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Real-time polymerase chain reaction tests versus antenatal culture tests for the screening of maternal group B Streptococcus colonisation in labour (Protocol)

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[Diagnostic Test Accuracy Protocol]

Real-time polymerase chain reaction tests versus antenatal culture tests for the screening of maternal group B Streptococcus colonisation in labour

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A B S T R A C T

This is a protocol for a Cochrane Review (Diagnostic test accuracy). The objectives are as follows:

The primary objective is to compare the diagnostic accuracy of commercially available real-time polymerase chain reaction (PCR) tests and antenatal culture tests for diagnosing group B *Streptococcus* (GBS) colonisation in pregnant women during labour.

B A C K G R O U N D

Target condition being diagnosed

Group B *Streptococcus* (GBS), or *Streptococcus agalactiae*, was first identified as a serious child health concern in the 1960s, when it was found to be the leading cause of neonatal sepsis (Baker 1973). GBS is a naturally occurring Gram-positive *Streptococcal* bacterium that intermittently colonises the gastrointestinal and genitourinary tract in 30% of healthy adults (Edwards 2010;

Edwards 2011; Rodriguez-Granger 2012). As such, GBS colonises the vagina in 10% to 30% of pregnant women (Daniels 2009). If a pregnant woman is vaginally colonised with GBS when she is in labour, there is approximately a 50% risk that GBS can be transmitted to the newborn, either through the newborn passing the colonised birth canal, or GBS ascending in utero (Brocklehurst 2005; Colbourn 2007a). Most of these GBS colonised babies will be asymptomatic, however approximately 1% to 2% will suffer from invasive GBS disease (Boyer 1985), and approximately 10% of babies with invasive GBS disease will die as a result of it (Heath 2004; Verani 2010a).

Invasive GBS disease is separated into early-onset GBS (EOGBS) and late-onset GBS (LOGBS). EOGBS occurs during the first seven days of life, with 90% of cases presenting within 24 hours (Heath 2004). EOGBS cases progress rapidly, presenting with sepsis in 63% of cases or pneumonia in 26% (Daniels 2009; Heath 2004). LOGBS presents between seven and 90 days after birth and is less progressive; it is associated with localised infections, particularly meningitis (43%), pneumonia or focal infections (Daniels 2009; Heath 2004). EOGBS is associated with higher morbidity and mortality than LOGBS (Edmond 2012; Feldman 1992). In the 1970s, case fatality rates from EOGBS were 20% to 50%; these have substantially declined to 4% to 10%, a decrease attributed to treatment (Rodriguez-Granger 2012; Verani 2010a).

Current global incidence of neonatal GBS is estimated to be 0.53 per 1000 live births (95% confidence interval (CI) 0.44 to 0.62) with case fatality at 9.6% (95% CI 6.2 to 18.3), although this incidence is likely to be an underestimate (Edmond 2012). GBS burden varies geographically, with the highest incidence per 1000 live births found in Africa (1.21), followed by the Americas (0.67), Europe (0.57), Eastern Mediterranean (0.35), Western Pacific (0.15), and very low estimates in Southeast Asia (0.016) (Edmond 2012). EOGBS incidence is estimated at 0.43 per 1000 live births, with 12.1% case fatality, while LOGBS incidence is 0.24 per 1000 live births with 6.8% case fatality (Edmond 2012). Incidence of GBS also varies widely by country. For example, during the 1980s, the incidence of EOGBS in the USA was between one and three per 1000 live births (Boyer 1985; Rodriguez-Granger 2012; Schrag 2002), which decreased to 0.24 per 1000 live births with the introduction of prevention (CDC 2013), whereas in the UK the incidence of EOGBS is approximately at 0.48 per 1000 live births with a case fatality of 5% to 10% (Heath 2004; Lamagni 2013). To prevent EOGBS, many countries recommend offering intrapartum antibiotic prophylaxis (IAP) to mothers to prevent vertical transmission (Boyer 1986; Verani 2010a). IAP was first demonstrated to be effective in reducing EOGBS in 1986 (Boyer 1986). An updated Cochrane review has also found that IAP substantially decreases the incidence of culture confirmed and probable EOGBS, compared to no treatment (Ohlsson 2014). However, the authors found a high risk of bias across the randomised controlled trials (RCTs) and concluded that the results could therefore be due to the bias in methodology. They did not find any evidence that IAP reduces neonatal mortality from GBS compared to no treatment. Different prevention strategies have been proposed to identify women at risk of having a baby with EOGBS, so that targeted IAP can be offered (RCOG 2012; Rodriguez-Granger 2012; Verani 2010a). One of these strategies involves assessing women for GBS risk factors during labour, while another involves actively screening all women for GBS carriage during pregnancy (RCOG 2012; Verani 2010b). A universal antenatal screening strategy was first adopted in the US in 1996 by the Centers for Disease Control and Prevention (CDC), and many European countries followed this example, including France, Germany, Spain and Italy

