapartum Antibiotic Prophylaxis (IAP) during labour. In countries where use of IAP is widespread, the incidence of EOGBS has decreased, but GBS remains one of the most important causes of severe early onset infection in newborn infants in most industrialised countries. In the USA, the incidence of neonatal EOGBS disease has fallen from 1.7/1000 in the early 1990s to 0.34-0.37/1000 between 2003 and 2008.[22] Likewise in Australasia the incidence fell from 1.43/1000 in 1993 to 0.25/1000 in 2001. Enhanced surveillance in the UK and Ireland between 2000 and 2001 showed an incidence of culture-proven neonatal EOGBS disease of 0.48 per 1000 live births. It is highly likely that some cases of serious neonatal sepsis caused by GBS are unrecognised because cultures of blood and CSF are negative. By taking into account superficial swab culture results from all neonates who underwent a septic screen in the first 72 hours of life, Luck *et al* concluded that the true incidence of neonatal EOGBS disease in the UK may be as high as 3.6/1000 live births, over seven times higher than previously estimated. [23]

In the 1970s, mortality rates from EOGBS as high as 50% were reported, but with advances in intrapartum and neonatal care these have fallen. In 2001 a national UK surveillance study identified 376 cases of whom 39 (10.4%) died.[24] Mortality is much higher in pre-term babies. Oddie and Embleton found that preterm infants comprised 38% of all cases and 83% of the deaths from EOGBS.[25] Information on

morbidity amongst survivors is less clear, but significant long-term morbidity, including impaired psychomotor development, has been reported in up to 30% of survivors. [26]

Risk factors

Epidemiological studies have suggested that various factors present at the time of birth are associated with the neonate having an increased risk of developing GBS disease, presenting as either an early or late onset infection. A systematic review estimated that 71% of deliveries had no recognised maternal risk factors for GBS disease.[15]

These risk factors have been suggested to include:

1) **Prematurity**

Colonised premature babies are at a high risk of developing early onset GBS disease as their immune system is immature and they are less likely to have received passive immunity transplacentally. The pooled incidence of early onset GBS from 5 UK studies [27-30], showed 40% were preterm deliveries; a 5.5 fold higher risk than for term babies. Birth weight is highly correlated with prematurity and inversely related to developing EOGBS disease. The surveillance study by Heath *et al* indicated an incidence of 4.0 early onset cases per 1000 deliveries in babies under 1500g, compared with 0.49 per 1000 overall.[31]

2) **Prolonged rupture of membranes (PROM)**

Premature or prolonged rupture of membranes (PROM) would be expected to lead to an increased likelihood of ascending infection and baby colonisation *in utero*, although there is debate as to what, if any, role the presence of GBS plays in the induction of PROM. Rupture of the membranes more than 18 hours before delivery is significantly associated with early onset GBS disease with an odds ratio 25.8 (95% CI 10.2 - 64.8) compared with non-infected infants. [25] Thus, babies born to mothers who experience preterm labour with prelabour rupture of membranes of any duration, or preterm labour if there is suspected or confirmed intrapartum rupture of membranes lasting more than 18 hours, are especially thought to be at risk of developing EOGBS.

3) **Maternal fever**

Pyrexia is a symptom of chorioamnionitis or endometritis and may be associated with a more intense maternal and baby colonisation.[32] Intrapartum fever is also highly associated with the development of EOGBS (odds ratio 10.0, 95% CI 2.4 - 40.8). [25]

4) Previous baby with GBS disease

Whilst suggested by some as being a significant risk factor in devleoping EOGBS disease in subsequent pregnancies, given the low incidence of GBS it is difficult to reliably estimate the size, if any, of this increased risk.

5) GBS detected in current pregnancy

Data from four studies of women with GBS bacteriuria in labour produced a pooled prevalence of maternal GBS colonisation of 78% (95% CI 63- 90%).[33-36] The association with GBS colonisation in labour given a previous positive urine or vaginal swab depends on the time interval between the two tests. Therefore the above prevalence is likely to be an over-estimate as screening for GBS was undertaken concurrently in urine and vaginal samples.

Detection of maternal GBS colonisation

There are several methods of detecting the presence of GBS from vaginal-rectal swabs, with bacterial culture regarded as the definitive approach to detection and discrimination. Several non-culture-based diagnostic systems are available commercially for GBS screening. The optimal test would accurately detect the presence of GBS within a timeframe that allows sufficient IAP to be adminstered to women in labour colonised with GBS.

As well as being rapid and accurate, the ideal test would require minimal preparatory steps and be easily interpretable to enable routine use in a busy clinical environment. Several of the diagnostic systems and technologies e.g. loop-mediated isothermal amplification and optical immunoassay require several preparative steps before the sample can be analysed. We therefore only considered polymerase chain reaction (PCR) tests as potentially suitable for point of care testing.

1) Bacteriological Culture

GBS grows on blood agar plates, forming characteristic glossy white colonies surrounded by areas of β-haemolysis after 24-48 hours. The use of a selective enrichment broth prior to plating increases the recovery of GBS from genital and anorectal samples by over 50%. [37] Lim broth, comprising of a Todd-Hewitt base with nalidixic acid and colistin to suppress gram-negative bacteria, is the most widely used enrichment media prior to plating on to chromogenic GBS agar plates, although the necessity of *selective* enrichment has been questioned.[22, 38] Obtaining swab specimens from both the vagina and rectum increases the incidence of detection of maternal GBS colonisation by 40% over swabs taken fron the vagina alone. [39, 40] A meta-analysis demonstrated a pooled sensitivity of 76% and pooled specificity of 95% for culture based tests.[41]

2) PCR based tests

PCR involves the repeated logarithmic amplification of specific areas of the bacterial chromosome using an iterative process of hybridisation of replication primers,

amplification from these primers of the target DNA and separation of the nascent DNA. Real-time detection of the amplified DNA is by the incorporation of a fluorescent marker which is quantitatively measured within the PCR thermocycler.

In GBS1, we found the highest levels of accuracy were obtained from combing the results from vaginal and rectal swabs, with this showing an 84% sensitivity and 87% specificity. However, this was considerably lower than the pooled estimates from a meta-analysis of all previous studies,[42] which reported a pooled sensitivity and specificity of 97% for PCR. This discrepancy may have arisen since, with more robust methodology, GBS1 avoided overestimation of accuracy associated with review bias.[43] Updating the meta-analysis to include results from GBS1 reduced the pooled estimates for PCR, with a new pooled sensitivity of 90% (95% CI 88-93%) and pooled specificity of 92% (95% CI 91-94%). The accuracy of the PCR, when considering samples from both the vagina and the rectum, compares favourably with that of screening by culture of swabs taken at 35-37 weeks gestation.

In GBS1, it was possible to train midwifery staff to undertake complex testing required by the Cepheid IDI-GBS and SmartCycler system, but it was not feasible to establish testing on demand. As the study progressed, fewer tests were done in real-time and were processed in batches, which was possible as prophylaxis was not directed by the results of the rapid test. Even when processing started immediately, there were considerable difficulties in ensuring the availability of results within the timescale that would have been required clinically. These mainly related to problems in ensuring the ongoing availability of sufficient staff who were competent to undertake testing, the fact that it was impossible when undertaking tests that require significant hands-on test time for staff to begin processing another sample when one was already in progress, as well as the conflicting demands on the midwifery staff.

Current NHS Policy and Practice

The current approach to minimise the development of EOGBS disease is that of prevention of mother to child transmission during labour by administering intrapartum antibiotic prophylaxis (IAP). In many countries around the world, culture based screening at 34- 37 weeks is used to identify women in labour who are colonised with GBS and informs intrapartum antibiotic prophylaxis. The UK adopts a risk based approach.

Introducing culture based screening into the UK healthcare system has been considered alongside other testing and vaccination strategies, and was cost-effective if all women in premature labour were also provided with IAP. [44] In another analysis, extension of the current practice to offer IAP to all women in preterm labour and at high risk was the most cost-effective option.[15] The National Screening Committee reviewed the evidence for universal and risk factor based screening in 2012 and concluded there was insufficient evidence against their standardised criteria to justify a change from the current risk factor based screening approach to guide administration of IAP.

The Royal College of Obstetricians and Gynaecologists have produced two Greentop guidelines (one in 2003, the other in 2012) recommending a screening strategy based on maternal risk factors for EOGBS disease. [45, 46]

The risk factors to consider in this approach, and the management options available, in the original and revised guidance are summarised below:

- Women with a previous baby with neonatal GBS disease
  2003 guidelines: offer IAP; 2012 guidelines: offer IAP

- Women with GBS bacteriuria in the current pregnancy
  2003 guidelines: consider IAP; 2012 guidelines: offer IAP

- Women with an incidental finding of vaginal GBS colonisation in the current pregnancy
  2003 guidelines: consider IAP; 2012 guidelines: offer IAP

- Prematurity < 37 weeks
  2003 guidelines: discuss IAP; 2012 guidelines: do not offer IAP in women presenting in established preterm labour with intact membranes with no other risk factors for GBS, unless they are known to be colonised with GBS

- Prolonged rupture of membranes > 18 hours
  2003 guidelines: consider IAP; 2012 guidelines: states that for women at term with prelabour rupture of membranes the evidence for IAP is unclear

- Fever in labour > 38°C
  2003 guidelines: discuss IAP; 2012 guidelines: offer IAP

NICE issued guidance in 2012 on antibiotics for the prevention and treatment of early-onset neonatal infection.[47] It recommends that IAP should be offered to women who have had:

- A previous baby with an invasive GBS infection

- GBS colonisation, bacteriuria or infection in the current pregnancy

It suggests that IAP is considered for women:

- In preterm labour if there is prelabour rupture of membranes of any duration

- In preterm labour if there is suspected or confirmed intrapartum rupture of membranes lasting more than 18 hours

For women with prelabour rupture of membranes at term, including prolonged (>24 hours) rupture, the use of prophylactic antibiotics is not recommended.[48]

Current management protocols

Intrapartum antibiotic prophylaxis for maternal GBS colonisation

Where IAP is indicated for prophylaxis against GBS transmission, the standard treatment is the intravenous administration of three grams of benzylpencillin as soon as possible after the onset of labour, and half that dose at four hourly intervals until delivery, with equivalent doses of clindamycin for those allergic to penicillin. The RCOG suggests that to optimise the efficacy of IAP, the first dose should be given at least two hours before delivery, although a minimum of four hours is considered ideal. [49] Benzylpenicillin levels in cord blood appear to exceed the minimum inhibitory concentration for GBS as early as 1 hour after maternal administration, [50] but it is not known how this relates to prevention of transmission.

Management of neonates

Around 94% of neonates who develop EOGBS do so within 12 hours of delivery. Two thirds of these had mothers with one or more risk factors during labour, with a significant number exhibiting foetal distress and low Apgar scores at delivery.[31, 51] Where clinical signs and symptoms of sepsis are observed, the baby should be evaluated and antibiotics prescribed. Other infants without clinical signs but whose mother had risk factors should be observed closely in their first 24 hours after birth. Routine administration of antibiotics to babies deemed low risk is not recommended.

Rationale and Risks/Benefits

The women taking part in GBS2 in the control arm (units where usual practice is followed) will face no additional risks above that associated with standard practice. Women taking part in GBS2 in units where the rapid test is placed should quickly and confidentially know their GBS colonisation status. This has a number of benefits including:

- *reducing antibiotic use* – by better identifying and only targeting those women colonised with GBS, Intrapartum antibiotics will only be given where they are needed

- *reducing time spent in hospital* – by knowing the GBS colonisation status of the mother with considerable certainty, babies born to mothers with risk factors for, but who are not colonised with GBS can be discharged home sooner and with less medical intervention

- *reducing readmissions* – Should a baby be born to a mother who has tested positive for GBS colonisation then this will aid clinical staff in judging if the mother has received sufficient Intrapartum antibiotics to prevent vertical transmission of GBS to her baby. By taking any necessary action earlier, the need to readmit at a later date will be reduced

4. TRIAL OBJECTIVES

Primary Objective

1. To evaluate if rapid GBS testing reduces maternal and neonatal antibiotic usage compared with usual care where intrapartum antibiotic prophylaxis is directed based on maternal risk factors alone, in a cluster randomised trial

2. To establish the real time accuracy of the Cepheid GeneXpert system rapid point of care test for GBS colonisation among women presenting to a labour ward with risk factors associated with GBS colonisation. The results of the Cepheid test will be compared against the reference standard of selective enrichment culture in a prospective cohort study

Secondary Objectives

1. To establish a standard operating procedure for use of a rapid, point-of-care test for GBS colonisation (GeneXpert) on a labour ward

2. To determine if the turnaround time (from taking the sample from the patient to obtaining a result) is compatible with the provision of a suitable duration of antibiotic administration to test positive mothers

3. To explore the impact of testing strategies on the rates of antibiotic administration to the woman with risk factors associated with GBS colonisation

4. To determine the cost and cost-effectiveness of rapid GBS testing for preventing EOGBS disease in babies born to women with risk factors for GBS transmission

5. To determine the GBS2 colonisation rate of neonates born to mothers who have risk factors associated with GBS colonisation, and to explore the rates of antibiotic administration and neonatal outcomes

6. To determine the antibiotic resistance profile of any GBS isolated from the rectal vaginal swab taken from the mother around or during her time of labour, and to compare this with the antibiotic resistance profile from any GBS isolated from a faecal sample taken from the woman's baby at six weeks of age

7. To estimate the carriage rate of three groups of antibiotic resistant bacteria of current public health concern in rectal samples from women recruited to the GBS2 study from centres in London and the South-East who are assigned a rapid test system. The groups are meticillin-resistant Staphylococcus aureus

(MRSA), vancomycin-resistant enterococci (VRE), and extended-spectrum β-lactamase producing (ESβL) Enterobacteriaceae

8. To confirm that there is vertical transmission of MRSA, VRE or ESβL -producing Enterobacteriaceae from mothers to their infants

9. To gather some information on peri-partum risk factors for transmission (mode of delivery, maternal co-morbidities, colonising species)

We will explore appropriate ways to use this evidence, if deemed relevant, in the economic model.

Primary Endpoint

The primary aim of GBS2 is to evaluate whether the proportion of women prescribed IAP for prevention of vertical GBS transmission differs between screening strategy groups. In statistical terms, the null-hypothesis of no difference can be tested using a mixed logistic regression model with prescription of IAP as the dependent variable, study screening strategy group as the independent variable, and maternity unit as a grouping (random effect) variable. These models will be fitted using population averaged models (similar to multilevel models) using GEE methods in STATA. Population averaged models, as opposed to random effects models, will be used as within the framework of cluster randomised trials: random effects models both lack appropriate interpretation and might be biased.[52] Similar models will be fitted for secondary outcomes and appropriate link functions used for outcomes which are not binary. The primary outcome will be considered significant at the 5% level (and so 95% CIs reported).

Secondary Endpoint

Secondary outcomes will be interpreted cautiously as the CRT is not necessarily powered to detect a difference in these.

5. METHODOLOGY

Centre eligibility

Maternity units will be eligible to participate in GBS2 if they are prepared to accept a policy of rapid test directed IAP administration as their standard practice of treating GBS colonisation for the duration of the study period. The Trusts hosting the maternity units will need to have microbiology facilities which are able to perform bacteriological culture.

Participant Inclusion Criteria

Presence of one or more of the following risk factors will define inclusion of the mother and baby into the study:

- Previous baby with early or later onset neonatal GBS disease as reported by the mother and documented in the maternal notes

- GBS bacteriuria during current pregnancy, as documented in the maternal notes, irrelevant of whether the GBS bacteriuria was treated at the time of diagnosis with antibiotics

- GBS colonisation of the vagina and / or rectum (determined from a vaginal / rectal swab) in current pregnancy, as documented in the maternal notes

- Maternal pyrexia (>38°C) observed at any point in labour, or clinically suspected / confirmed chorioamnionitis

- Preterm labour (<37 weeks' gestation) with prelabour rupture of membranes of any duration

- Preterm labour (<37 weeks' gestation) if there is suspected or confirmed intrapartum rupture of membranes lasting more than 18 hours

The first three risk factors are apparent from the women's history and will be evident before the start of labour, enabling women to be immediately approached for a vaginal and rectal swab. Preterm labour with prelabour rupture of membranes will be evident at presentation in labour.

Pyrexia, chorioamnionitis and prolonged rupture of membranes in preterm labour are emerging risk factors associated with maternal GBS colonisation and labouring women should be monitored for these signs.

Exclusion Criteria

Women who will automatically be excluded from screening will be:

- Those under 16 years of age

- Women in labour at a gestation age of <24 weeks

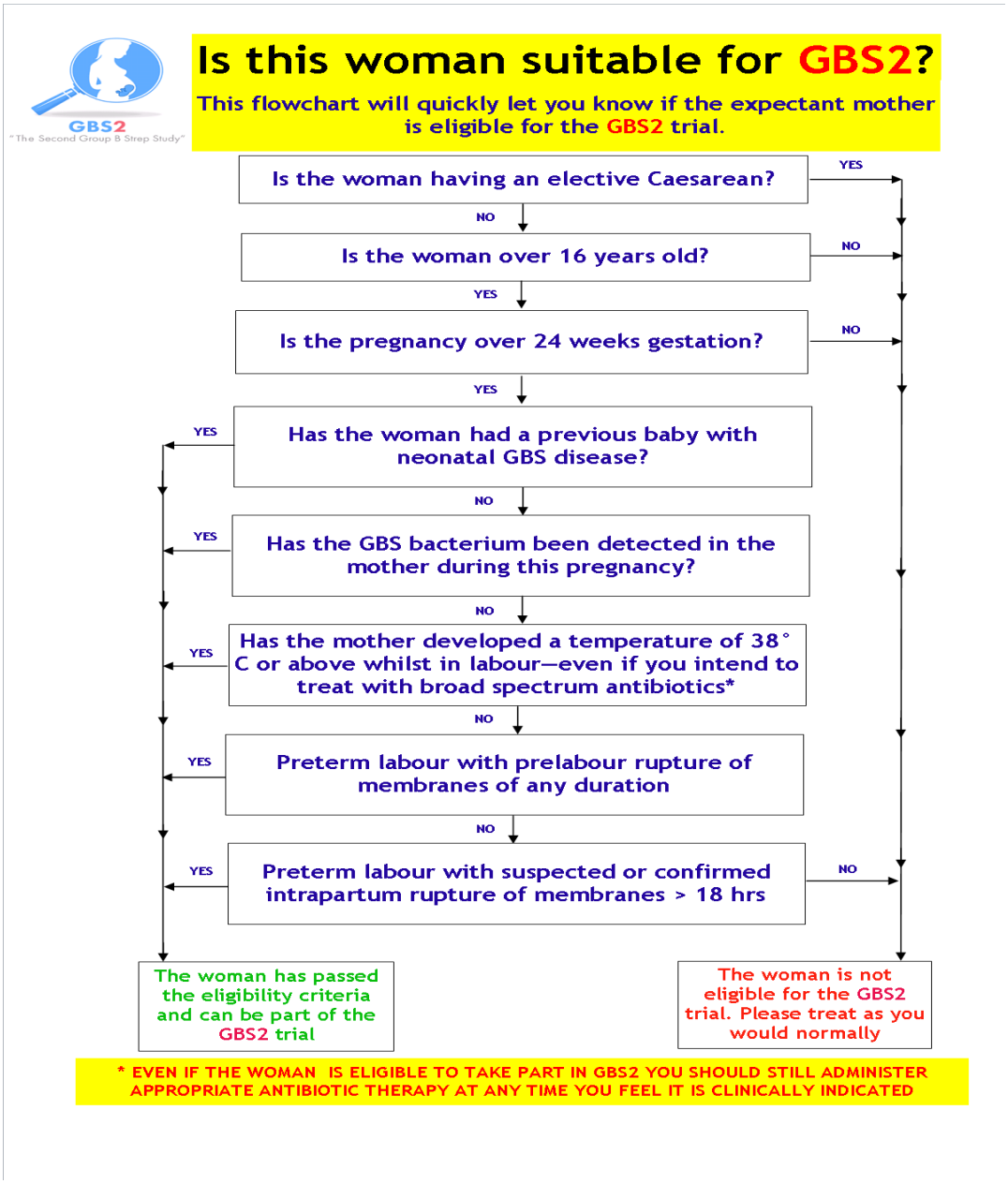
- Women who, on arrival at the maternity unit, are already in second stage of labour or who are considered likely to deliver their baby imminently

- Women whose baby is known to have died in utero or who has a congenital anomaly incompatible with survival at birth

- Women having an elective Caesarean delivery

All these exclusion criteria should be apparent when the woman arrives at the maternity unit labour ward or delivery suite.

The common eligibility criteria are shown in **Figure 2** (below):



Study Design / Plan – Study Visits

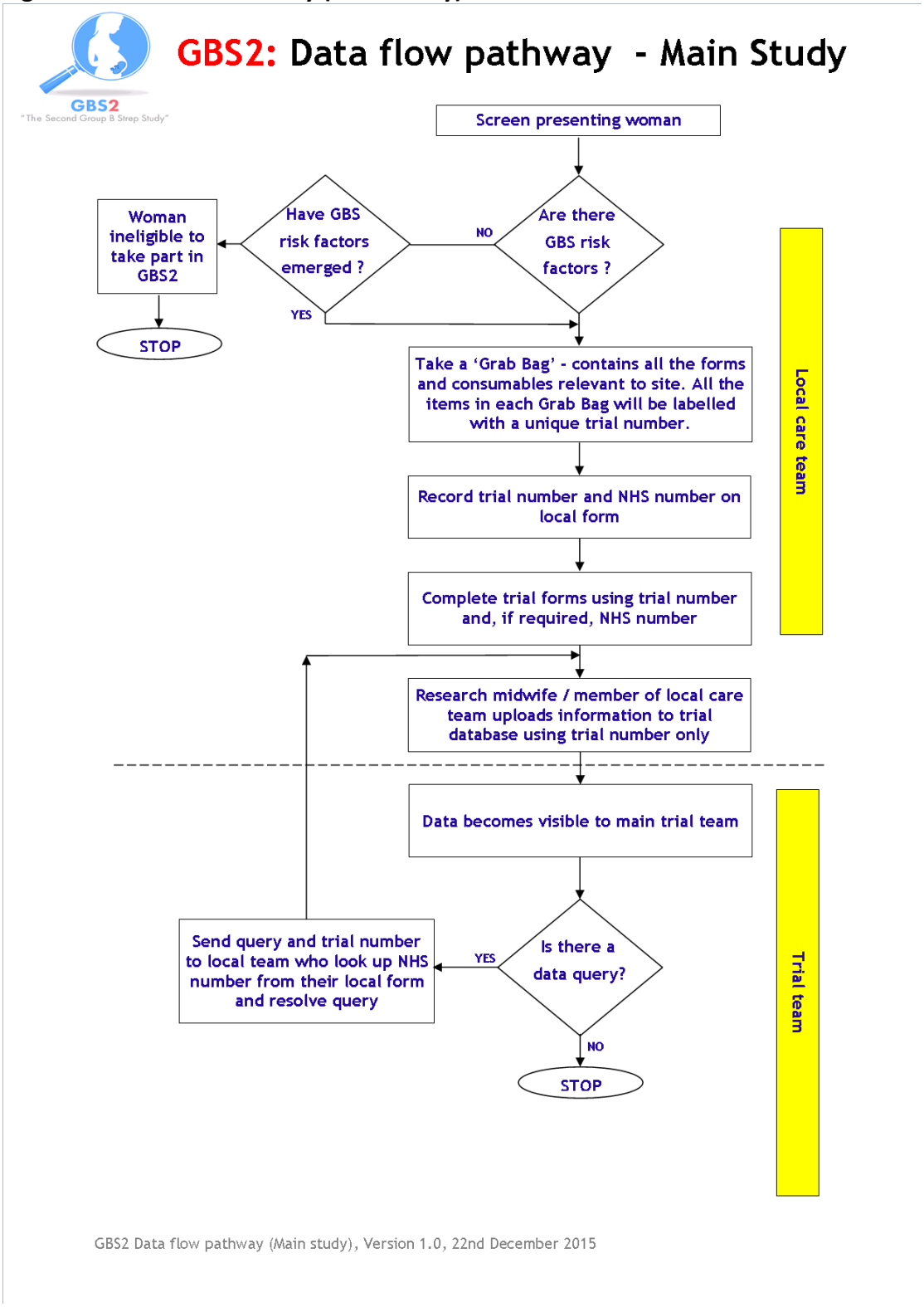
The data flow for the main study is shown in Figure 3.

A paper checklist will be used by delivery suite midwives to screen all women presenting to the labour ward to record the presence or absence of maternal risk factors that determine eligibility. This eligibility checklist will simply implement the RCOG guidelines which are, or inform, the standard policy for GBS screening in most participating hospitals. This checklist will be inserted into maternal handheld notes for women delivering during the study period.

If the woman does have risk factors associated with GBS colonisation then the midwife will take a GBS2 Grab Bag. This is a Ziploc back which contains all the forms and consumables relevant to the site. All the items in this bag will be labelled with

the same unique trial number. The midwife will record this trial number alongside a number of identifiers such as the woman's name, NHS / Hospital number and Date of Birth on a central form held at the local site. Should risk factors not be present then the woman will be monitored during her labour for any emerging risk factors.

Figure 3: Data Flow Pathway (Main study)



The time of the start of labour, timing of the swab, test results, and any antibiotics received will be recorded alongside other factors. The checklists will be collected

centrally at the local site and compiled on a daily basis by a designated midwife. The trial forms will be completed as labour progresses and the woman gives birth. Personal identifiers will be recorded on any internal documents if demanded by Good Clinical or Good Laboratory practice, however these will not be made available to the trial team. Once the forms are completed then either a member of the local care team or the research midwife will transpose the information from the forms onto the secure trial database. Only the local trial number will be used to link the data together – no other patient identifiers will be entered on to the trial database. Paper copies of the data collection forms will be stored securely at the local site.

In order to ensure accuracy of the data the Trials team will monitor the uploaded information. Should they have a query then they will approach the local care team with the patient's trial number. By checking the woman's trial number against their local records the local care team will be able to retrieve the NHS number and other identifiers and check any query against the source data and, if necessary, make any changes on the main trial database. Using this method, no identifiable information will leave the local site and participant confidentiality will be ensured.

At the conclusion of the study period the trial team ask the local care team to supply their IT department with list of NHS numbers from women who have been included in the GBS2 trial from that site. The local IT team will be asked to download relevant data items (such as antibiotics prescribed for GBS prophylaxis, number of cases of EOGBS diagnosed during the trial period etc) from their electronic records and, after ensuring that all identifiers have been removed, to securely transfer these records to the main trial office. Upon receipt, these electronic records will be reconciled with the paper records to check their accuracy and any discrepancies investigated.

No consent model

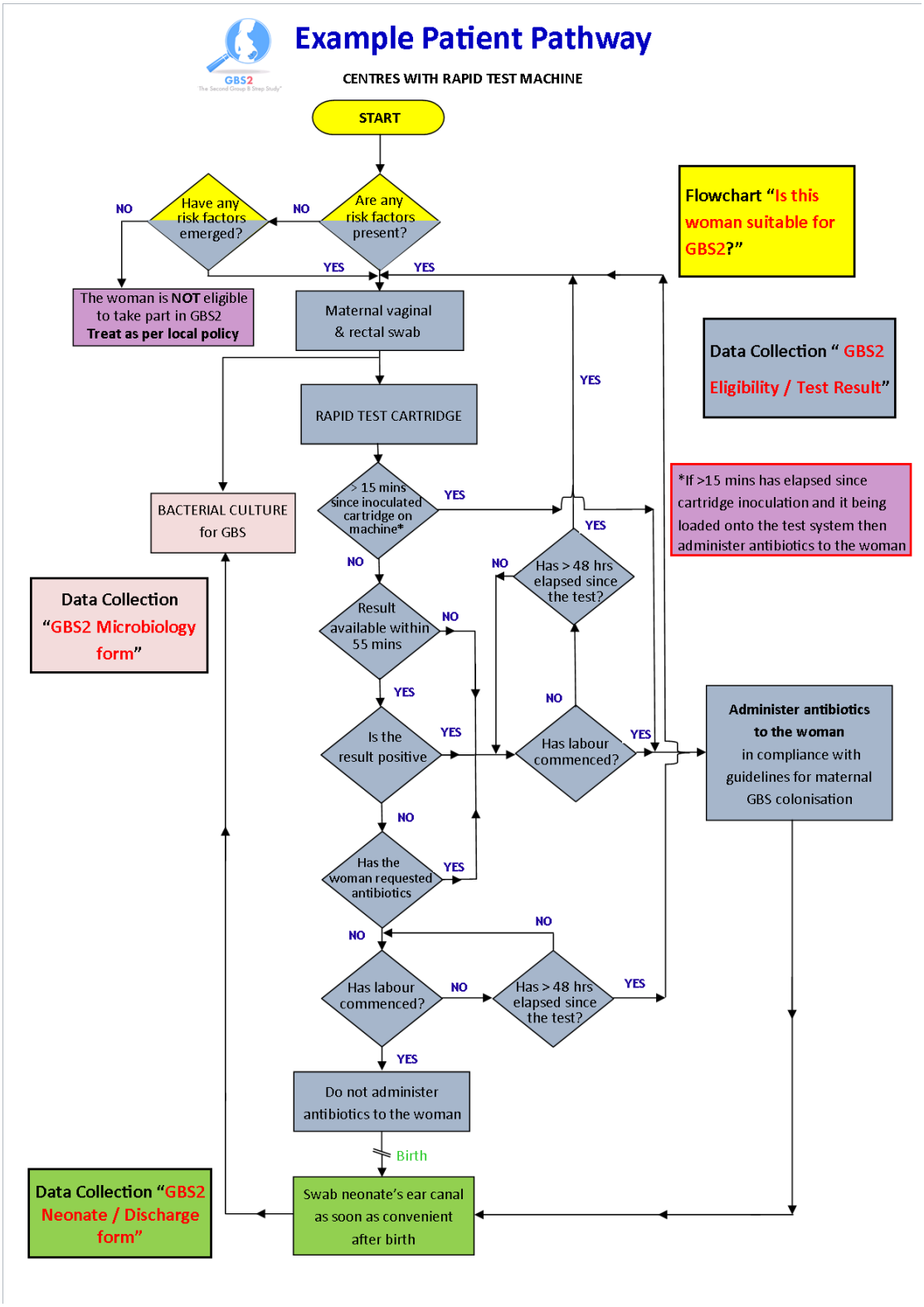
The principal difference to an individually randomised trial is that individual written consent for participation in the main GBS2 cluster trial and test accuracy study will not be sought. The rationale for this is that in the maternity units allocated to risk based screening, usual practice is being followed and all women would be screened and treated in the same manner had the study not existed. In the maternity units allocated to rapid test based screening, rapid tests will be considered to be standard practice for the duration of that unit's study participation and offered to all women identified as having risk factors for GBS colonisation, using the same criteria as for risk based screening i.e. the women attending these centres will have a second level of test. In this situation, participation in the cluster trial is not something that they can chose Women delivering in the rapid test strategy units will be supplied with a REC approved information sheet informing them of why the swab is being taken and how to raise any concerns they may have, before verbal clinical assent to have a vaginal-rectal swab taken is obtained. This consent will be obtained in the same way that the woman would be asked for verbal consent for a vaginal examination or cardiotocograph, and the woman can decline.

Given the uncertainty remaining around the accuracy of the PCR test for GBS colonisation in real-life situations, i.e. when performed by labour ward staff, and the feasibility of using the test result to direct IAP, a classical test accuracy study is required. The rapid test performed on vaginal-rectal swabs using the GeneXpert system is the index test. The results of this test will be compared against microbiological enrichment culture of duplicate swabs, which will act as the reference standard.

To compare screening strategies, a randomised comparison provides the most reliable data. We have considered and rejected individual randomisation due to the risk of contamination, which we believe will come from two sources. Firstly once the GeneXpert system becomes available on a maternity unit, given its presumed high accuracy, it would be difficult not to offer this test to all women in labour. Secondly, previous experience from GBS1 suggests that women in labour are not approached to take part in GBS diagnostic studies, as explaining the trial and taking consent significantly delays the onset of IAP administration, a situation which is clinically unacceptable.

To reduce these factors as far as possible GBS2 will randomise at the level of the maternity unit. Each unit will be randomised to follow either their standard risk factor based strategy, or a strategy of administering IAP to women with risk factors associated with GBS colonisation based on the result of the rapid, point of care, PCR test.

Figure 4: An example patient pathway



Within cluster trials, it is important that all eligible participants are identified prior to the unit being randomised. As intrapartum risk factors can only be identified at the time when the screening strategy needs to be applied we need to include all women in this group. If consent was sought there would be selection by midwife (overtly or

unintentionally, due to time pressures), and as a result of women declining to provide swabs or data for research. The selection bias caused by the need to approach and individually consent participants within a cluster leads to unreliable estimates of screening effectiveness.[53] However, if the screening strategy is adopted as standard practice by the maternity unit, and anonymous routinely collected data is retrieved, consent for research is unnecessary (although clinical consent for vaginal / rectal and neonatal swabs would prevail).

If a rapid point of care test improves the detection of GBS then it is likely that important cost implications will be seen for the health care sector. For example, inappropriate IAP will be avoided for many women who test negative for GBS colonisation. Furthermore, the rapid administration of appropriate antibiotic treatment for those who test positive should lead to a reduction in admission to neonatal intensive care. The accuracy of the test must be carefully examined and established for its impact on both false positive and false negative results, and the costs and outcomes that follow decisions based on the result of the test must be evaluated. For example, the rapid test may detect additional cases of GBS compared to standard risk factor based screening. This will increase the use of antibiotics prescribed in the intra-partum period, although it could ultimately reduce the risk of developing EOGBS disease and avoid costly admissions to higher cost specialist care units. Alternatively, the replacement of risk factor screening with the rapid test may lead to an increased number of false positives resulting in the administration of unnecessary IAP, or it could lead to an increased number of false negative test results and consequential increase in adverse outcomes. Given this, a model based economic evaluation is required to extend the time frame of the analysis beyond the pathway of the GBS2 test accuracy study and CRT. This model based analysis should adopt the perspective of the NHS.

Whilst the main, cluster trial examining test accuracy and antibiotic prescription rates will be run without maternal consent one of the sub-studies embedded in GBS2 will estimate the relatedness of certain bacterial strains of public health concern which may be colonising the mother during the birth of her baby, and in her child when it reaches six weeks of age. This sub-study will only run in participating centres in London and the South-East who are randomised to receive a rapid test system.

In these centres the vaginal-rectal swab will be taken from women who have risk factors associated with GBS colonisation using a triple headed swab. The first swab and second swab heads will follow the same pathway as in other centres assigned a rapid test, i.e. the first swab head will be used to inoculate a Cepheid rapid test cartridge to detect GBS, whilst the second will be sent to microbiology where it will be used to inoculate a media selective for GBS. The third swab will be sent to the microbiology laboratory at Barts Health NHS Trust and will only be processed further if notification of written consent has been obtained from the woman is given to the sub-study trial co-ordinator who will pass this on to the Microbiology laboratory.

Following the swabs being taken the woman will be provided with an information sheet and asked to consider participating in the sub-study examining if certain bacteria colonise the baby directly from the mother or if they colonise the baby from its environment. As women in the throes of labour may be deemed to temporarily lack capacity, consent to participate in the sub-study can be requested at any time between the woman being admitted to the labour ward and her discharge home.

The Research Assistant at Barts Health NHS Trust who is overseeing the sub-study will be informed by the local midwifery team once written consent has been obtained. The sub-study Research Assistant will simply be informed of the unique trial number associated with each participant and that they have consented. No other information will be passed to the sub-study trial office. The date this information is received by the sub-study trial office will be noted.

Should the woman consent to joining the sub-study then the third swab will undergo selective enrichment for GBS, meticillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), and extended-spectrum β-lactamase producing (ESβL) *Enterobacteriaceae* and the presence of any bacteria of interest profiled by a variety of techniques (including, but not necessarily limited to, antibiotic resistance, molecular / genetic characterisation, and matrix-assisted laser desorption ionization–time of flight mass spectrometry). Any bacteria of interest will be stored for later work. The presence of any bacteria of interest will be stored against the woman's unique trial number, no personal information which links the stored bacterial sample to the donor will be kept.

Once a period of five weeks has elapsed from when the trial office was notified that consent had been given the sub-study Research Assistant will supply the maternity unit with a faecal sample pot labelled with a unique number. The sub-study Research Assistant will record the number on the pot alongside the mother's trial number.

After checking with the Community Midwifery team that nothing untoward has happened to the child then maternity unit staff will send out a follow-up sample collection pack. This pack will consist of a faecal sample pot accompanied by a covering letter and a suitable prepaid, addressed transport container to the woman's home address requesting a sample of her child's expelled faecal material be collected from its nappy.

Following receipt of this faecal sample the Microbiology Laboratory at Barts Health NHS trust will inform the sub-study Research Assistant who will record this against the woman's unique trial number. If this sample is not marked as received then at nine weeks after notification the sub-study Research Assistant will request that the mother is sent another follow-up sample collection pack. Should this faecal sample fail to be returned within a period of 12 weeks after notification of consent has been received by the sub-study trials office then the sample will be marked as 'not received' and no further attempts to obtain a faecal sample will be made.

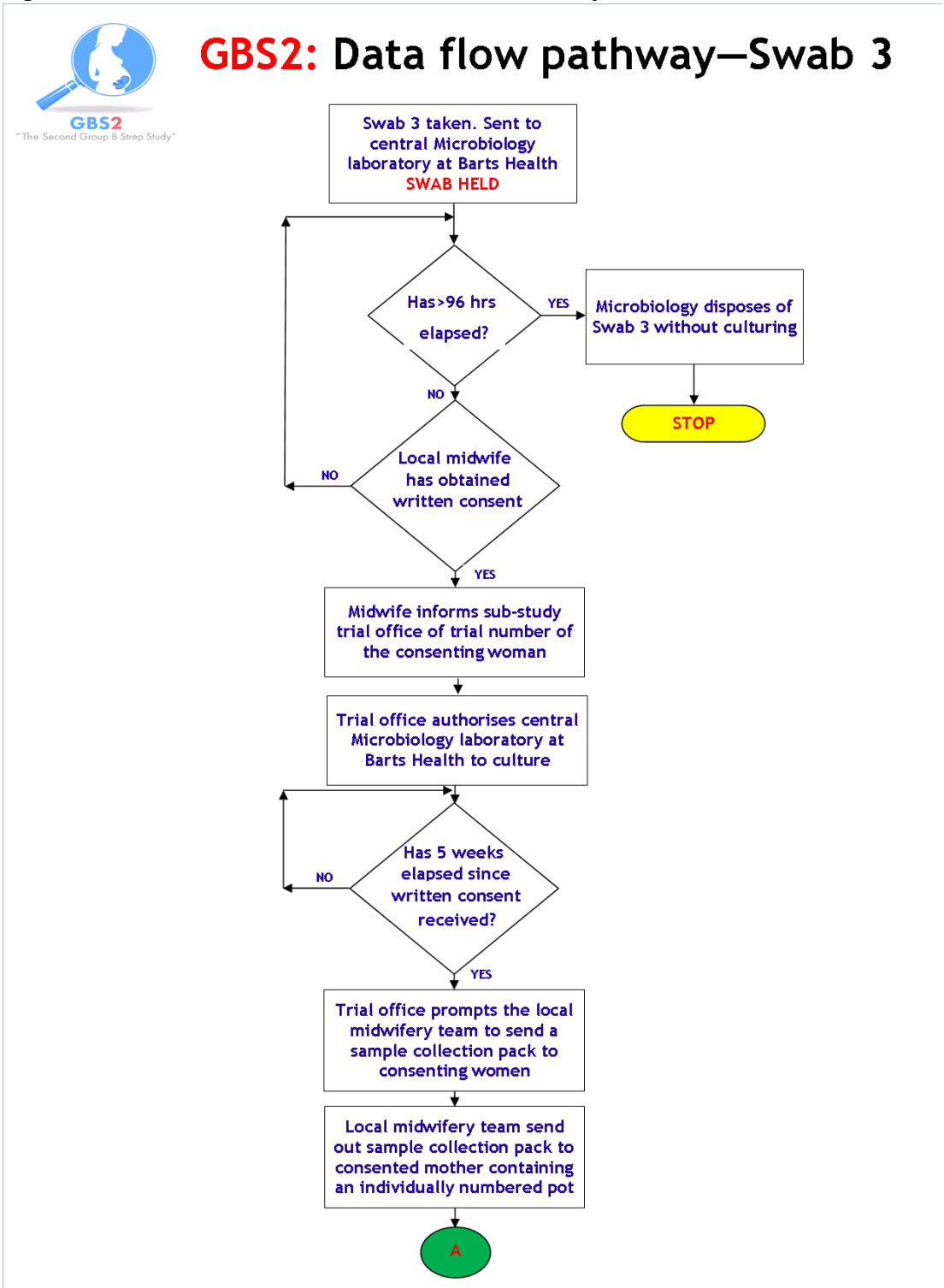
The pot will be placed in a suitable transport container and returned to the central microbiology lab at Barts Health NHS Trust. The post room at Pathology and Pharmacy Building will sort through all deliveries and any samples labelled for the GBS2 sub study will be directed to the appropriate lab for analysis.