(Rodriguez-Granger 2012). No RCT evidence is available on GBS screening, but observational evidence from countries that have implemented screening has shown that screening is associated with a lower incidence of EOGBS compared to risk-based prevention or no prevention (CDC 2013; Taminato 2011). Without RCT evidence, it is difficult to calculate the impact of antenatal screening due to confounding factors.

Index test(s)

It is important to note that the aim of GBS testing is to prevent GBS disease in newborn babies. However, tests that are currently available do not discriminate between colonised mothers who will or will not transmit GBS to their babies, or between babies who will or will not suffer from GBS disease. Instead there are several methods for identifying GBS maternal colonisation in labour, and some of these GBS-positive women will not give birth to GBS infected babies. The gold standard for detecting GBS colonisation in labour is considered to be intrapartum bacterial culture. However, because bacterial culture takes 24 to 48 hours to process, culture is not feasible to use in labour because results cannot be available in time to treat. Instead, bacterial culture has to be performed antenatally. Traditionally, screening programmes culture vaginal or vagino-rectal swabs at 35 to 37 weeks, as this has been identified as the optimal time to test for GBS, to take into account changes in colonisation status and provide sufficient time to obtain results (Schrag 2002; Verani 2010a). However, a systematic review published in 2010 demonstrated that in prospective studies, around 30% of women with a positive GBS culture at 35 weeks or more had changed to negative by birth (Valkenburg-van den Berg 2010).

Culture testing involves plating vaginal or vagino-rectal (more sensitive) swabs on blood agar plates where, if a woman is colonised, GBS grows, forming white colonies surrounded by β-haemolysis areas (Daniels 2009). This woman will therefore be diagnosed as positive for GBS colonisation. If the GBS colonies do not grow, the woman is diagnosed as negative for GBS colonisation. Selective enrichment broth before plating is recommended to improve the isolation of GBS from swabs by 50%, although its necessity has been questioned (CDC 1999; Daniels 2009). The most widely used enrichment media is Lim broth with Todd-Hewitt base, nalidixic acid, and colistin before plating on blood agar (Daniels 2009). The site of swab, timing of swab and use of selective media can vary the detection of GBS colonisation (Schrag 2002). As GBS colonisation may be transient, antenatal culture is not very accurate at predicting GBS vaginal colonisation in labour. The systematic review in 2010 found sensitivity ranging from 42.8% to 100% and specificity ranging from 49% to 100%, a mean positive predictive value (PPV) of 69% (ranging from 43% to 100%) and a mean negative predictive value (NPV) of 94% (ranging from 80% to 100%), using intrapartum culture as the reference standard (Valkenburg-van den Berg 2010). The mean antenatal GBS

prevalence was 18% and the mean intrapartum GBS prevalence was 20%.

As a result of the limitations in culture methods, rapid testing methods have been developed, where women found to be GBS carriers during labour can be offered IAP. In addition to being accurate, rapid tests need to be timely enough to allow prompt and effective IAP treatment, and need to be easy to use in routine practice in busy maternity wards. A systematic review (Honest 2006), and a subsequent study (Daniels 2009), found that out of all the rapid tests available, real-time polymerase chain reaction (PCR) testing was the most promising. The remaining rapid tests (see alternative tests below) took too long to achieve a result or did not have adequate test accuracy (Daniels 2009; Honest 2006). Similarly, a literature review for the development of European consensus guidelines reported that the other rapid tests showed poor sensitivity, ranging from 33% to 65% (Di Renzo 2014).