Upon receipt the number on the pot will be recorded and the sub-study Research Assistant notified. The bacterial flora present in the supplied faecal material will undergo selective enrichment for GBS, meticillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), and extended-spectrum β-lactamase producing (ESβL) *Enterobacteriaceae*. If GBS is present its antibiotic resistance profile will be determined, whilst any MRSA, VRE and ESβL will undergo typing as described above. As before, any bacteria of interest will be stored for later work. No personal information which links the stored bacterial sample to the donor will be available to any member of the sub-study team.

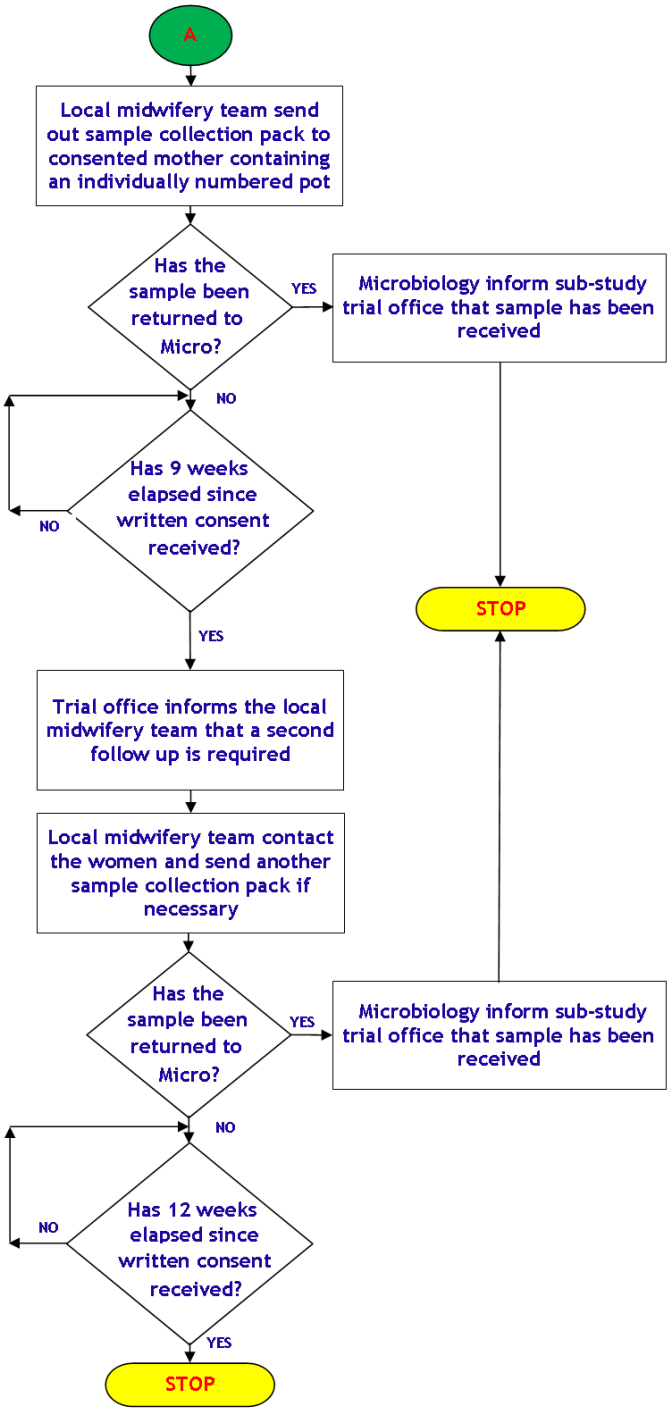
By linking the number on the pot with that of the mother the profile of any GBS, MRSA, VRE, and ESβL obtained from the mother's vaginal - rectal sample will be compared with that of any GBS, MRSA, VRE, and ESβL obtained from the baby's faecal sample at around six weeks old. Once the last faecal sample has been processed the local midwifery staff will be supplied with a list of trial numbers of those mothers who returned samples of their child's faecal material and asked to supply data on the mode of delivery the woman experienced alongside any maternal co-morbidities. These data will be combined to see if there is any correlation between the colonising species and the mode of delivery, and / or maternal co-morbidities.

The data flow for the relatedness sub-study is shown in Figure 5 on the next two pages.

Figure 5: The data flow for the relatedness sub-study



GBS2: Data flow pathway—Swab 3



The study schema in centres assigned a rapid test in 1) The West Midlands [Figure 6], and 2) London and the South-East [Figure 7], followed by 3) those centres without a rapid test [Figure 8] are shown below:

Study Schema

Figure 6: Centres in the West Midlands assigned a rapid test

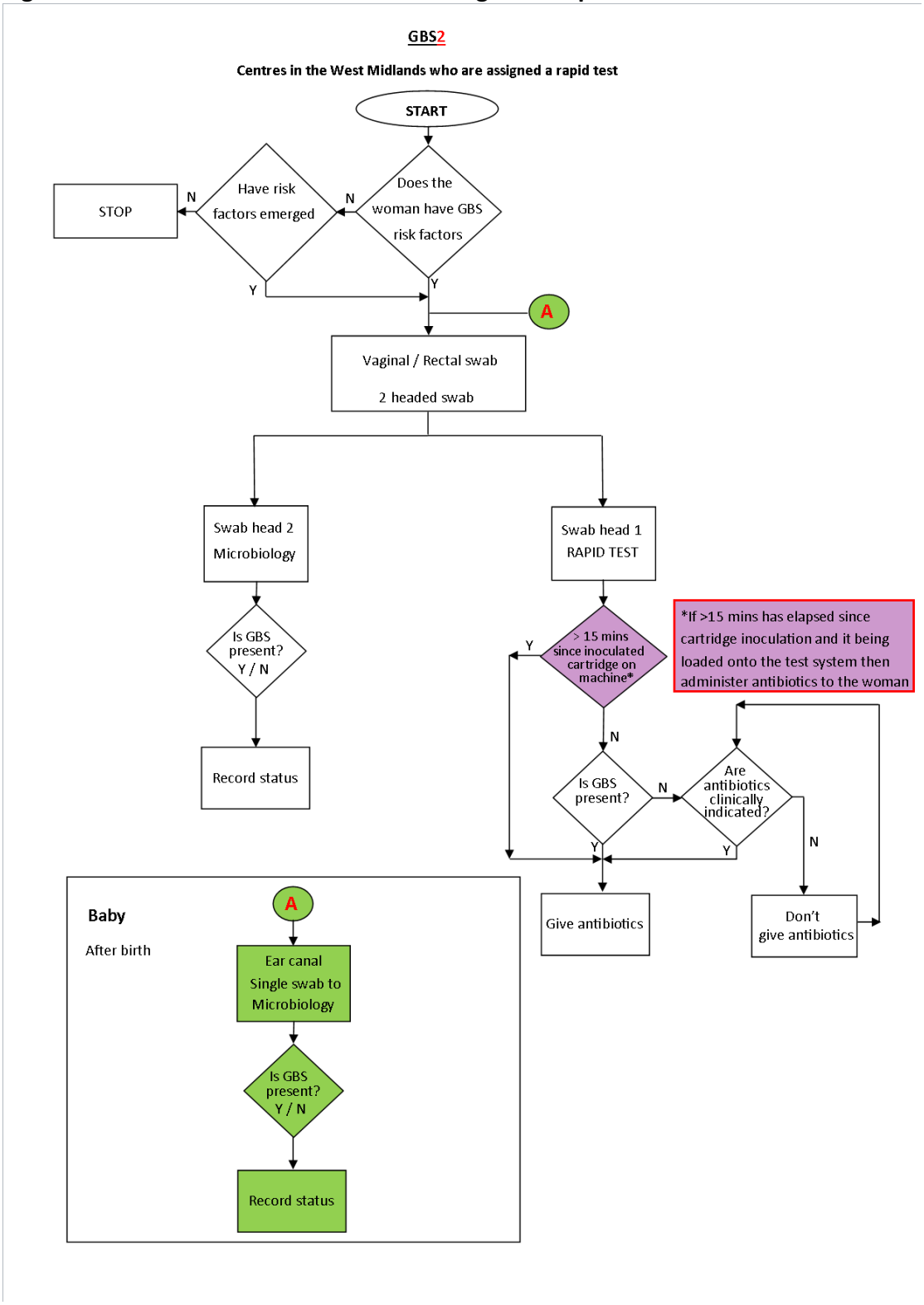


Figure 7: Centres in London and the South-East assigned a rapid test

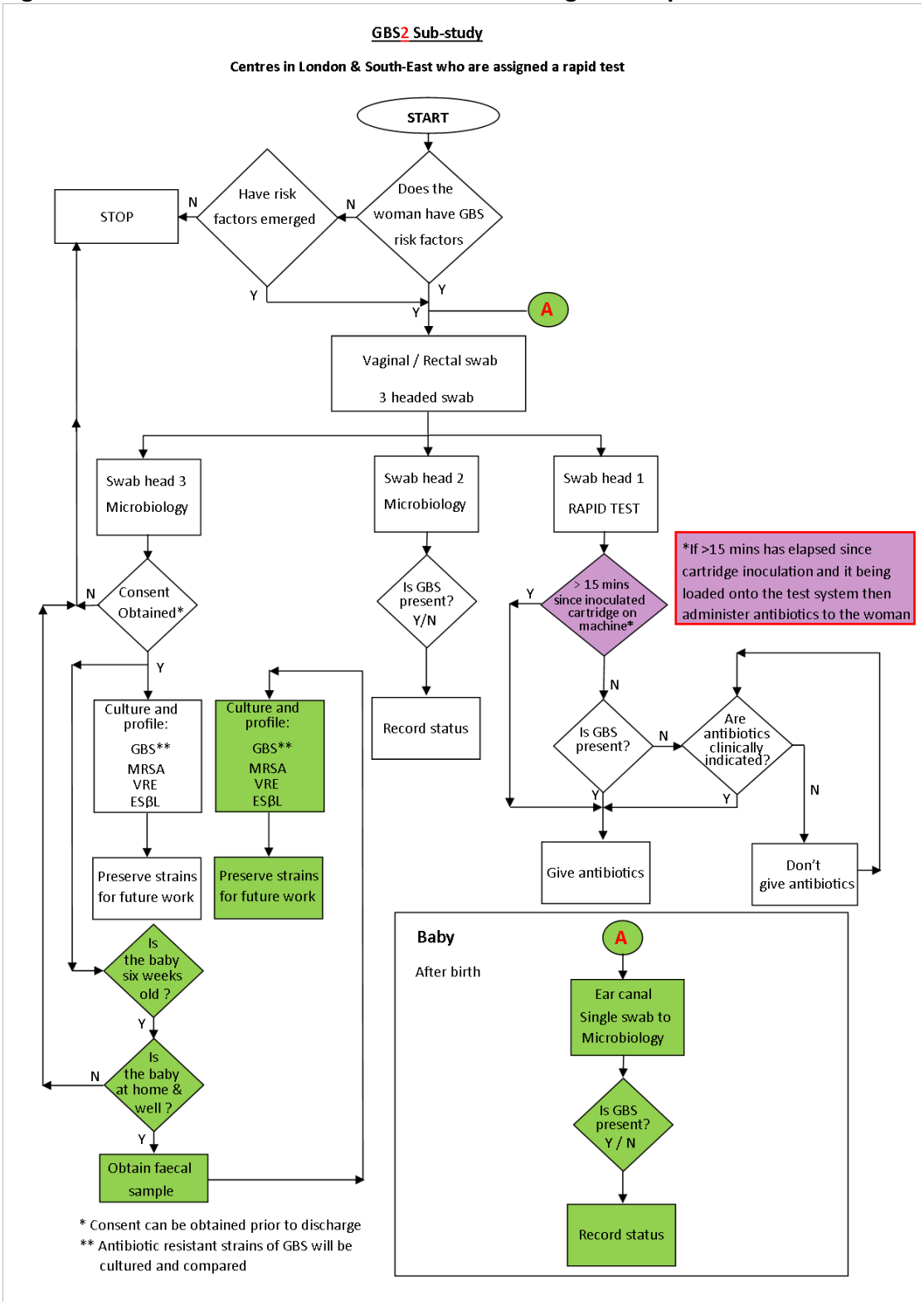
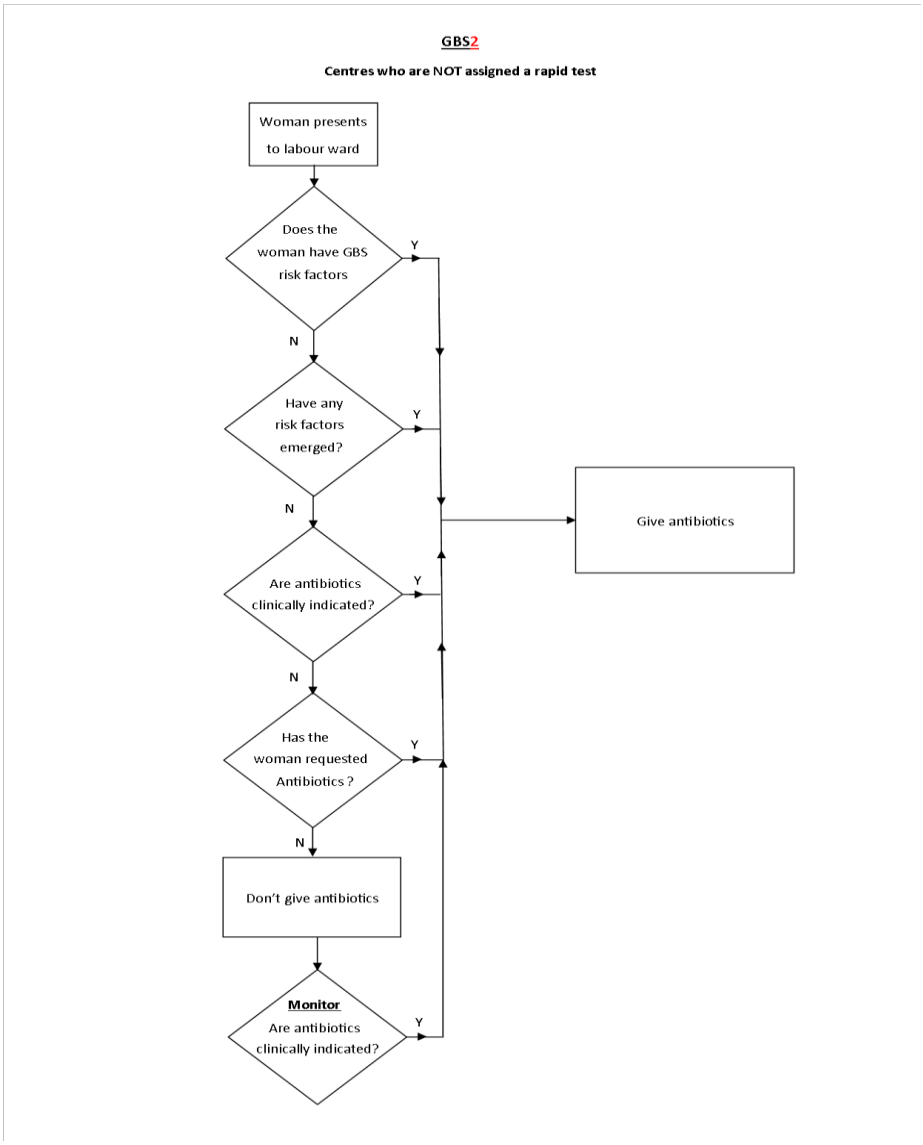


Figure 8: Example pathway in centres not assigned a rapid test



Current rates of intrapartum prophylaxis in participating maternity units

Information will be sought from all participating units regarding their current rate of administration of IAP for GBS transmission. This can be derived from audit, interrogation of hospital records or pharmacy prescribing databases.

Randomisation of maternity units

The purpose of the obtaining the baseline current IAP usage rates is to stratify the randomisation of clusters. With a relatively small number of clusters it may be inappropriate to attempt to pair clusters. As clusters will be randomised in up to four waves, it is more appropriate to stratify the randomisation of clusters within each wave by baseline IAP rate. Maternity units will be randomised to follow either the risk factor based screening strategy or the rapid test strategy to direct the decision to offer IAP.

Risk based screening units (i.e. those not assigned a rapid test system)

All women considered eligible for the study according to the risk factors for GBS transmission described should be offered IAP.

If a woman is pyrexic, or suspected or confirmed to have chorioamniotis, broad spectrum antibiotics (e.g. fourth generation cephalosporins) should be given immediately.

The use of IAP will be collected, including information of whether IAP was declined or deemed too late given the progression of the labour, along with the date and time the IAP was initiated.

Rapid test screening units

Supply of trial consumables

Units randomised to receive the rapid point of care test will be supplied with a Cepheid GeneXpert GBS rapid testing system plus a sufficient number of GeneXpert GBS test cartridges and other consumables to cover the number of women projected to have GBS risk factors presenting to their unit during the study period. Should stocks of consumables or test cartridges start to run low (to a level of around 25% of that initially supplied) then the centre should contact the Trials Office in Birmingham who will arrange re-supply.

Obtaining a vaginal-rectal swab

The use of the rapid test for screening will be restricted to women who present to the labour ward and who exhibit at least one of the risk factors associated with GBS colonisation.

Depending on the stage of labour the swabs will be obtained by either the woman herself, or a suitably qualified, trained, and locally approved member of the woman's care team. This could be on admission to the labour ward, before a vaginal examination is performed, or after a risk factor is detected - for example, maternal fever is observed.

At all sites assigned a rapid test, a testing kit will be taken from the pre-packed Grab Bags kept on the labour ward. This Grab bag will contain a multi-headed swab, the test cassette for determining the presence of GBS, the relevant data collection forms, numbered stickers to alert the microbiology lab that the swabs are part of the GBS2 trial. In sites in the West Midlands assigned a rapid test this test kit will contain a double headed swab and REC approved information sheet. In centres in London and the South – East this test kit will contain triple headed swabs, an ethics approved information sheet, and a consent form. In centres who are not assigned a rapid test these Grab Bags will just contain a copy of the GBS data collection forms. All the items in each individual Grab Bag will be labelled with the same trial number to allow linking of all the results.

Swabs will be taken from lower vagina first and then from rectum, using the same multi-headed swab for each. Vaginal specimens for testing will be obtained by gently rotating the swabs across the mucosa of the lower vagina. Rectal swabs will be obtained by inserting the swabs through the anal sphincter and then gently rotating. After withdrawal, the shafts of the swabs will be separated carefully and placed into a transport tube to avoid any risk of inadvertent contamination.

In all sites the first swab will be applied to the Cepheid GeneXpert rapid test to determine the colonisation status of the woman and thus guide the clinician if prophylactic antibiotics should be administered. Should more than 48 hours have elapsed since the test result has become available and the woman has still to deliver then the test result shall be regarded as invalid. In this situation the woman should be re-swabbed and the presence of GBS tested for again using both the rapid test and microbiological techniques.

The second swab will be sent to the local microbiology department where it will be used to inoculate a selective enrichment media prior to plating to detect the presence of any GBS.

Additional swabs will be available on the ward in case of inadvertent contamination.

In centres in London and the South-East assigned a rapid test kit a triple headed swab will be used to perform the vaginal – rectal swab. As before, the first swab will be used to inoculate a Cepheid GeneXpert rapid test cartridge to detect the presence of GBS. The second swab will be sent to microbiology where it will be placed in a growth medium selective for GBS. The third swab will be sent to the main GBS2 microbiology laboratory at Barts Health NHS Trust where it will be held pending the notification of receipt of informed, written consent. Ideally consent for this third swab will be obtained before the woman's labour becomes distractingly intense which may impair her ability to provide informed consent. However, it may well be the case that the woman's labour is at such a stage that she is unable to give truly informed consent, a situation which may well continue until several hours postnatally. In order to ensure that these women are not denied the opportunity to participate in research the third swab will be held for a period of 96 hours in the Microbiology Department of Barts Health NHS Trust to allow the woman to receive an invitation to participate in research and give informed consent. Should notification of consent not be forthcoming within 96 hours of this swab being received in the Microbiology Department then the swab will be destroyed without being processed.

In centres in London and the South-East assigned a rapid test, women who have provided a swab will be given an ethics approved information sheet and the profiling sub-study explained to them. The woman will have sufficient time to consider participation in this sub-study and, if they agree, their consent will be taken. Consent for this study can be taken at any point between admission to the labour ward and

when the woman and her child are discharged home. Once notification that consent has been obtained by the microbiology laboratory the third swab will be tested for the presence of GBS, MRSA, VRE and ESβL-producing Enterobacteriaceae by a variety of techniques (including, but not necessarily limited to; antibiotic resistance, molecular / genetic characterisation, and matrix-assisted laser desorption ionization–time of flight mass spectrometry). Any bacteria of interest will be stored for later work. No personal information will be available to the members of the trial team.
Comparing the isolate types from the mothers and their infants will allow an estimate to be made of the extent of vertical transmission of antibiotic-resistant isolates between a colonised mother and her child.

Obtaining a neonatal ear canal swab

If the mother has risk factors associated with colonisation by GBS, as soon as convenient after birth a single swab should be gently rotated in the neonates' ear canal. This swab should be put into a transport tube, labelled with a numbered sticker and sent to microbiology for culture using the hospital's usual request system.

This neonatal ear canal swab will only be sent to microbiology to determine the presence of GBS. The swab taken from the neonate's ear canal will not be applied to the rapid test machine.

Obtaining a faecal sample from the infant (six weeks post-delivery)

This will only occur in centres in London and the South-East who have been assigned a rapid test system, and to the children born to women who have consented to be part of the sub-study.

Once a period of five weeks has elapsed from when the trial office was notified that consent had been given the sub-study Research Assistant will supply the maternity unit with a faecal sample pot labelled with a unique number. The sub-study trial co-ordinator will record the number on the pot alongside the mother's trial number.

After checking with the Community Midwifery team that nothing untoward has happened to the child then maternity unit staff will send out a follow-up sample collection pack. This pack will consist of a faecal sample pot accompanied by a covering letter and a suitable prepaid, addressed transport container to the woman's home address requesting a sample of her child's expelled faecal material be collected from its nappy.

Should this sample of the baby's faecal material not be forthcoming after the first request then one repeat and final request will be sent to the mother at around nine weeks after her baby is born.

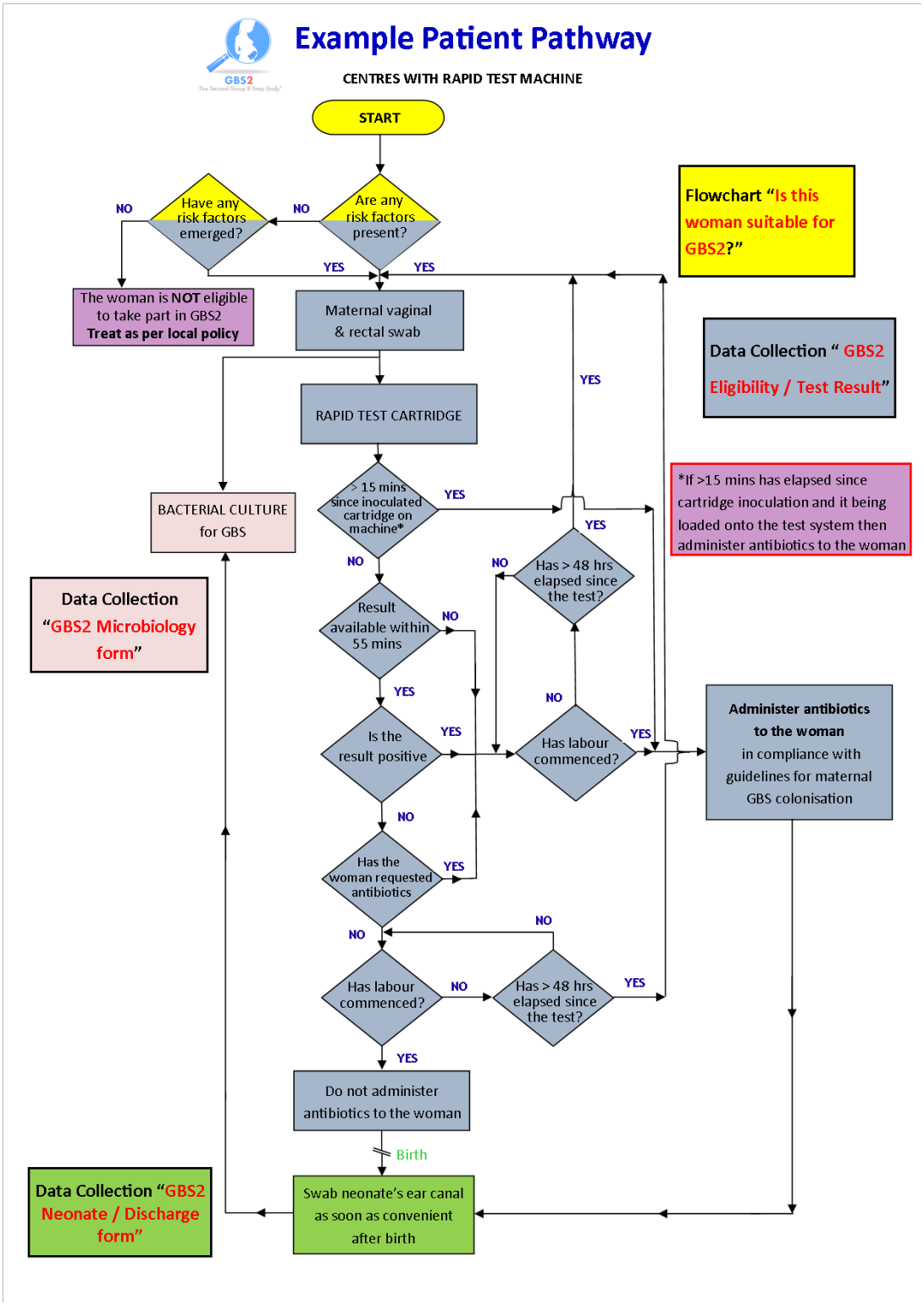
GeneXpert rapid test platform

Each unit allocated to the rapid test screening policy will be required to locate the GeneXpert machine and computer centrally within the unit and have it plugged in and operational at all times. Immediately after the vaginal – rectal swab has been taken, one exposed swab head should be inserted into the GeneXpert GBS test cartridge. Should more than 15 minutes have elapsed from when the swab was inserted into the test cartridge to the test cartridge being loaded onto the machine and the analysis commenced then the test will be deemed to have failed and the woman should be administered prophylaxic antibiotics in compliance with RCOG guidelines. On the computer, the start test icon is selected, the NHS or hospital number entered alongside the patient's unique trial number. This trial number will be identical on each item of study material associated with this woman and her child. Once the start button has been clicked a blinking light on the machine will indicate which bay the cartridge should be loaded into. Once the door on the bay is shut, the test starts automatically. When the test is finished, the door opens and the result is displayed on the computer screen. Two printouts of the results will be available. To comply with Good Clinical Practice the first printout will contain identifiers to ensure that the patient receives the most appropriate treatment and results recorded in the correct set of notes. The second print out will show the unique number and the colonisation status and will be used for trial purposes.

It takes on average 35 minutes to give a result if GBS is present, 55 minutes to confirm if no GBS present, and an error message is presented if the test has failed.

If more than 48 hours have elapsed since the test result has become available and the woman has still to deliver then the test result shall be regarded as invalid. In this situation the woman should be re-swabbed and the presence of GBS tested for again using both the rapid test and microbiological techniques. This pathway is shown in the flow chart **in Figure 4**.

Figure 4: Example Patient Pathway (Centres assigned a rapid test)



Used test cartridges and swabs should be disposed of according to local policies for clinical waste.

The outcome of the rapid test will be recorded on the GBS2 checklist. Periodic downloads of the data held on the computer will supply data on the start time, duration and outcome of each test performed.

Microbiological culture for the reference standard

Swabs designated for bacteriological culture to determine the presence of GBS will be placed in a suitable transport tube and sent to a designated microbiology lab according to local practice. The swab from the neonates ear canal and the swab from the mother's vagina and rectum will be inoculated into separate aliquots of Todd Hewitt broth for overnight enrichment at 37°C. Following this overnight incubation samples of the broth will be subcultured onto chromogenic GBS agar plates for a second overnight aerobic incubation at 37°C. GBS grows as pink-red colonies on the chromogenic media.

Following a suitable time period, the microbiologist will interpret the plates to determine if GBS is present in the mother's vaginal – rectal sample, and in the neonates ear canal. Any viewing of the plates will be undertaken blind to the results of the rapid test. The outcome of the culture will be recorded on each unit's clinical systems by the reporting microbiologist. This information will be recorded on a trial specific data collection form before being transfered to the database by the research midwife or microbiologist.

Microbiological culture for the bacterial profiling (centres in London and the South-East who are assigned a rapid test system)

Upon receipt, the third maternal vaginal – rectal swab will be held in microbiology at Barts Health NHS Trust until notification has been received from the midwifery staff that written, informed consent has been obtained from the women. Should notification of this consent not be received by the microbiology laboratory at Barts Health NHS Trust within 96 hours (four days) of the swab being received, then it will be disposed of in a manner compliant with the local rules on clinical waste.

Upon receipt from the local midwifery staff that notification of written consent has been procured the third swab will be exposed to a range of selective and non-selective culture methods suitable to detect the presence of any GBS, MRSA, VRE or EsβL-producing Enterobacteriaceae. Any bacteria of interest will be profiled using a variety of methods, including, but not necessarily limited to; matrix-assisted laser desorption ionization–time of flight mass spectrometry, antibiotic susceptibility profile, and genetic analyses (including analysis of resistance mechanisms). Resistant isolates will be stored for subsequent molecular characterisation. These isolates will be stored for later work. No personal information on the donor will be available to either the members of the trial team or the microbiologists.

Women in the London and centres in the South-Eastern who have consented to participate in the relatedness sub-study will be sent a follow-up sample collection pack. This will contain a numbered faecal sample pot accompanied by a covering letter and a suitable prepaid, addressed transport container and will be sent to the woman's home address after the birth of their baby by the local midwifery team. The woman will be asked to place a sample of their baby's expelled faecal material into

the pot and return this to the Central Microbiology lab at Barts Health NHS Trust. Upon receipt in microbiology the baby's faecal material will be exposed to a range of selective and non-selective culture methods suitable to detect the presence of any GBS, MRSA, VRE or EsβL-producing Enterobacteriaceae. As before, any bacteria of interest will be profiled using a variety of methods, including matrix-assisted laser desorption ionization–time of flight mass spectrometry, antibiotic susceptibility profile, and genetic analyses (including analysis of resistance mechanisms). Resistant isolates will be stored for subsequent molecular characterisation.

Should this sample of the baby's faecal material not be forthcoming after the first request then the local midwifery team will make one repeat and final request which will be sent to the mother at nine weeks after her baby is born.

The profiles obtained from the gut bacteria colonising the infant's faeces will be compared with that obtained from the vaginal – rectal swab taken from the mother in labour.

Antibiotic administration

Units supplied with a rapid test will be asked only to offer IAP based on the results of the rapid GBS test or if the woman specifically requests them, not the presence of maternal risk factors alone. If the rapid test is positive, the mother will be presumed to be colonised with GBS (given the anticipated high sensitivity) and offered IAP.

If the rapid test is negative, no IAP should be offered for prevention of GBS transmission, unless the women is pyrexic, suspected or confirmed to have chorioaminotitis, or directly requests antibiotic prophylaxis.

If more than 15 minutes have elapsed from when the swab was inserted into the test cartridge and the test cartridge being loaded onto the machine and analysis commenced, or the rapid test fails to deliver a result within 55 minutes of loading the sample on the machine the test will be designated as 'failed'. As the women has risk factors associated with GBS colonisation they will be given intrapartum antiobiotic prophylaxis as per the RCOG guideline. We do not anticipate that more than 10% of tests will fail to deliver a timely result.

Should more than 48 hours have elapsed since the test result has become available and the woman has still to deliver then the test result shall be regarded as invalid. In this situation the woman should be re-swabbed and the presence of GBS tested for using both the rapid test and microbiological techniques.

The use of any IAP will be collected, including information of whether IAP was declined or given the progression of the labour deemed too late to be administered. The date and time the administration of IAP was initiated will be recorded.

1. ANTIBIOTIC REGIMENS AND NEONATAL MANAGEMENT

Maternal antibiotics

Antibiotic regimen for prevention of vertical GBS transmission

In maternity units using either risk factor or rapid test based screening, the IAP regimen will be identical. IAP should be administered as soon as possible after the decision is made and the woman has given assent. Intravenous benzylpenicillin (3 grams) should be infused and an additional 1.5 grams of intravenous penicillin given every four hours until delivery. In those women who are allergic to penicillin, 900mg of Clindamycin should be administered intravenously every eight hours, until delivery.

Antibiotics for maternal infection

If a woman is pyrexic or is suspected or confirmed to have chorioamniotitis, broad spectrum antibiotics (e.g. fourth generation cephalosporins) should be given immediately.

Collection of maternal antibiotic data

The offer and prescription of IAP or any other antibiotics for maternal infection will be recorded on the GBS2 checklist, alongside details of date and time of first dose, dosage and number of subsequent doses.

Neonatal Management

Management of well infants at risk of early onset sepsis

Well infants born to women who had one risk factor for GBS transmission during labour should be monitored hourly for the first two hours after birth, then at two hourly intervals until 12 hours after birth, with assessment made of their general wellbeing, heart rate, respiratory rate and temperature. Clinical judgement should be used to determine whether the appearance of one clinical indicator of potential sepsis necessitates further investigations and administration of antibiotics.

Regardless of the status of the infant, if intravenous antibiotics were administered to the mother for confirmed or suspected invasive bacterial infection e.g. sepsis at any time during labour or in the 24 hours before or after birth, the baby should be evaluated and antibiotic treatment started immediately, before the test results are available if necessary.

Collection of neonatal antibiotic data

The prescription of antibiotics for neonatal infection will be recorded on the GBS2 checklist, with details of date and time of first dose, dosage and number of subsequent doses.

Management of infants with clinical signs of EOGBS disease

Many infants with EOGBS disease will have signs at or soon after birth. Whilst initially subtle these can progress quickly.[31] Respiratory distress, seizures and signs of shock are particularly important indicators for treatment. Whether the mother

received IAP or not, clinical acumen should be used to determine if any baby with clinical signs and symptoms of sepsis should be administered antibiotics immediately, or if investigations should be undertaken before the start of antibiotic administration. If investigations are undertaken then these should include a blood culture to measure the level of C-reactive protein. A lumbar puncture to obtain cerebrospinal fluid should be undertaken if thought safe and timely, and there is a strong clinical suspicion of infection with or without symptoms or signs of meningitis.

Urine microscopy or culture, and skin culture, unless there are localised signs of infection, are not indicated.

Intravenous benzylpencillin with gentamycin is the first choice antibiotic regimen for empirical treatment of suspected infection. The suggested starting dose of benzylpencillin is 25mg kg^{-1} every 12 hours. The suggested starting dose of gentamycin is 5mg kg^{-1}, with further doses as required.[47] Clinicians should manage the situation according to the NICE Guidelines on early onset neonatal infection and their local policies, with the engagement of clinical microbiologists or infectious disease doctors with specific experience in neonatal infection.

In multiple births where one sibling has suspected or confirmed EOGBS disease, the other siblings should be considered at high risk, evaluated and treated with antibiotics.

Collection of neonatal treatment and outcome information

Information on the immediate destination of the baby, the date of discharge, any signs and symptoms of infection, and any antibiotic used will be collected from the neonatal notes using dedicated data collection forms and transcribed to a trial specific database by the research midwife. The unique trial number will be used to link the information from the baby with that obtained from its mother.

After the study period

The rapid test screening units will have the GeneXpert system and any remaining test kits retrieved by the study team, and will revert to their previous clinical policy for screening for GBS.

Schedule of Assessment (in Diagrammatic Format)

Assessment	Screening	On-study treatment phase	End of Study	Follow up visits
Risk factor associated with GBS colonisation	x			

End of Study Definition

The main study will be deemed complete once all babies born to mothers in the study have reached six months of age.

The sub-study will be deemed complete when the last woman in the last maternity unit has delivered and her baby has reached 12 weeks of age. This will ensure that sufficient time has passed for any follow-up faecal samples to be received in the central microbiology laboratories at Bart's Health NHS Trust.

STATISTICAL CONSIDERATIONS

Test accuracy study

The sample size of the test accuracy study is dependent on the sensitivity of the rapid test. For the test to be proven useful we need to show that it will detect a higher proportion of GBS infections than other tests, but not at the cost of low specificity and/or unnecessary administration of IAP.

Results from GBS1 suggested that the most cost-effective test (if untargeted universal IAP was excluded) was antenatal culture for GBS at 35-37 weeks' gestation. In GBS1 the sensitivity of this test was 75.8% (95% CI 47.2% to 91.5%). Thus if we could prove that the sensitivity of rapid test was higher than 90% the results of the GBS2 study would be convincing. This is a stringent test – a lower threshold might also be adequate. We will not be comparing the rapid test with antenatal GBS culture testing within the study, but comparing with this result from external literature. Thus we will be undertaking a "one sample, sample size" computation comparing against a fixed value.

We have data from a systematic review on the performance of the new GBS test. From the meta-analysis of 9 studies, the pooled accuracy of the test was estimated, giving a sensitivity of 96.4% (90.8-98.6) and specificity of 98.9% (97.5-99.5). Sample size calculations are thus based on showing a test with sensitivity of 96.4% is greater than a fixed value of 90%. With a power of 90% to demonstrate this sensitivity, 167 cases of maternal GBS colonisation are required (or 136 at 80% power).

A sample size of 676 would provide 90% chance of us accruing enough GBS colonised women to have 90% power to show the sensitivity of the GBS test to be statistically significantly (with $p<0.05$) greater than 90% should the meta-analytical estimate of its performance (96.4%) be correct whilst allowing for 10% loss from failed tests, based on the GBS prevalence observed in GBS1, which was 29.8% (89/299, 95% CI: 24.6%-35.2%). Of the 606 participants with data we would expect 167 to be GBS carriers and 439 to be negative for GBS colonisation. If the prevalence of GBS colonisation is actually at the lower 95% confidence interval from GBS1, namely 24.6%, then 673 total women will give 90% chance of observing 136 cases of GBS colonisation, including 10% lost tests.

The 95% Cis we would observe on sensitivities and specificities of 85%, 90%, 95% and 98% with a sample size of 676 (606 with data) are shown in **Table 1** and have adequate precision (sensitivity within 10%, specificity within 6%) for modelling.

Point estimate	CI for sensitivity (*n* = 167)	CI for specificity (*n* = 439)
85	78.7 – 90.1	81.3 – 88.2
90	84.2 – 94.0	86.8 – 92.7
95	90.9 – 97.9	92.5 – 96.8
98	94.8 – 99.6	96.2 – 99.1

Table 1: Confidence Intervals for sensitivity and specificity at various point estimates

Cluster randomised trial

The focus of this sample size calculation is on the effectiveness of the rapid GBS test within a cluster randomised trial (CRT) in which the number of clusters, in this case maternity units, is limited by the by the willingness of NHS Trusts to participate, but the number of potential participants is only limited by the duration of study period.

The CRT requires a minimum of 8 maternity units per screening strategy i.e. a total 16 units. Based on assumptions cited below, we estimate that the sample size per cluster will be approximately 83. This equates to a total sample size of approximately 664 participants per arm, and so therefore allows sufficient numbers of women to be recruited for the test accuracy study too.