Real-time PCR for GBS involves amplification and detection of GBS-specific DNA. A vaginal or vagino-rectal swab is taken (enriched or standard) and DNA extracted. The specific areas of the bacterial chromosome are targeted by primers and undergo logarithmic iterative amplification, in order to identify whether there is any evidence of GBS DNA (Daniels 2009). In some older real-time PCR tests, such as LightCycler (Idaho Technology), Smart-Cycler (Cepheid), and IDI-Strep B (Somagen), a swab has to be prepared first, before it can be placed into the real-time PCR machine for analysis. Preparation includes extracting the bacterial DNA from the swab and adding the primer and polymerase. Positive and negative controls also have to be prepared for each kit to ensure biases and false results are avoided (Alfa 2010; Daniels 2009). The samples are then inserted into the PCR machine where the target DNA for GBS is amplified, and the presence of GBS is detected from fluorescent signals (Alfa 2010; Daniels 2009). More recently, the GeneXpert GBS (Cepheid) automatically integrates the whole process of DNA extraction, amplification, and detection in the GeneXpert GBS machine. A vagino-rectal swab is inserted into a single-use cartridge in the machine that contains the PCR reagents and controls to process and analyse the swab (Helali 2009; NICE 2015; Park 2013). The results for any of the real-time PCR tests present as positive, negative or inhibitory (i.e. results are inconclusive and the patient needs to be re-tested). Each commercially available GBS real-time PCR assay can target different genes for GBS, use different methods for DNA extraction, and have a different number of cycles for DNA amplification. Any rapid intrapartum test needs to be rapid enough for sufficient time to deliver IAP in labour. The average time to complete a real-time PCR test is 40 to 50 minutes (Honest 2006; NICE 2015).

The previous systematic review found real-time PCR to have a median sensitivity of 96% and specificity of 98% across two studies when a) anal, b) lower vaginal, and c) anal and lower vaginal intrapartum culture were used as a reference (Honest 2006). Since the report, there have been varying estimates of diagnostic accuracy for real-time PCR, with sensitivity ranging from 79% to 92% and

specificity from 95% to 98% (Bazian Ltd 2012). The disadvantage of real-time PCR is that it is unable to determine antibiotic susceptibility, which directs the choice of antibiotic to use for IAP in women who are allergic to penicillin. Methods to determine antibiotic susceptibility are culture-based, and as indicated earlier, cannot be conducted in sufficient time to direct antibiotic selection if GBS colonisation was only identified by real-time PCR in labour.

Clinical pathway

To prevent EOGBS disease and mortality in newborn babies, the current recommendation in the UK is to asses women for known maternal GBS risk factors, and offer IAP to those who have one or more risk factors (NICE 2012; RCOG 2012). Risk factors include; intrapartum fever, incidental GBS, GBS bacteriuria, and a previous baby with GBS disease (NICE 2012; RCOG 2012). However, a third of babies with EOGBS disease are born to women with no GBS risk factors, who therefore would not have been offered IAP (Bazian Ltd 2012). Likewise, not all women with GBS risk factors will transmit GBS to their newborn babies. Screening for GBS is not recommended in the UK (Bazian Ltd 2012; NICE 2012; RCOG 2012).

In the US, and other countries that actively screen all pregnant women for GBS, the most commonly used test is antenatal culture, typically administered between 35 to 37 weeks of pregnancy, and before the onset of labour (Schrag 2002). IAP is then offered to all women with positive antenatal culture results, though not all women will be positive at labour when IAP treatment would be given (Valkenburg-van den Berg 2010). The most recent recommendation from the CDC on GBS screening suggested that rapid tests, such as real-time PCR, could be combined with antenatal culture testing in settings where it is available (Verani 2010b). A European consensus group has expanded this into a recommendation for intrapartum GBS screening (Di Renzo 2014). In such a programme, real-time PCR would be administered in labour, to all women who do not present with risk factors. Those who are positive would be offered IAP treatment. Women who present with risk factors would not be screened, and would be treated with IAP immediately. Antenatal culture testing would only be offered to women with a history of penicillin allergy, in order to assess susceptibility to antibiotic agents. In this way, an effective antibiotic can be selected for IAP treatment, should the patient go on to receive a positive real-time PCR result in labour.

As real-time PCR can be administered at the time of treatment, it is possible that it is more accurate than antenatal culture and can therefore reduce the number of women receiving IAP unnecessarily. If so, real-time PCR could potentially replace antenatal culture for the majority of pregnant women, with only those women who are allergic to penicillin requiring antenatal culture. Real-time PCR may also benefit the risk-based prevention pathway implemented in the UK. By administering real-time PCR to women

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with risk factors, and only offering IAP to those with a risk factor and a positive real-time PCR result, it may be possible to reduce the number of women who receive IAP for GBS (NICE 2015). Safely reducing unnecessary IAP would be especially useful, as a number of potential harms have been associated with widespread IAP usage, including Gram-negative infections, antimicrobial resistance, gut microbiota alterations which may be associated with long-term health problems, maternal anaphylaxis, and the medicalisation of labour (Bazian Ltd 2012; Colbourn 2007b; RCOG 2012). However, real-time PCR would need to demonstrate superior test accuracy, feasibility, and timeliness before it could be implemented in practice.

For a visual representation of where these tests fit into the clinical pathway, see Figure 1.

Figure 1. Clinical and research pathwayBlue arrows show the clinical pathway. Red arrows show the research pathway to assess the diagnostic accuracy.Footnotes:EOGBS: early-onset GBSGBS: group B StreptococcusPCR: polymerase chain reaction

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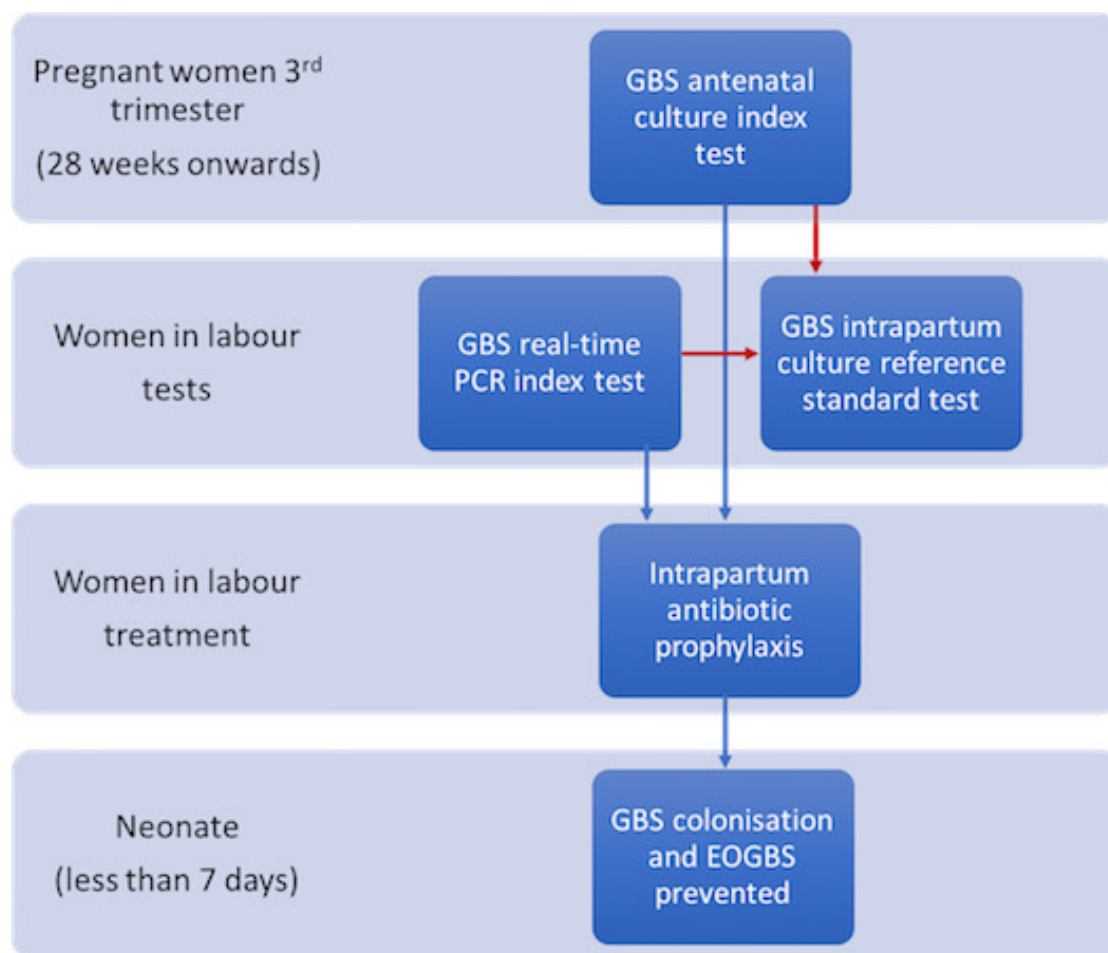
Alternative test(s)