As there are two arms (Standard Practice / Rapid Test), each of at least 664 participants this gives a projected sample size of 1,328 women. This has been rounded up to a target sample size of 1,340 women.

For the sub-study looking at antibiotic resistance, of the 1,340 study sample size, we estimate that around 650 will be recruited from centres in London and the South-East. Of these, 325 will be assigned to the rapid test arm. Assuming that one out of five women decline their invitation to participate in the sub study, we estimate that the total sample size will be around 260 participants.

The proportion of women receiving IAP for prevention of GBS transmission under a risk factor based strategy is expected to be in the region of 50% to 75%. In GBS1, only 47% of women with risk factors received IAP. We expect that greater understanding and implementation of the RCOG and NICE guidelines will have improved this figure in contemporary practice, but it is unlikely that there will be perfect compliance with RCOG/ NICE guidelines. We thus estimate an IAP rate of 75% to be reasonable. This primary outcome is a process outcome and so the within-cluster correlation of this outcome (the ICC) is expected to be higher than it would be for a clinical outcome. We have therefore considered the sensitivity of our

calculations to a range of proportions in the risk factor-based screening strategy group and a range of ICC values which we believe to be quite conservative. All of our calculations allow for 90% power and 5% significance.

Guided by the need to have at least 676 women to fulfil the needs of the test accuracy study, and under a range of values for the risk-factor based strategy IAP rates and values of ICC, we have worked out the difference detectable and equivalent relative risk reduction (RRR), shown in **Table 2**.

IAP rate in risk-factor screening strategy	ICC=0.2	ICC = 0.1	ICC = 0.05	ICC = 0.01
75	38% (RR = 0.51)	48% (RR = 0.64)	55% (RR = 0.73)	63% (RR = 0.84)
60	22% (RR = 0.37)	32% (RR = 0.53)	39% (RR = 0.65)	47% (RR = 0.78)

Table 2: Range of relative risk reductions achieved with a range of important parameters

The CRT would have around 90% power to detect a reduction in the proportion of women prescribed antibiotics from 75% to 63% (RRR of about 20%) for a low value of the ICC; to a reduction from 75% to 38% for a very conservative value of the ICC (0.2), equating to a relative risk reduction of 50%.

We have not acknowledged varying cluster sizes in our calculations, as we consider the coefficient of variation of unit delivery sizes to be small (approximately 0.21 based on annual delivery rates for 2013) and can be altered by varying the study duration at maternity units. Given our conservative assumptions, and that the impact of the rapid test on the rate of IAP provided is expected to be large, it is expected that the impact of any varying cluster sizes will be minimal.

Analysis methods

Test accuracy study

The accuracy of the rapid test for detection of GBS colonisation will be expressed as sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios, along with their associated 95% confidence intervals.

Cluster randomised trial

The baseline characteristics, for example parity, ethnicity, risk factors and gestational age at onset of labour will be summarised as means and standard deviations, or medians and inter-quartile ranges, grouped by unit allocation (risk factor based screening or rapid test based screening).

Analyses of outcomes will be by intention to treat. As randomisation will be at the maternity unit level, appropriate statistical methods to account for the clustering within units will be used in the analysis.

The primary aim of the study is to evaluate whether the proportion of women prescribed IAP for prevention of GBS transmission differs between the screening strategy groups. In statistical terms the null-hypothesis, that of no difference can be tested using a mixed logistic regression model with prescription of IAP as the dependent variable, study screening strategy group as the independent variable, and maternity unit as a grouping (random effect) variable. These models will be fitted using population averaged models (similar to multilevel models) using GEE methods in STATA. Population averaged models, as opposed to random effects models, will be used as within the framework of cluster randomised trials: random effects models both lack appropriate interpretation and might be biased.[52] Similar models will be fitted for secondary outcomes and appropriate link functions used for outcomes which are not binary.

Primary analysis will be unadjusted, but secondary analyses will adjust for pre-specified and clinically important baseline covariates. These will include gestational age, and presence of individual risk factors, and maternity unit level covariates such as size. We will allow for clustering at the maternity unit level. The primary analysis will be a complete case analysis. However, missing data will be reported and associations between outcomes explored. Depending on the nature of these associations and the extent of the missing data, sensitivity will be explored by means of multiple-imputation.

The primary outcome will be considered significant at the 5% level (and so the 95% Cis reported); whereas other secondary outcomes will be interpreted cautiously as the CRT is not necessarily powered to detect a difference in these. Reports of interim analysis will be supplied in confidence to the DMEC during the recruitment period. Final analysis will be performed once all babies born to mothers in the study have reached six months of age.

Relatedness sub-study

The proportions of mothers and infants carrying antibiotic resistant bacteria will be documented and the degree to which the strains are indistinguishable when characterised using techniques which include; matrix-assisted laser desorption ionization–time of flight mass spectrometry, antibiotic susceptibility profile, and genetic analyses (including analysis of resistance mechanisms) will be documented. These data will allow an estimate of the extent to which mothers and infants at around six weeks of post-natal life carry indistinguishable strains of resistant bacteria. This data will be used to inform the design of more detailed studies aimed at better understanding transmission pathways with the objective of defining potential interventions.

Accrual and study initiation roll-out

We will not attempt to start recruitment in all sites simultaneously but instead plan on opening equal numbers of control and experimental sites in staggered waves. The burden of supporting sites will be spread over 12 months and lessons learnt in the first wave will be carried forward to the management later units.

A conservative estimate of the number of eligible deliveries per 4 weeks in the participating Trusts indicates an average of 71 deliveries per site, so a study period of 4-5 weeks would be sufficient at all sites. We consider this period the minimum needed should a Trust be assigned a rapid test to implement its use as policy.

Economic analysis

Resource use data will be collected within GBS2 to estimate the costs associated with the diagnosis of women using the rapid test compared to risk factor alone based screening. The main resources to be monitored include:

- The resources required to perform the GeneXpert rapid test and act on its results
- Any resource use associated with identifying risk factors, and establishing they exist before acting on the result
- The administration of IAP and associated resource use (midwife time, monitoring patient)
- Monitoring, investigation and management of infants born to women exhibiting risk factors for GBS transmission
- Any extended stays on post-natal ward for neonatal monitoring and any admissions to neonatal unit (special, high or intensive care)

Estimates of the incidence of EOGBS disease, treatment effects of maternal antibiotics on EOGBS disease, and mortality rates will be based on secondary sources.[54]

Information on unit costs or prices will be obtained and assigned to each resource item in order that an overall cost per patient can be calculated. Cost data will be collected from two principle sources:

Firstly, the test accuracy study will provide resource use data to estimate costs incurred in administering the rapid test. Where possible cost data, such as cost of midwife time etc will be collected from routine sources, including those obtained from Curtis and Netten[55] and hospital finance departments. Many cost sources are already identified and available in recently published sources including a systematic

review and the GBS1 study.[56] These cost data will be appropriately updated by revisiting the relevant sources.

The overall approach to the economic analysis will take the form of a model based economic evaluation and will build on that used in GBS1,[44, 56] whilst maintaining all the alternative screening and treatment pathways. We will refine and develop the previous decision tree model using the data from GBS2 and from up-to-date secondary sources. The test accuracy study will provide data on sensitivity and specificity of the GeneXpert rapid test and allow the treatment pathways followed by women on the basis of the tests results to be modelled. The CRT will provide clear data on feasibility of the woman receiving antibiotics before the baby is delivered, and provide clarity for the treatment pathways based being compared in the study.

A decision based analytic model will be used to allow the extrapolation of cost and effectiveness parameters beyond the data observed in the clinical study (and to allow extrapolation to other settings, for example, an unselected population of women in labour).

A limitation in the economic model on which the GBS1 evaluation was based is that this was an accuracy only study. This meant that treatment pathways had to be modelled and could only suggest what the comparator pathway should look like on the basis of the test result. There was concern that, in reality, clinical practice was likely to be very heterogeneous compared to what our model assumed. The advantage of the GBS2 CRT is that it will provide absolute clarity on the comparators upon which the economic evaluation is based. Thus, the previous economic evaluation will be refined using GBS2 data and compared with many of the previously modeled alternatives and combinations.

The two main strategies compared in GBS2 will be i) screening based on the presence of risk factors, and ii) screening based on the results of the rapid test during labour. These strategies will be compared against each other and with a 'do nothing' strategy using many of the 'modelled' pathways utilized in the GBS1 study. Comparing the interventions to 'do nothing' will again require a calibration process, but as this is not a strategy used in clinical practice it is not included as an arm in the trial.

The data available from the GBS2 study will be patient-specific resource use and costs adjusted for clustering. Given the skewness inherent in most cost data and the concern of economic analyses with mean costs, we shall use a bootstrapping approach in order to calculate confidence intervals around the difference in mean costs.[57, 58].

Using data from GBS2 it will be possible to estimate the additional cost per case of IAP avoided. An incremental economic analysis will be conducted. The base-case analysis will be framed initially in terms of cost-consequences, reporting data in a disaggregated manner, adjusted for cluster, and report on the incremental cost, the

important consequences including use of IAP, alongside data on the number of true positive cases of GBS detected, etc.

The model based analysis will consider treatment over the total disease duration for an infected infant and will include appropriate consideration of medical and/or surgical treatments provided in the longer term, depending on availability of appropriate data. Dependent on data reported in published sources, the model-based analysis will adopt either a cost-effectiveness (i.e. cost per life year gained) or a cost-utility (i.e. cost per quality-adjusted life year [QALY]) approach. In GBS1, results were presented in terms of cost per episode of EOGBS disease avoided, and EOGBS associated infant death avoided, because there were insufficient data available to estimate a QALY based on infection with GBS. If QALY data are now available the results will be presented in terms of cost per QALY.

The model based analysis will allow projection of costs and benefits beyond the endpoint of the study which will be when the infant reaches six weeks age of age, to consider a lifetime impact. Given the relatively long time horizons being considered in these analyses, many of the costs (and benefits) will be incurred (and experienced) in future years. Using discounting, adjustments will be made to reflect this differential timing. The base-case analysis will follow recommendations of NICE and discount costs and benefits at 3.5%.

The results of these economic analyses will be presented using cost-effectiveness acceptability curves to reflect sampling variation and uncertainties in the appropriate threshold cost-effectiveness value. Both simple and probabilistic sensitivity analyses will be used to explore the robustness of these results to investigate plausible variations in key assumptions and variations in the analytical methods used, and to consider the broader issue of the generalisability of the results.

6. ETHICS

GBS2 will be conducted according to the principles of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, and Good Clinical Practice Guidelines.

All centres will be required to sign an Investigator's Agreement, detailing their commitment to strategy implementation, accrual, compliance, Good Clinical Practice, confidentiality and publication. Deviations from the agreement will be monitored and the Trial Steering Committee (TSC) will decide whether any action needs to be taken, e.g. withdrawal of funding, or suspension of the centre.

The Trial Office will ensure researchers not employed by an NHS organisation hold an NHS research passport that includes the maternity units in their region.

Regulatory and Ethical Approval

Ethical and Trust Management Approval

The GBS2 study has a favourable ethical opinion from the West Midlands - Edgbaston Multi-centre Research Ethics Committee (MREC) approval Ref: 16/WM/0036, confirming that the study design respects the rights, safety and wellbeing of the participants.

The Comprehensive Research Network and NHS Trust R&D managers will conduct governance checks and assess the facilities and resources needed to run the GBS2 study in order to grant host site permission to start collecting data. The Trial Office in Birmingham is able to help the local Principal Investigator in the process of the site specific assessment by completing as much of Site Specific Information section of the standard IRAS form as possible. The local Principal Investigator will be responsible for liaison with the Trust management with respect to locality issues and obtaining the necessary signatures at their Trust.

After Trust approval has been obtained, the Trial Office will arrange for the maternity unit to be randomised to either a standard risk factor, or rapid test based screening strategy and this will be conveyed to the local Principal Investigator. A start date and duration for the study period will then be agreed.

Funding and Cost implications

The research costs of the trial are funded by a grant from the National Institute of Health Research Health Technology Assessment Programme awarded to Queen Mary College, University of London.

The GBS2 study will have minimal extra 'service support' costs for participating hospitals, due to the no-consent model of trial recruitment. Excess treatment costs associated with the rapid tests have been estimated from the quotations received from Cepheid and the ability of the Trust's to underwrite these costs will be discussed prior to randomisation, on the understanding that there will be a 50% chance of the Trust being called upon to fund such costs. On top of this Trusts assigned the rapid test will find that there are hidden savings. These include:

- *reducing antibiotic use* – by better identifying and only targeting those women colonised with GBS, Intrapartum antibiotics will only be given where they are needed

- *reducing the time spent in hospital* – by knowing the GBS colonisation status of the mother with considerable certainty, babies born to mothers with risk factors for, but who are not colonised with GBS can be discharged home sooner and with less medical intervention

- *reducing readmissions* – Should a baby be born to a mother who has tested positive for GBS colonisation then this will aid clinical staff in judging if the mother has received sufficient Intrapartum antibiotics to prevent vertical transmission of GBS to her baby. By taking any necessary action earlier, the need to readmit at a later date will be reduced

The costs of the swabs and transport tube and the microbiology culture for GBS are research costs and will be reimbursed pro-rata from the research grant.

Indemnity
Queen Mary, University of London will act as a Sponsor, as defined by the Research Governance Framework for Health and Social Care (April 2005) for the project. The project will also be covered by the sponsor's insurance brokers on a "No Faults Compensation for Clinical Trials and/or Human Volunteer Studies". This policy will indemnify/cover the insured in respect of their legal liabilities arising out of the insured's activities.

Publication
A meeting will be held after the end of the study to allow discussion of the main results among the collaborators prior to publication. The success of the study depends entirely on the wholehearted collaboration of a large number of doctors, nurses and others. For this reason, chief credit for the main results will be given not to the committees or central organisers but to all those who have collaborated in the study. Centres wishing to publish local data obtained from participants in the GBS2 Trial should submit a request outlining their audit or research project to the GBS2 TSC who will give any request due consideration.

7. SAFETY CONSIDERATIONS:

There are no safety concerns above those associated with standard care for any participants in the GBS2 trial. It goes without saying that the clinical staff must take any measures they see fit at any time to ensure the safety and protection of the clinical trial subjects from any immediate hazard to their health or safety. In this instance, the approval of the REC prior to implementing these safety measures is not required.

Should such an intervention be necessary it is the responsibility of the local PI to inform the co-ordinating unit in writing within 3 days of any such intervention taking place. The sponsor (care of the Joint Research Management Office) must be sent a copy of the correspondence with regards to this matter by the co-ordinating unit.

8. DATA HANDLING AND RECORD KEEPING:

Electronic data transfer and paper data collection forms

The National Maternity Information System database and / or the dedicated data collection sheets will be the source for routinely recorded demographic and delivery data. Data will be collected in batches using the mother's unique trial number as anonymous linkers. Where data is requested from the National Maternity Information System each individual's unique trial number will be returned to the site accompanied by a request to download this woman's data from the Trust's electronic systems, anonymise this, then securely return these data to the Main Trial office. Antibiotic prescription data will be extracted from either maternal prescription charts or pharmacy records for the labour ward, which will be cross-referenced with data already collected using the unique trial number. The GBS2 Trial Office will work with the unit's information systems managers in obtaining these data. The availability of, and access to, electronic data will be negotiated with each unit's information systems manager and agreed during the local research governance approval process.

Most maternity units do not collect data such as risk factors electronically, so some paper data collection forms will be required. The GBS2 checklist described above will collate all this information and be linked by the woman's and her baby's unique trial number.

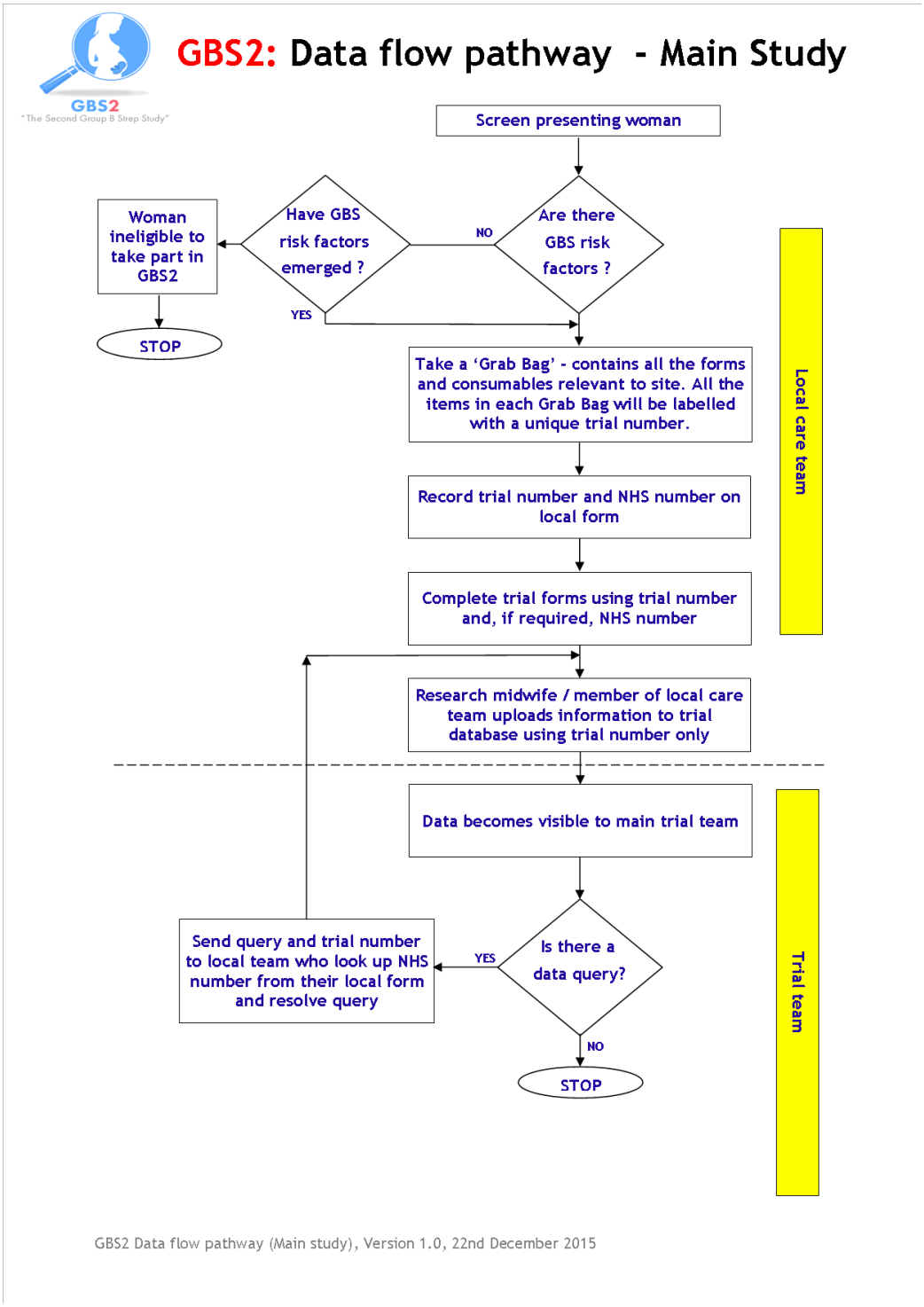
Information on the rapid tests will be downloaded from the computer attached to the GeneXpert machine at intervals throughout the study period and securely transferred using secure socket layer (SSL) encryption technology to the GBS2 Trials Office. Depending on the local site this transfer will either take place via a secure electronic transfer. If local conditions do not allow this then local Trust staff will download the data onto a suitably encrypted and locked USB device which will be securely transported to the trials office.

Confidentiality of personal data

Electronic data retrieved from Trusts and from the GeneXpert machine will not contain any identifiers such as names or address, but will require the use of a unique linker such as the unique trial number. As the results from the rapid test machine will be used to direct patient care, Good Clinical Practice demands that a copy of the results are lodged in the patients notes. To maintain confidentiality but still ensure that trial data is collected the machine will produce two print-outs stating the result of the test. The first will contain a number of identifiers plus the result of the test. This print out will be placed in the patient's notes by a member of the staff responsible for the woman's care and the result acted on appropriately. The second print out will be produced which just displays the woman's unique trial number and various anonymous parameters such as the time the test started, the time the test completed, if the test failed the reason why, and the colonisation status of the mother. The results displayed on this print out will be uploaded onto the trials system by a local member of staff responsible for the care of the woman or the trial research midwife. This print out will be placed with the paper trial records which will be stored securely at the local site.