Other rapid tests available for GBS detection include optical immunoassay (OIA), DNA hybridisation, enzyme immunosorbent assay (ELISA), latex agglutination as well as conventional PCR (Daniels 2009). However, we will not evaluate the clinical performance of these tests in this review; as real-time PCR and culture tests are the most used and relevant in clinical practice, we will focus our review on these tests. Latex agglutination, DNA hybridisation, and ELISA tests are not used in practice, and real-time PCR was found to be most accurate and effective in a previous review (Honest 2006), and more accurate than OIA in a previous diagnostic accuracy study (Daniels 2009). Compared with real-time PCR, conventional PCR is more time-consuming and less sensitive.

Rationale

GBS is a significant health problem which can cause fatal complications in newborn babies (Heath 2004; Verani 2010a). Currently, the only strategies available to prevent EOGBS disease in newborns is to identify and treat pregnant women with antibiotics in labour (NICE 2012; RCOG 2012; Verani 2010b). Screening, in particular, is complicated by transient colonisation which means that over 30% of women could be unnecessarily treated with antibiotics in a climate of increasing antibiotic resistance (Bazian Ltd 2012; Valkenburg-van den Berg 2010).

Real-time PCR tests are available and their use in pregnant women may increase diagnostic accuracy in screening and risk-based programmes, and therefore reduce unnecessary antibiotic usage and its potential harms across countries (Daniels 2009; Honest 2006; NICE 2015). However, studies on real-time PCR have shown varying results and the search for the last systematic review on the diagnostic accuracy of real-time PCR was implemented over 10 years ago in 2005 (Honest 2006). There is a need to update the systematic review and compare real-time PCR with antenatal culture. This systematic review will aim to incorporate new evidence to compare the clinical performance of commercially available real-time PCR tests and antenatal culture tests to diagnose GBS colonisation in pregnant women in labour.

It is important to note that the outcome of most interest for a GBS screening programme is not intrapartum culture, but GBS disease in the newborn. However, we could not investigate this as neonatal GBS is a prognostic outcome that likely depends on many factors, including conditions during birth and maternal treatment.

O B J E C T I V E S

The primary objective is to compare the diagnostic accuracy of commercially available real-time polymerase chain reaction (PCR) tests and antenatal culture tests for diagnosing group B *Streptococcus* (GBS) colonisation in pregnant women during labour.

Secondary objectives

To investigate potential sources of heterogeneity in the diagnostic accuracy of the real-time PCR tests and antenatal culture tests. This will include variations in: type of intrapartum culture reference standard used, prevalence rate, and high or low GBS risk population.

M E T H O D S

Criteria for considering studies for this review

Types of studies

We will include retrospective and prospective cohort and cross-sectional studies that evaluated the diagnostic accuracy of commercially available real-time polymerase chain reaction (PCR) tests or antenatal culture tests, or both, compared with the reference standard of intrapartum culture. We will include relevant studies irrespective of whether they assessed PCR and antenatal culture alone or in combination with other tests.

We will exclude diagnostic case-control studies assessing healthy versus group B *Streptococcus* (GBS) colonised individuals, as results from such studies may overestimate diagnostic accuracy (Lijmer 1999).