Once transferred to the GBS2 Study Office, data will be stored in a master database and linked using the unique trial number. This system also provides an effective archive which will allow results to be traced by the participating Trust should any queries arrive or if they are required in the future e.g. to answer the threat of any legal action.

The dataflow pathway for this is shown in Figure 3 (below)



As noted, data collected on paper, for example the GBS checklist, will be entered onto a secure computer database. Paper derived records will be entered directly by the local site *via* the internet using secure socket layer (SSL) encryption technology. Built in access controls will ensure that local trials staff will only be able to view anonymised patient information derived from their site, and will prevent disclosure of rapid test results to the person entering microbiological data on to the database.

Results derived from participants in the sub-study examining the relatedness of any bacterial transmission between the mother and her infant after its birth will be stored against their trial number on a secure, independent database system at a site separate to that where the information on the main (cluster) trial is held. Patient identifiable information will not be available to any members of the trial team.

Specimen identification, processing and storage

The specimens collected will depend upon the location of the trial centre. In centres in Birmingham and the West Midlands who are assigned a rapid test, specimens will be collected from the woman presenting to the labour ward and her neonate.

Women attending units using the rapid test, and who are identified as having factors associated with GBS colonisation will be asked to provide a swab of their vaginal – and rectal mucosa using a multi-headed swab. One of these swab heads will be sent to the local microbiology department using the local test request procedure. The other swab head will be applied to the Cepheid GeneXpert rapid test cartridge.

Neonates born to women who have risk factors associated with GBS colonisation in centres assigned a rapid test kit will have a swab taken from their ear canal at the earliest possible opportunity after birth. This will be sent to the local microbiology lab where it will undergo selective enrichment. The presence of any GBS will be recorded.

In centres in London and the South-East assigned a rapid test kit, women with risk factors associated with GBS colonization, will be swabbed with a triple headed swab. After exposure, as in centres in the West Midlands, the first swab will be applied to a rapid test cartridge, whilst the second swab will be sent to microbiology where it will undergo a culture process suitable to detect the presence of GBS. In centres in London and the South-East who have a rapid test system the third swab head will be being sent to the microbiology department at Bart's Health NHS Trust labelled only with the woman's trial number.

The third maternal swab will be held for a period of up to 96 hours for written, informed consent to be granted. Once the midwifery staff inform the sub-study Research Assistant that written informed consent has been granted this will be passed to the microbiology department who will commence microbiological culture on Swab 3.

If consent is not granted then the third swab will be disposed of in a safe and secure manner.

At five weeks post-delivery the sub-study trial office will request that the local midwifery team send out a follow-up sample collection pack consisting of a faecal sample pot accompanied by a covering letter and a suitable prepaid, addressed transport container to the woman's home address. The woman will be asked to take a sample of her baby's expelled faecal material, place it in the pot and return it in the prepaid transport container provided. The unique trial number displayed on the pot will allow the lab to identify the infant's sample and link it to that of the mother. Should this sample of the baby's faecal material not be forthcoming after the first request then one repeat and final request will be sent to the mother at nine weeks after her baby is born. If no faecal sample has been returned by twelve weeks after the sub-study trial office has logged notification of consent being received then the sample will be marked as 'not expected' and no further follow-up attempts will be made.

Good laboratory practice demands that any swabs sent for clinical microbiology are accompanied by at least two unique identifiers to ensure that the results are recorded in the correct set of notes. Any swab not accompanied by these identifiers are simply disposed of without processing. To ensure that the GBS2 swabs are processed, all clinical microbiology requested on Swabs 2 (the mother's vaginal / rectal swab) and Swab 4 (the neonatal ear swab) will follow the established local procedure for requesting and reporting microbiological tests. The request form or bag will be flagged with a GBS2 sticker to alert the microbiology department to follow the culture protocol and reporting arrangements.

The other maternal vaginal-rectal swab (Swab 1) will be used immediately to inoculate the test cartridge for the rapid point of care test for GBS and will not need to be labelled.

Swabs sent to microbiology lab for the determination of the GBS colonisation status will be introduced into Todd Hewitt broth for overnight enrichment at 37°C. This enriched broth will be subcultured onto chromogenic GBS agar plates where it will undergo a second overnight aerobic incubation at 37°C. GBS will be identified by the presence of pink-red colonies on this chromogenic media.

Results of the microbiological culture will need to be recorded in the patients' notes according to local practice, mindful that these results will not be available until after delivery of the baby. Microbiological data will need to be transferred to the trial database by either a member of the microbiology department, a local research nurse or if possible, by direct data transfer from the sites' information management system. Where manual data entry is required, the data entry screen will not allow review of the rapid test results for that patient by those outside of the study office, thus reducing the risk of review bias. All data transfers, no matter how they are performed will use only the unique trial number as a linker. No identifiable data will be available to the trial team.

Upon notification from the local midwife that written consent has been acquired the third vaginal / rectal swab obtained from the mother during labour, or the faecal sample obtained from the infant after it is born will be exposed to a range of selective and non-selective culture methods suitable to detect the presence of any GBS, MRSA, VRE or EsβL-producing Enterobacteriaceae. Any antibiotic resistant isolates of these bacteria will be stored for subsequent molecular characterisation.

Record Retention

Archiving will be undertaken by the Sponsor following submission of the end of trial report to the funding body. GBS2 is not a clinical trial of an investigational medicinal product so no minimum retention periods are specified. The trial dataset (which is anonymised) will be put into a form allowing the computer database to be legacy archived indefinitely.

Since GBS2 is sponsored by Queen Mary (University of London), the approved repository for long-term storage of local records is the Barts Trust Modern Records Centre where paper records will be kept for a minimum of twenty years.

Principal Investigators are responsible for the secure archiving of essential trial documents for their site according to the local policy.

No personal identifiable information on the woman and her offspring participating in the sub-study will be available to the trial team. The local midwifery staff will inform the sub-study Research Assistant of the woman's trial number once written consent has been obtained. This trial number will be used to link the data collected and no information which can be used to identify the woman or her child will be passed to any member of the trial team.

9. LABORATORIES (if applicable)

Central/Local Laboratories

In centres randomised to receive a rapid test system, the participating trust's microbiology laboratory will be requested to culture any bacteria adhering to the mother's vaginal / rectal swab, and that taken from the neonate's ear canal on a selective media, and to report on the presence or absence of GBS.

Lab Procedures

Women attending units using the rapid test, and who are identified as having factors associated with GBS colonisation will be asked to provide a vaginal / rectal swab using a multi-headed swab. One of the swab heads will be sent to the local microbiology laboratory for GBS culture using the established local systems for requesting microbiological tests. The request form or bag will be flagged with a GBS2 sticker to alert the microbiology department to follow the culture protocol and

reporting arrangements. The other vaginal – rectal swab will be used in the rapid point of care test for GBS and will not need to be labelled.

As soon as is convenient after its birth, a swab will be gently rotated in the neonate's ear canal. This swab will be placed in a transport tube and sent to the local microbiology laboratory for GBS culture using established local systems for requesting microbiological tests. The request form or bag will be flagged with a GBS2 sticker to alert the microbiology department to follow the culture protocol and reporting arrangements. The neonatal ear swab will not be applied to the rapid test system.

Upon receipt in Microbiology the mother's rectal-vaginal swab and that taken from the neonate's ear canal will be introduced into a Todd Hewitt broth for overnight enrichment at 37°C. This enriched broth will be sub-cultured onto chromogenic GBS agar plates where it will undergo a second overnight aerobic incubation at 37°C. GBS will be identified by the presence of pink-red colonies on this chromogenic media.

The sub-study determining the degree of relatedness between certain bacteria colonising the vaginal tract and lower gut of the mother and the lower gut of her baby at six weeks of age will only take place in centres in London and the South-East which are assigned a rapid test machine. In these centres, women joining the relatedness sub-study examining the source of new born colonisation by three types of bacteria will give written, informed consent for their child to be to be followed-up. Should a woman decline to provide consent, or if 96 hours have passed since the receipt of the third swab in Bart's and the London's Microbiology Department, then this third swab will not be processed but disposed of in a suitable manner compliant with local policies.

Upon notification from the local midwife that written consent has been obtained from the participant this third swab vaginal / rectal swab will be exposed to a range of selective and non-selective culture methods suitable to detect the presence of any GBS, MRSA, VRE or EsβL-producing Enterobacteriaceae. Any bacteria of interest will be profiled using a variety of methods, including matrix-assisted laser desorption ionization–time of flight mass spectrometry, an antibiotic susceptibility profile will be undertaken, and genetic analyses (including analysis of resistance mechanisms). No personal identifiable information will be available to either the sub-study Research Assistant or microbiology staff.

Once a period of five weeks has elapsed from when the trial office was notified that consent had been given the sub-study Research Assistant will supply the maternity unit with a faecal sample pot labelled with a unique number. The sub-study trial Research Assistant will record the number on the pot alongside the mother's trial number.

After checking with the Community Midwifery team that nothing untoward has happened to the child then maternity unit staff will send out a follow-up sample collection pack. This pack will consist of a faecal sample pot accompanied by a covering letter and a suitable prepaid, addressed transport container to the woman's home address requesting a sample of her child's expelled faecal material be collected from its nappy.

Following receipt of this faecal sample the Microbiology Laboratory at Barts Health NHS trust will inform the sub-study Research Assistant who will record this against the woman's unique trial number. If this sample is not marked as received then at nine weeks after notification the sub-study trial co-ordinator will request that the mother is sent another follow-up sample collection pack. Should this fail to be returned by twelve weeks after notification of the receipt of consent then the sample will be marked as 'not received' and no further attempts to obtain a faecal sample will be made.

The pot will be placed in a suitable transport container and returned to the central microbiology lab at Barts Health NHS Trust. The post room at Pathology and Pharmacy Building will sort through all deliveries and any samples labelled for the GBS2 sub study will be directed to the appropriate lab for analysis

Upon receipt the number on the pot will be recorded and the sub-study trial co-ordinator notified. The bacterial flora present in the gut bacteria colonising the infant's faeces will be compared with that obtained from the vaginal – rectal swab taken from the mother in labour.

Data Preparation and Collection

Results of the microbiological culture for the presence of GBS on Swab 2 (Mother's vaginal / rectal) and Swab 4 (Baby's ear canal swab) will need to be recorded in the patients' notes according to local practice. Results will need to be transferred to the trial database by either a member of the microbiology department, a local research nurse or if possible, by direct data transfer from the sites' information management system. Where manual data entry is required, the data entry screen will not allow review of the rapid test results for that patient, thus reducing the risk of review bias.

10. PRODUCTS, DEVICES, TECHNIQUES AND TOOLS

Devices

PCR based tests

PCR involves the repeated logarithmic amplification of specific areas of the bacterial chromosome using an iterative process of hybridisation of replication primers, amplification from these primers of the target DNA and separation of the nascent DNA. Real-time detection of the amplified DNA is by incorporation of a fluorescent marker which is quantitatively measured within the PCR thermocycler.

In GBS1, we found the highest levels of accuracy were obtained from combing the results from vaginal and rectal swabs, this showing an 84% sensitivity and 87% specificity. However, this was considerably lower than the pooled estimates from a meta-analysis of all the previous studies[42] which reported a pooled sensitivity and specificity of 97% for PCR. This discrepancy may have arisen since, with more robust methodology, GBS1 avoided overestimation of accuracy associated with review bias and other features prevalent in previous studies.[43] Updating the meta-analysis to include results from GBS1 reduced the pooled estimates for PCR to a new pooled sensitivity of 90% (95% CI 88-93%), and pooled specificity of 92% (95% CI 91-94%). The accuracy of the PCR, when considering samples from both sites, compares favourably with that of screening by culture of swabs taken at 35-37 weeks gestation.

In GBS1 we found that it was possible to train midwifery staff to undertake complex testing required by the Cepheid IDI-GBS and SmartCycler system, but it was not feasible to establish testing on demand. As the study progressed, we found that fewer tests were done in real-time but were instead samples were stored before being processed in batches. This was possible since, in GBS1, prophylaxis was not directed by the results of the rapid test. In GBS2, even when processing started immediately, there were considerable difficulties in ensuring the availability of results within the timescale that would have been required clinically. These mainly related to problems in ensuring the ongoing availability of sufficient staff who were competent to undertake testing, the fact that it was impossible when undertaking tests that require significant hands-on test time for staff to begin processing another sample when one was already in progress, as well as the conflicting demands on the midwifery staff.

Development of molecular methods that allow rapid bedside detection of microorganisms offers the potential to target antibiotic use more specifically than was previously possible. GBS1 showed that implementation of complex point of care (POC) tests is technically feasible. However the practical value of any POC test depends on accurate results being reliably available within a clinically relevant timeframe. To this end, careful consideration is required of a number of factors including the expected frequency of testing, achievable result turnaround times, the amount of hands-on test time, strategies to deal with test failure, and assurance of the ongoing availability of sufficient staff able to undertake testing when required.

The majority of the commercially available test systems require multiple preparation and incubation steps comparable to the previous generation Cepheid Smart-Cycler system. In light of the limitations found with this system in GBS1, the technology that we propose to investigate is the Cepheid GeneXpert system. The GBS Xpert test for this platform allows accurate detection of GBS within 35 minutes of placing an appropriate sample in the machine, with a hands-on preparation time of less than 2 minutes.

Existing research related to Cepheid GeneXpert GBS system

The Cepheid GeneXpert GBS system has been available since 2008 and several groups have assessed its accuracy in studies, although with different swabbing strategies and reference standard comparators. A systematic search of test evaluation studies and randomised comparisons of screening strategies using the Cepheid GeneXpert GBS system has been undertaken. Searches were made of Medline, Embase, Cochrane Clinical Trial Register, Cochrane Database of Systematic Reviews and the International Clinical Trial Registry Portal (ICTRP), from 2008 to the present (the GeneXpert GBS system received a CE mark in 2009) using search terms (*group B streptococcus* or *streptococcus agalactiae*) and *(GeneXpert* or *pcr*) and (*identif$* or *screen$* or *diagnos$* or *test$*).

The search identified 15 test evaluation studies, from which data for meta-analysis could be extracted from 11 studies. There is one ongoing single centre study registered on ICTRP, at a UK private hospital, of which we were already aware. There were no randomised comparisons of strategies.

The majority of studies excluded pre-term and precipitate deliveries. Double or triple swabs were invariably used to minimise sampling differences and 8 took recto-vaginal swabs. From a meta-analysis of 9 studies that used an enriched culture method for the reference standard, we obtained estimates of the average accuracy of the test of sensitivity 96.4% (90.8-98.6) and specificity 98.9% (97.5-99.5). Overall, the quality of the studies was adequate, but the potential for bias remained, mainly from a lack of blinding, use of only vaginal swabs or an inappropriate reference standard that could underestimate colonisation rates, remains.

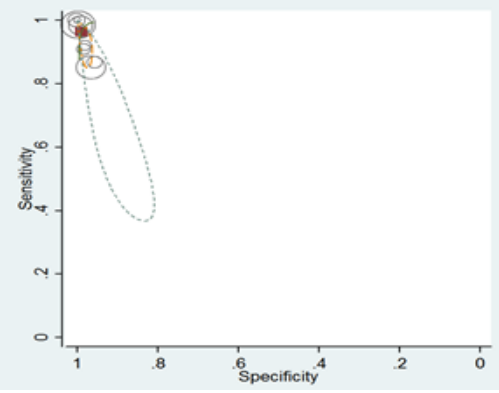


Figure 9. Receiver-operative curve for 8 GeneXpert accuracy studies

Better targeting of IAP

Existing UK guidelines (RCOG, NICE) and the National Screening Committee advocate a risk based approach to the prevention of neonatal early-onset GBS sepsis. This approach results in 20-30% of women receiving antibiotics. GBS1 showed that only two thirds of women with risk factors are colonised with GBS and compliance with national guidelines varies in practice.[59] Thus antibiotic exposure could be considerably reduced if targeted only at those women in labour who are shown to be colonised with GBS.

A study in France assessed whether rapid intrapartum screening for GBS using the Cepheid GeneXpert system reduced the proportion of women unnecessarily administered IAP in comparison with a hypothetical situation where the French national screening programme standard prevailed. [60] The investigators assessed the diagnostic performance of the rapid test to detect the presence of GBS on vaginal swabs taken during the intrapartum period. These results were compared with microbiological screening at 34-38 weeks' gestation and a reference standard of microbiological culture, albeit using a non-standard method. Preterm deliveries (less than 35 weeks' gestational age) were excluded and the rapid tests were performed by laboratory technicians and junior doctors.

Administration of IAP was directed during the study by the result of the rapid test, and the adequacy of this strategy compared against that which would have been hypothetically followed by use of the 34-38 week culture result. This matched pair design allows each woman to act as her own control, but overestimates the adequacy of the antenatal screening strategy.

Only 4.5% of the women in the study arm using the rapid test were found to have received IAP unnecessarily. This rose to 13.6% of the women where the results of the 34-38 week culture were used to direct antibiotic administration. This study demonstrates that the use of a rapid test can substantially reduce the administration of unnecessary IAP in comparison with a tissue culture technique.

A more rigorous, randomised evaluation of the rapid test against the current UK policy of risk-factor directed IAP is required. An assessment of diagnostic performance of the same rapid test performed by midwives in a clinical rather than laboratory setting, would verify whether the results of the meta-analysis described above remain applicable. In their study, Poncelet-Jasser *et al* [60] potentially underestimated the prevalence of GBS (and hence the predicative values of the test) by taking only vaginal swabs, and excluding pre-term deliveries.

Furthermore, assessment of neonatal outcomes, to include impact on EOGBS disease and neonatal death, are needed to inform an economic evaluation that goes beyond the outcome of cost per case of unnecessary IAP avoided.

Finally, providing paediatricians with the maternal GBS colonisation status during labour has the potential to reduce the number of babies who are treated with unnecessary antibiotics in the postpartum period, as well as shortening the duration of antibiotic treatment in those babies who commenced prophylaxis antibiotic administration after receiving insufficient exposure during their mother's labour.

The need to reduce the use of antibiotics and spread of resistance

Currently, at least 10% of all babies born in the UK are treated with intravenous antibiotics, despite the fact that the incidence of early-onset neonatal sepsis is very low at 0.48 per 1000 births.[32] As a result, tens of thousands of babies are kept in hospital at considerable cost to the NHS and receive intravenous antibiotics that may be unnecessary. As well as encouraging the emergence of an increasing number of antibiotic resistant superbugs, recent research has highlighted a number of not insignificant risks arising from exposure of the foetus and new-born infant to antibiotics. These may include a heightened risk of developing necrotising enterocolitis, [60, 61], inflammatory bowel disease,[62] fungal infection,[63] and cerebral palsy.[64]

Antibiotic resistance is regarded by many as an imminent threat to human health. The international importance of this issue is reflected by the call for action from the World Health Organisation[61] and recent reports by the Centre for Disease Control in the US[62] and Public Health England in the UK[63].

Antibiotic (treatment) resistant bacteria have been increasingly shown to cause early and late onset neonatal sepsis[65, 66], and neonatal intensive care unit (NICU) sepsis outbreaks.[67, 68] Strategies for control have focused on reducing the selection pressure (antibiotic stewardship) and control of the spread of the resistant strains. Carriers of antibiotic resistant microbes can be identified by screening and actions taken to prevent spread to others include the implementation of contact precautions and decontamination of the colonised individual. By contrast with methicillin-resistant *Staphylococcus aureus* (MRSA), there is currently no reliable method of decontamination of individuals colonised with antibiotic resistant *Enterobacteriaceae* including those with extended spectrum β-lactamases (ESBLs). ESBLs have recently emerged in community acquired *Escherichia coli* and *Klebsiella pneumonia*, and their identification as causal agents of infections in neonatal units and the lack of effective therapeutic options is a worrying development.

Transmission of bacteria from mothers to their infants has been well documented. Studies have predominantly focused on the causative agents of early neonatal sepsis – *Streptococcus agalactiae*, GBS, and *E. coli*.[69, 70] Risk factors for neonatal colonisation with ESBLs have been described but the relative contribution of perinatal vertical transmission as opposed to horizontal acquisition is uncertain.[71]

The reported prevalence of *E. coli* vaginal colonization in pregnant women ranges from 7% to 20%.[72, 73] One study from Argentina reported peri-anal colonization with ESBL-producing *E.coli* in 5.4% of pregnant women.[74]

As part of an Olympics surveillance project (in collaboration with Public Health England) ESBL-producing *Enterobacteriaceae* were isolated from 20% of women of child bearing age (15-45 years) in North-East London who submitted a faecal sample for laboratory examinations.[75] At the same time colonisation with ESBL-producing

Enterobacteriaceae has been demonstrated in 7% of NICU infants of less than 31 weeks gestational age recruited into a multi-centre, double blind, placebo-controlled randomised probiotic feeding study in South-East England (*PIPS* study, personal correspondence). The extent to which NICU infant colonisation reflects perinatal vertical transmission is currently unclear.

11. SAFETY REPORTING

GBS2 is a study comparing two screening policies and is not a clinical trial assessing the efficacy of an investigational medicinal product. This is reflected by the degree of study specific adverse event reporting in GBS2.

Direct risks of screening policies

The risk based screening approach involves the noting of historical risk factors and the monitoring of women for emerging risk factors such as chorioamnionitis. This presents no risk to the women other than a failure to identify and act upon these risk factors.

The rapid test screening requires a vaginal / rectal swab to be collected during labour, which is benign and presents no foreseeable risk of harm. As with almost any diagnostic test, there is a risk that testers may suffer an inoculation injury (most likely mucous membrane exposure) with clinical material. Every effort will be made to minimise this risk through training and provision of appropriate personal protective equipment and a safe working environment. In the event of such an incident, the local Trust policy for the management of inoculation injuries will be followed.

Risks arising from antibiotic regimens

There may be adverse events as a result of administering benzylpencillin, either to the mother or baby. The most significant of these is an anaphylactic reaction which requires immediate treatment with adrenaline. Lesser degrees of hypersensitivity are common and a rash (all forms), fever or serum sickness observed in 1-10% of recipients. Depending on their underlying cause, these adverse reactions may be treated with antihistamines.

There may be significant consequences of the failure to offer IAP to a woman with GBS risk factors, or who the rapid test identifies as being colonised with GBS, in terms of the potential vertical transmission of GBS to their baby and its increased risk of developing EOGBS disease. Conversely, there is a risk of overtreatment if women without risk factors, or with a negative rapid test, have IAP administered. These instances are considered outcomes of interest within the study, and not adverse events.

Despite either screening approach, there may be babies who develop a superficial or systemic infection, including EOGBS disease. Again, these are defined as outcomes, rather than adverse events, within the study.

Reporting adverse events of testing or antibiotic administration

The following need to be reported as adverse events:

- Inoculation injury to the clinical staff either whilst taking the swab or using the GeneXpert system
- Anaphylaxis in the mother following IAP or her child following antibiotic treatment

Notification and reporting of Serious Adverse Events

Should either event occur then local hospital and Yellow Card reports should be submitted as per trust policy, and the event should be reported to the trials office using an SAE form within 24 hours of learning of the event. The sponsor will coordinate submission of the SAE to the main REC within 15 days in line with the required timeframe.

GBS2 is not assessing the efficacy of antibiotics *per se*, so the clinical trial concept of a suspected, unexpected serious adverse reaction is not applicable here. Serious adverse intrapartum and neonatal outcomes, including but not limited to birth asphyxia, post-partum haemorrhage, neonatal consequences of prematurity and neonatal death are not considered adverse events.

Annual Safety Reporting

The CI will send the Annual Progress Report to the main REC using the NRES template (the anniversary date is the date on the MREC "favourable opinion" letter from the MREC) and to the sponsor.

Overview of the Safety Reporting responsibilities

The CI/PI has the overall oversight responsibility for GBS2. The CI/PI has a duty to ensure that safety monitoring and reporting is conducted in accordance with the sponsor's requirements.

12. MONITORING & AUDITING

The CI will ensure that GBS2 is conducted in compliance with the principles of the Declaration of Helsinki (1996), and in accordance with all applicable regulatory requirements including but not limited to the Research Governance Framework, GCP, Trust and Research Office policies, procedures, and any subsequent amendments.

Non-Compliance is defined as a noted systematic lack of both the CI and the study staff adhering to the principles of the Declaration of Helsinki (1996), applicable regulatory requirements including, but not limited to, the Research Governance

Framework, GCP, Trust and Research Office policies and procedures and any subsequent amendments, which leads to prolonged collection of deviations, breaches or suspected fraud.

These non-compliances may be captured from a variety of different sources including monitoring visits, collected data, communications and updates. The sponsor will maintain a log of any non-compliance to ascertain if there are any trends developing or escalating. The sponsor will assess the non-compliance and action a timeframe in which they need to be dealt with. Each action will be given a different timeframe dependent on the severity. If the actions are not dealt with accordingly, the sponsor will agree an appropriate action. This may include an on-site audit.

Due to the nature of the source data, the use of transferred electronic data, and the low risk of harm to the women delivering in the maternity units, a central monitoring approach will be adopted.

Notwithstanding this principle, investigators and their host Trusts will be required to permit any study-related monitoring and audits to take place by the sponsor or their nominated monitor, providing direct access to source data and site file documents as requested. Trusts may also be subject to inspection by the Research and Development Manager of their own Governance Team and should do everything requested by the Chief Investigator in order to prepare and contribute to any inspection or audit.

Data transfer quality control

A bespoke computer database will be constructed for the GBS2 trial data and will include range and logic checks to prevent erroneous data entry. Independent checking of data entry will be periodically undertaken on small sub-samples. All data merging programs and macros will be tested prior to acceptance of the system.

To ensure the smooth running of the trial and to minimise the overall procedural workload, it is proposed that each participating centre should designate different individuals who would be chiefly responsible for local co-ordination of either the clinical, policy and administrative aspects of the GBS2 study.

All investigators are responsible for ensuring that any research they undertake follows the agreed protocol. This includes ensuring that women with GBS risk factors are screened according to the assigned strategy for that unit, that ‘at risk’ women and babies are provided with IAP according to NICE / RCOG guidelines, and to protect the integrity and confidentiality of clinical and other records and data generated by the research, and for reporting any failures in these respects, adverse events or suspected misconduct through the appropriate systems.

13. TRIAL COMMITTEES

Independent Trial Steering Committee (TSC)

The TSC provides independent supervision for the trial, providing advice to the funder, Sponsor, Chief and Co-Investigators on all aspects of the trial as well as affording protection for patients by ensuring the trial is conducted according to the MRC Guidelines for Good Clinical Practice in Clinical Trials.

If the Chief and Co-Investigators are unable to resolve any concern satisfactorily, Principal Investigators, and all others associated with the study, may write via the Trial Office to the chair of the TSC drawing attention to any concerns they may have about the implementation of the study or about any other matters thought relevant.

DMEC: determining when clear answers have emerged

If the use of a rapid test screening strategy for GBS really is substantially better or worse than risk factor alone based screening with respect to the rate of IAP administration then this may become apparent before the target number of participants has been reached or the total number of maternity units have been deployed. Alternatively, new evidence might emerge from other sources or NHS policy may change. To protect against this, during the rapid test implementation period of the study, interim analyses of the primary outcome will be supplied, in strict confidence, to an independent Data Monitoring and Ethics Committee (DMEC) along with updates on results of other related studies, and any other analyses that the DMEC may request. The DMEC will advise the chair of the Trial Steering Committee if, in their view, the randomised comparisons has provided both (a) "proof beyond reasonable doubt"[1] that for all, or for some, types of labouring women, a particular strategy is definitely indicated or definitely contraindicated, in terms of a net difference in the primary outcome, or that the rapid test was demonstrating unanticipated poor accuracy and (b) evidence that might reasonably be expected to influence the National Screening Committee. The TSC can then decide whether to close or modify any part of the trial. Unless this happens, however, the TMG, TSC, the investigators and all of the central study staff (except the statisticians who supply the confidential analyses) will remain unaware of the interim results.

Members of the various committees are shown in the appendix.

1 Appropriate criteria of proof beyond reasonable doubt cannot be specified precisely, but a difference of at least three standard deviations in an interim analysis of the primary outcome may be needed to justify halting, or modifying, the study prematurely. If this criterion were to be adopted, it would have the practical advantage that the exact number of interim analyses would be of little importance, so no fixed schedule is proposed.

14. FINANCE AND FUNDING

The research costs of the trial are funded by a grant from the National Institute of Health Research Health Technology Assessment Programme awarded to Queen Mary, University of London.

The trial will have minimal extra 'service support' costs for participating hospitals, due to the no-consent model of trial recruitment. Excess treatment costs associated with the rapid tests have been estimated from the quotations received from Cepheid and the ability of the Trust's to underwrite these costs will be discussed prior to randomisation on the understanding that there will be a 50% chance of the Trust being called upon to fund such costs, and the Trusts will be made aware of the potential savings in being assigned a rapid test system. The costs of the swabs and transport tube and the microbiology culture for GBS are research costs and will be reimbursed pro-rata from the research grant.

15. INDEMNITY

Queen Mary, University of London will act as a Sponsor, as defined by the Research Governance Framework for Health and Social Care (April 2005) for the project. The project will also be covered by the sponsor's insurance brokers on a "No Faults Compensation for Clinical Trials and/or Human Volunteer Studies". This policy will indemnify/cover the insured in respect of their legal liabilities arising out of the insured's activities.

16. DISSEMINATION OF RESEARCH FINDINGS

A meeting will be held after the end of the study to allow discussion of the main results among the collaborators prior to publication. The success of the study depends entirely on the wholehearted collaboration of a large number of doctors, nurses and others. For this reason, chief credit for the main results will be given not to the committees or central organisers but to all those who have collaborated in the study. Any publications shall acknowledge the role played by the NIHR (as the funders), Birmingham Clinical Trials Unit (as the co-ordinating centre), and QMUL (as the Sponsor).

Centres wishing to publish local data obtained from participants in the GBS2 Trial should submit a request outlining their audit or research project to the TSC.

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18. APPENDICIES

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Figure 2: Eligibility Flowchart

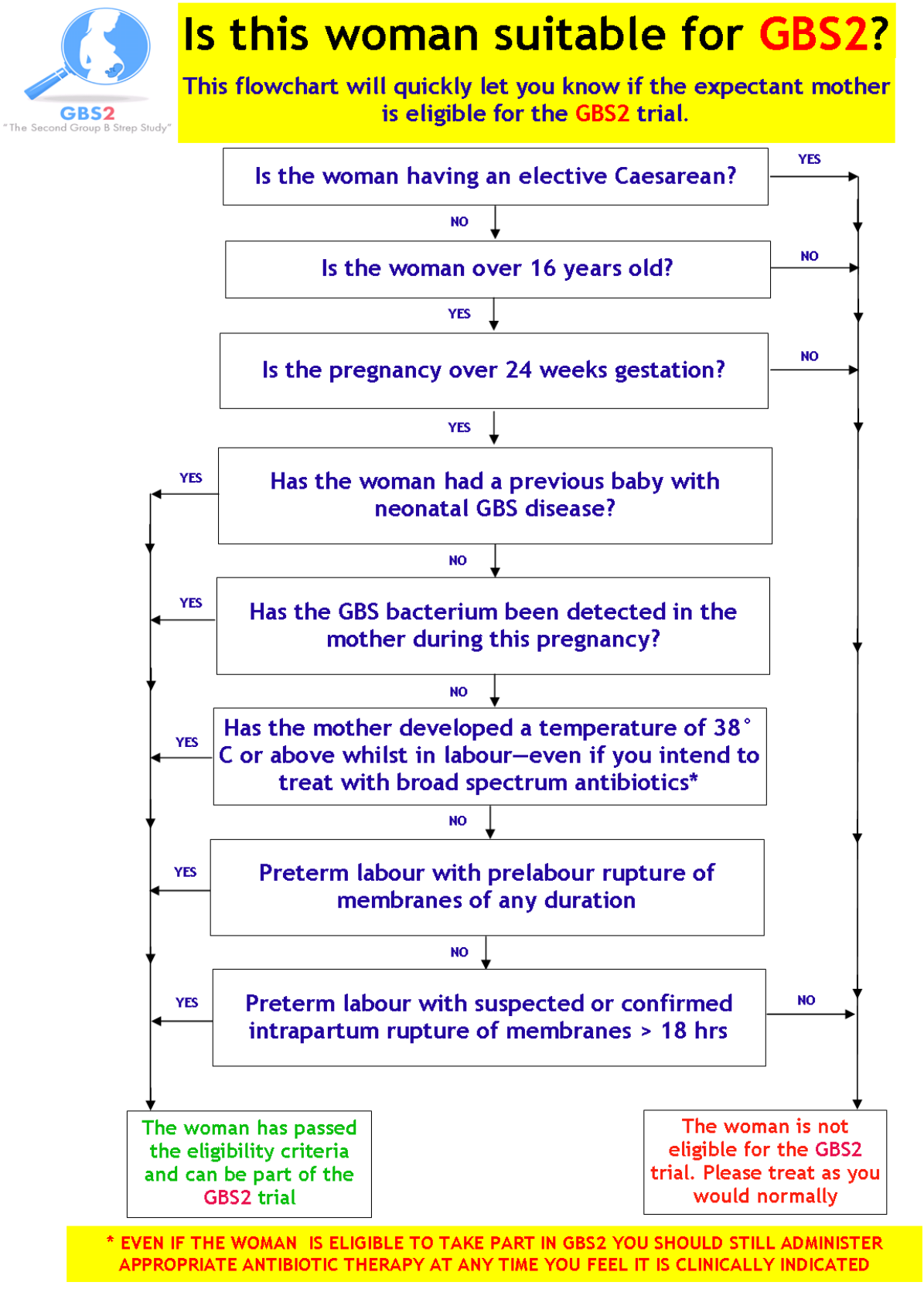


Figure 3: Data flow pathway – Main study

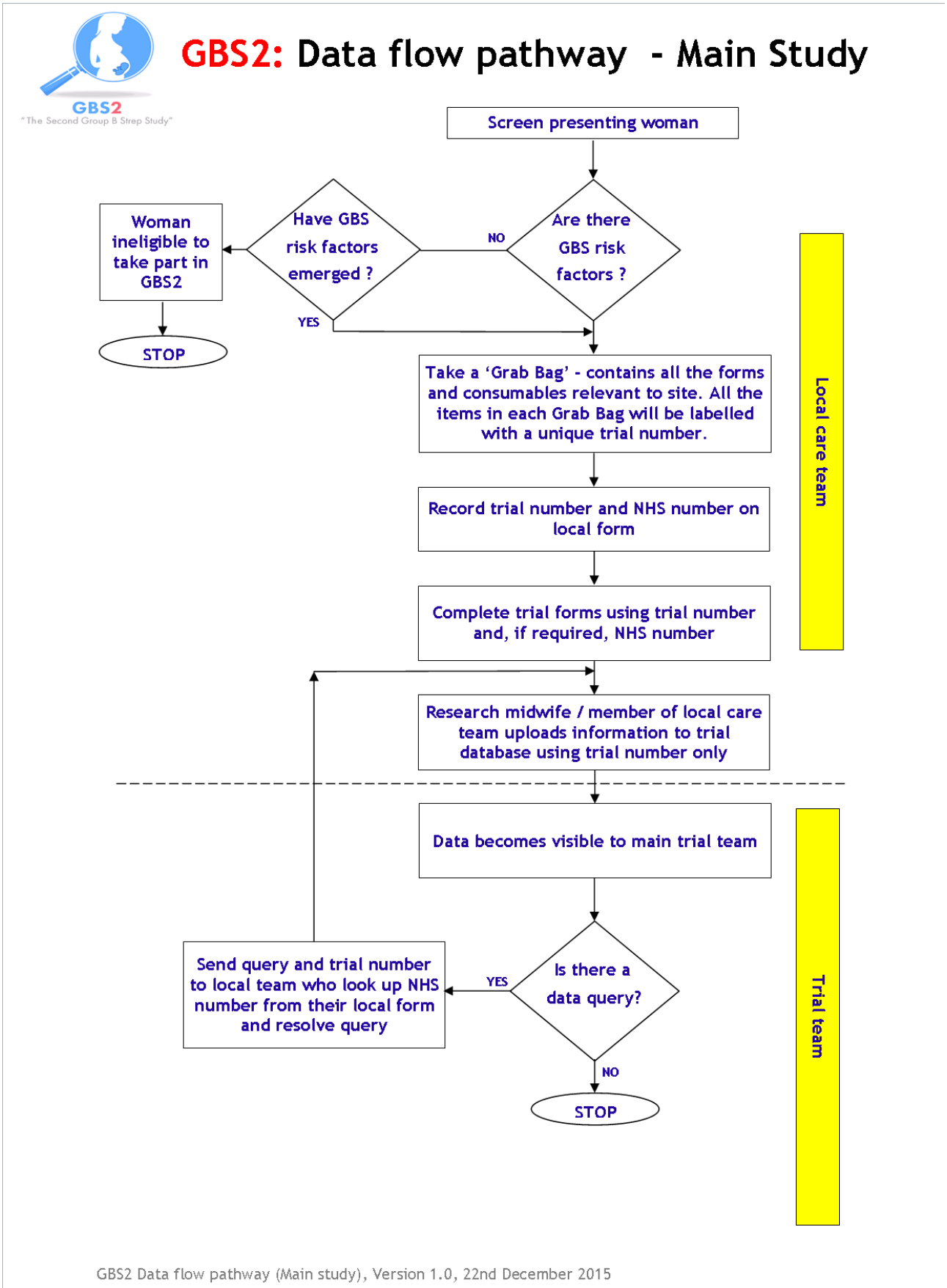


Figure 4: Example Patient Pathway (Centres assigned a rapid test)

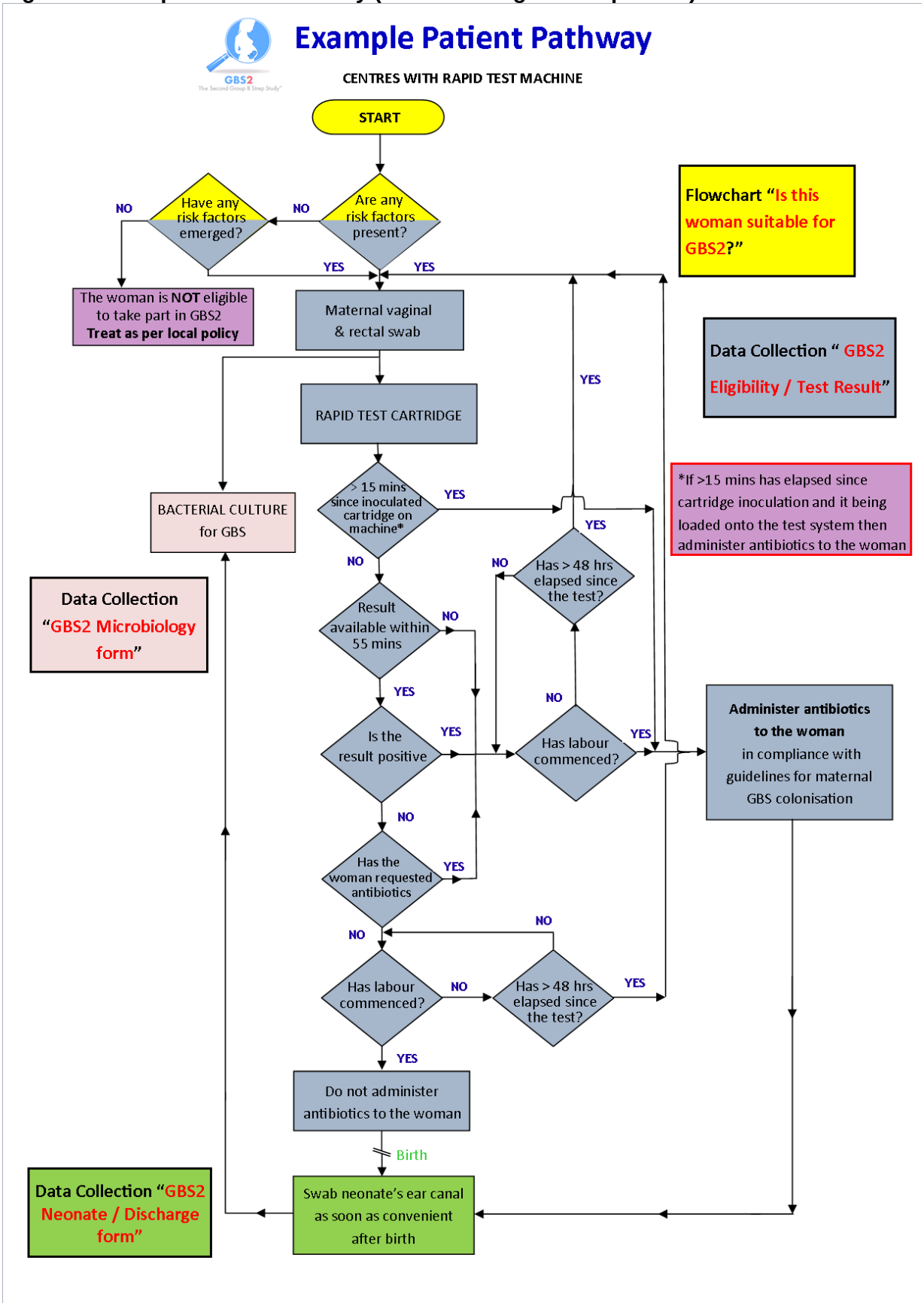
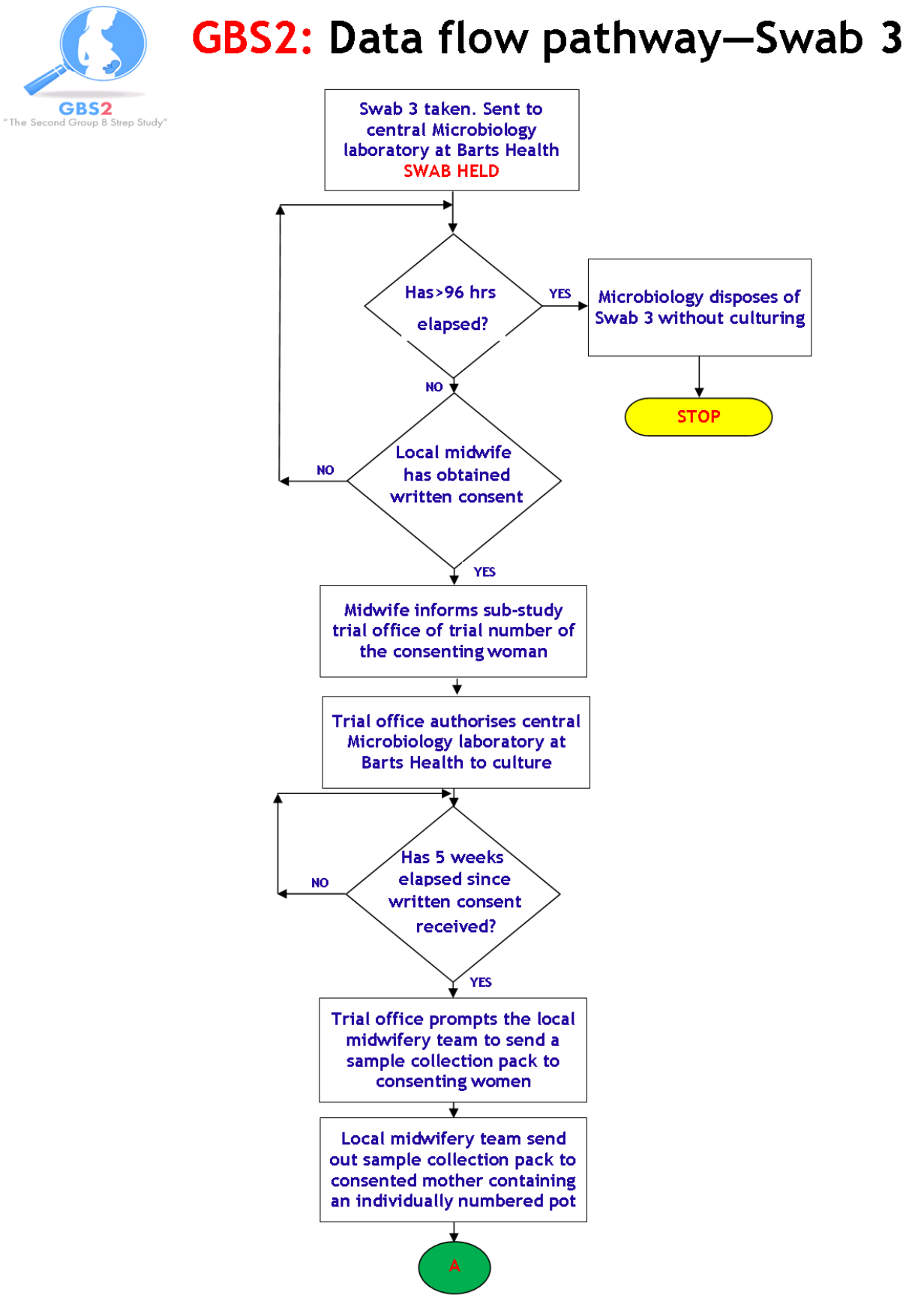
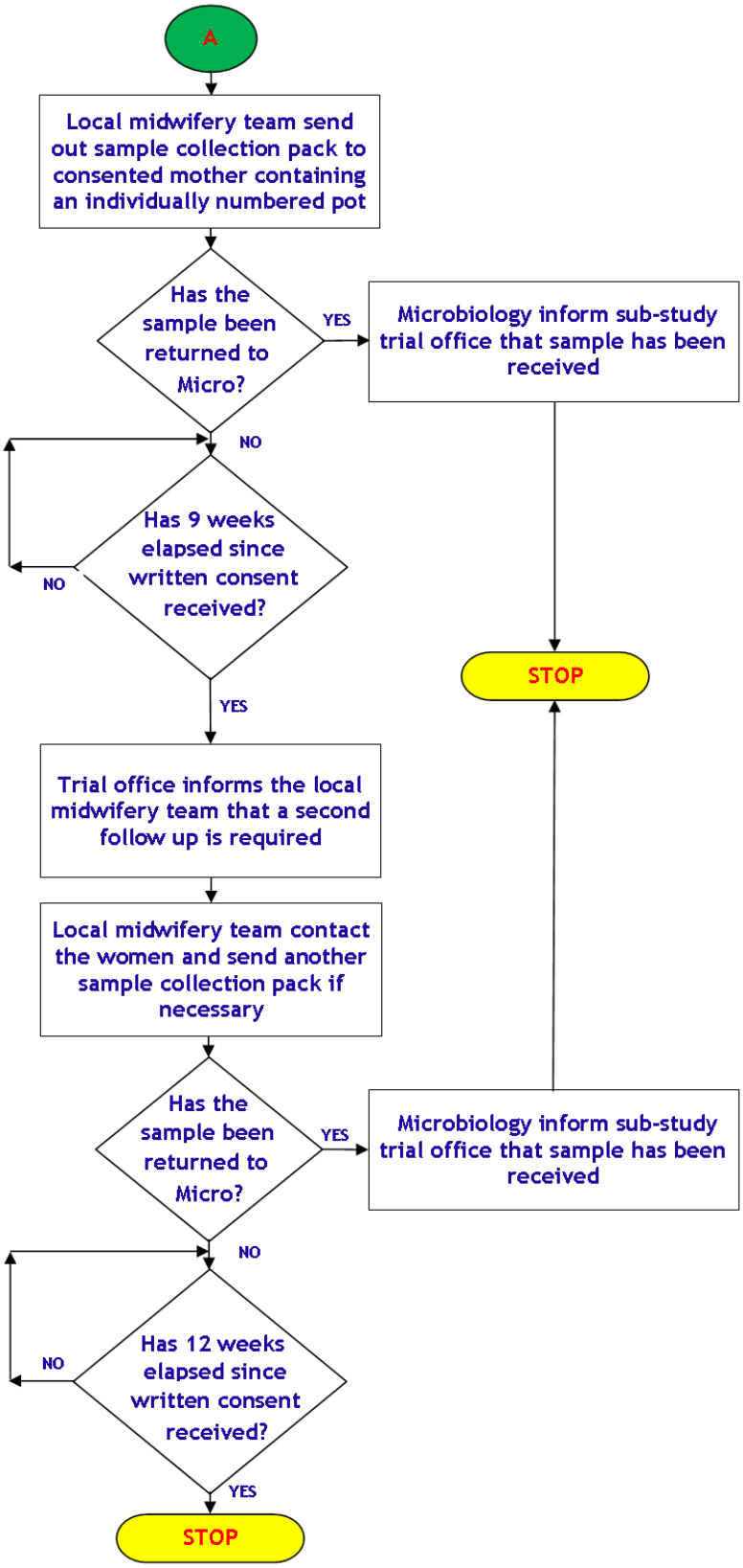


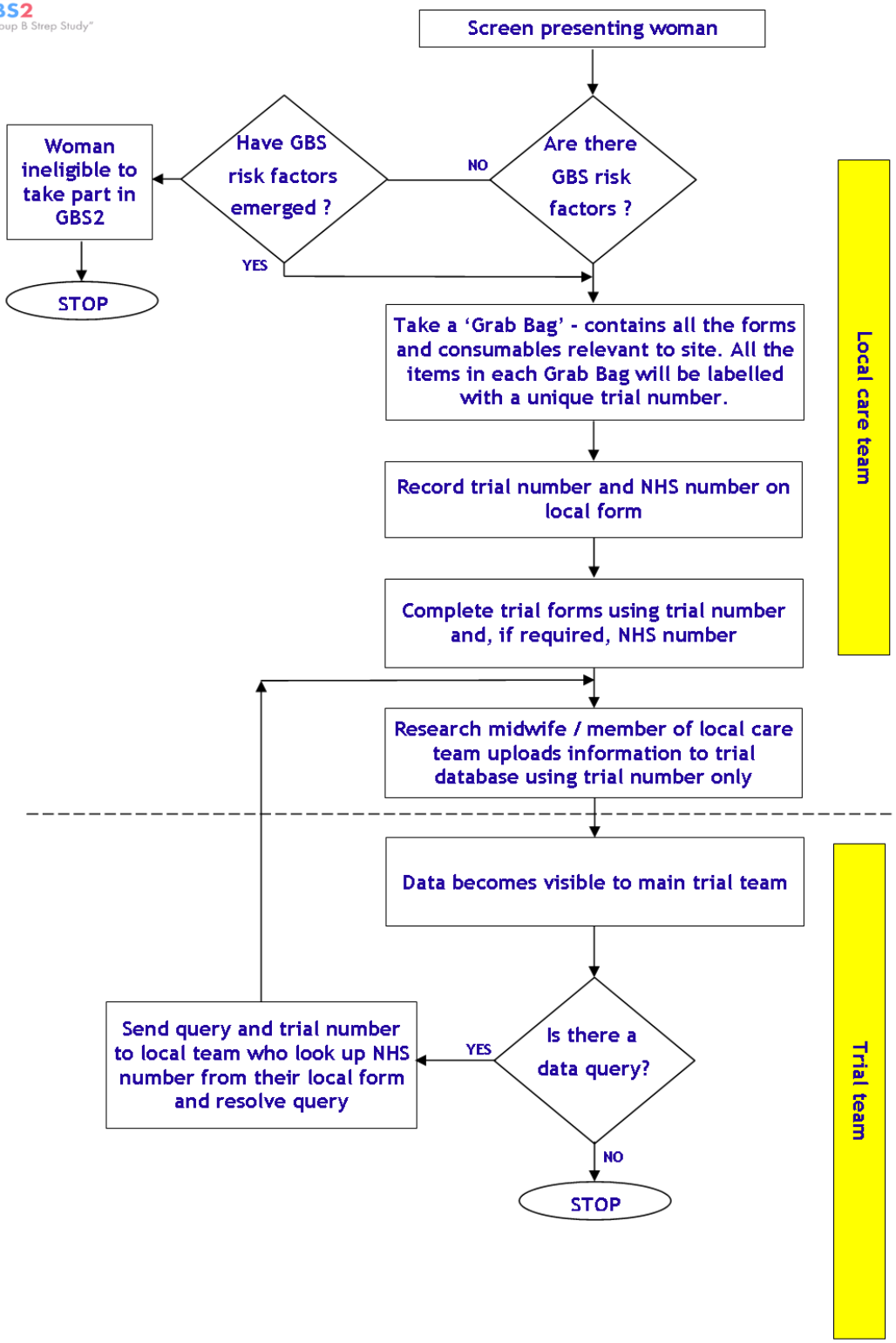
Figure 5: Data flow pathway – Swab 3 (sub-study)



GBS2: Data flow pathway—Swab 3



GBS2: Data flow pathway - Main Study



GBS2 Data flow pathway (Main study), Version 1.0, 22nd December 2015

Figure 6: Study Schema – Centres in the West Midlands assigned a test system

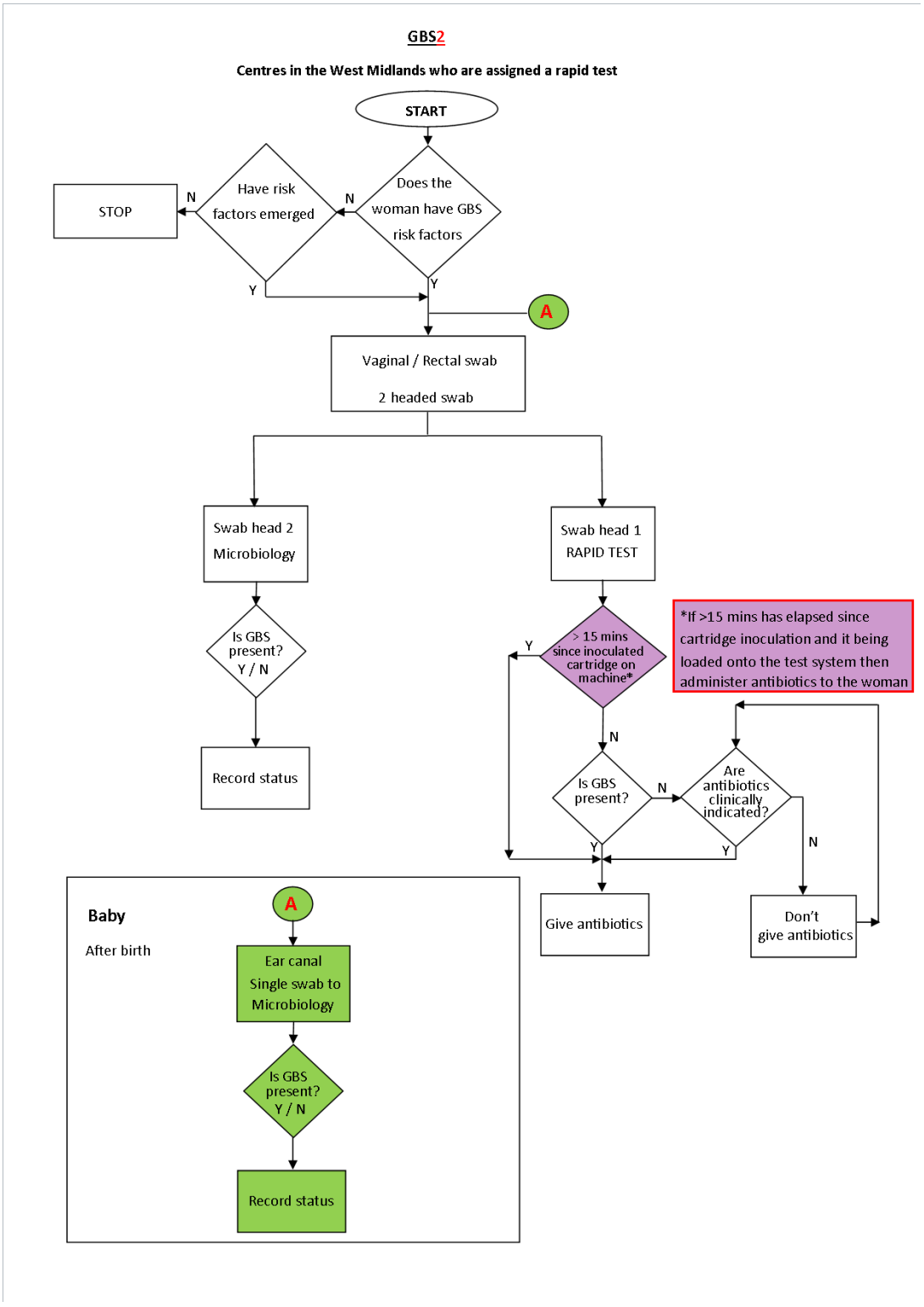


Figure 7: Study Schema – Centres in the South East assigned a test system

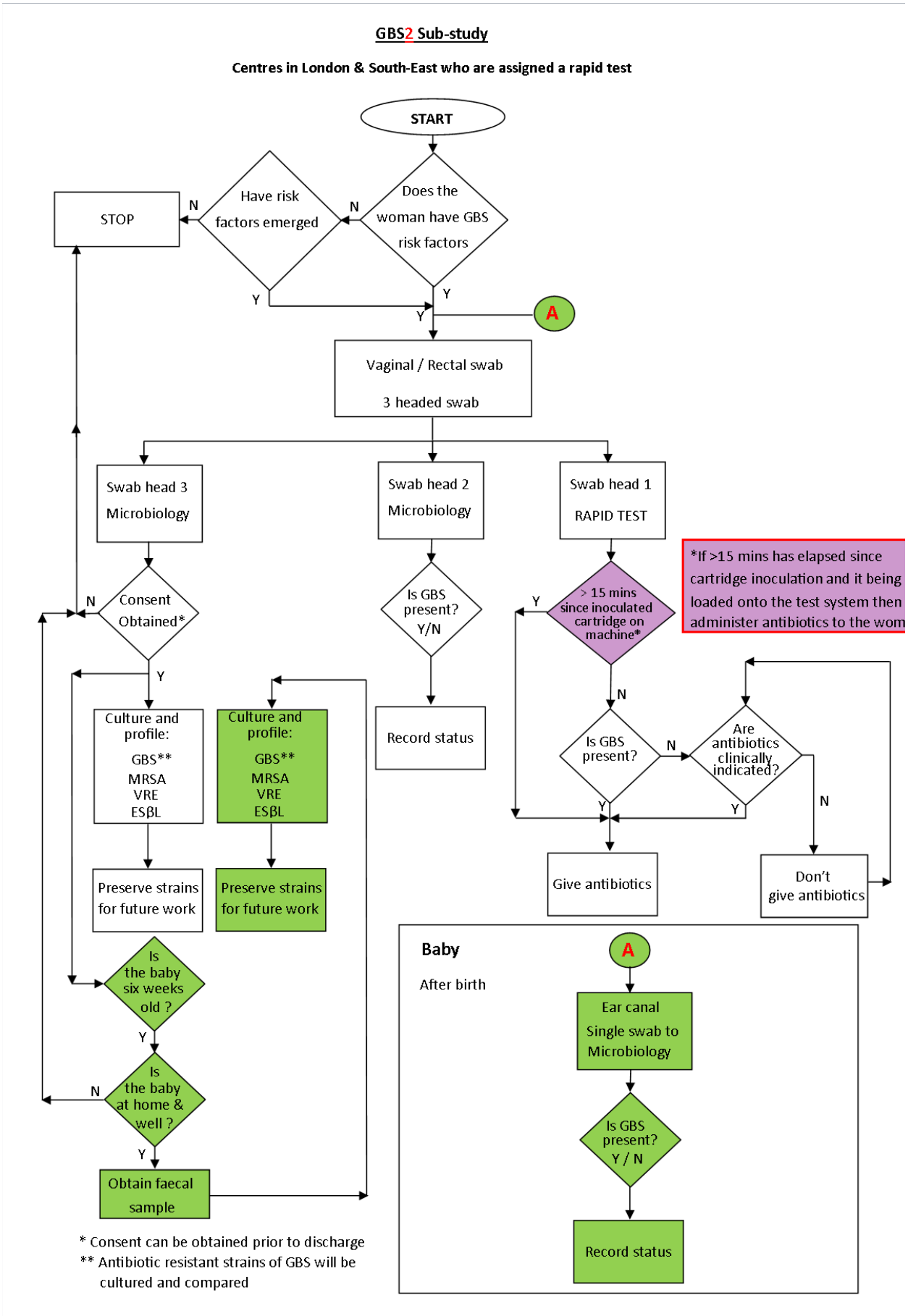


Figure 8: Study Schema – Centres NOT assigned a test system