Participants

We will include studies of women during pregnancy (for antenatal culture) and labour (for real-time PCR), regardless of age and ethnicity.

We will include studies conducted in any setting, typically antenatal care, labour and delivery care.

Index tests

We will include only studies of commercially available real-time PCR tests or antenatal culture tests. We will exclude studies in which antenatal culture tests were administered to pregnant women before the third trimester, as tests before the third trimester are too early to be used for detecting intrapartum GBS colonisation.

Target conditions

We will include studies that diagnose GBS colonisation in pregnant women in labour.

Reference standards

We will only include studies that used intrapartum culture to diagnose GBS colonisation in pregnant women in labour, with vaginal or vagino-rectal culture on a blood agar plate using standard or enriched media broth in a microbiology laboratory.

We will exclude studies in which intrapartum antibiotic prophylaxis was given prior to the index test or reference standard being administered.

Search methods for identification of studies

Electronic searches

Scoping searches have been undertaken to inform the development of the search strategy. An iterative procedure was used, with input from all the authors, a medical librarian, the Information Specialist from the Cochrane Pregnancy and Childbirth group, previous systematic reviews and the Cochane Handbook for Systematic Reviews of Diagnostic Test Accuracy (Deeks 2010). We will search the following bibliographic databases:

- MEDLINE (Ovid);
- MEDLINE In-Process & Other Non-Indexed Citations (Ovid);
- EMBASE (Ovid);
- Cochrane CENTRAL, DARE and HTA databases (Wiley);
- Science Citation Index and Conference Proceedings (Web of Science); and
- Cumulative Index to Nursing and Allied Health Literature (Ebscohost).

Auto alerts will be run in Medline and Embase from the date of the searches to identify relevant new studies.

The search strategy combines three sets of search terms using both text words and controlled terms through boolean operators OR within each set and then AND to combine the sets. The first set is made up of search terms for real-time PCR or culture, the second set is made up of search terms for GBS, the third set is made up of search terms for women who are pregnant or in labour. The search strategy is not limited to any date or language. Non-English language papers will be translated into English. We have adapted the search strategy to suit each database. The detailed search strategies can be found in Appendix 1. We will record the date of the search and the number of search results for each search line.

We will also search the following trial registers: ISRCTN registry, UK Clinical Trials Gateway, ClinicalTrials.gov, and the WHO International Clinical Trials Registry Platform (ICTRP).

Searching other resources

We will handsearch reference lists of included studies and relevant systematic reviews identified through the database searches. We will also invite international experts on GBS to provide further references or conference proceedings that may be additional to our included lists of references from the database searches.

Data collection and analysis

The following methods section of this protocol is based on a standard template used by the Cochrane Pregnancy and Childbirth Group.

Selection of studies

Two authors (FS, JC) will independently screen the titles and abstracts of all identified bibliographic records for relevance (screening level 1). Afterwards, the two authors will retrieve full-text reports of all potentially relevant records identified at screening level 1 and review them using the same study eligibility criteria (screening level 2). Any disagreements over inclusion or exclusion will be resolved by discussion between two review authors or arbitration by a third review author. We will document the study flow and reasons for exclusion of full-text papers in a PRISMA study flow diagram (Moher 2009).

Data extraction and management

For each included study, two authors (FS, JC) will independently extract relevant data using an a priori defined and piloted data extraction sheet using Google Forms. We will cross-check extracted data, and any disagreements will be resolved by discussion or arbitration by a third author. If information is unclear, we will contact study authors for further explanation. The data we extract will include the following.

- Study characteristics: first author, country, publication year, setting, number of sites, sources of funding, sources of trial funding, methodological quality, recruitment dates, trial authors' conflicts of interest
- Participants: method of participant selection, inclusion and exclusion criteria, number of eligible participants, number enrolled, number who received both tests, number results available, number of excluded participants, mother and gestational age, ethnicity, prevalence of GBS colonisation, number of high risk GBS, GBS case load, inclusion of elective caesarean section
- Reference standard: type of tests used for identification of GBS colonisation, timing of tests, site of tests, culture medium, laboratory transfer, definition of positivity/negativity, other methods of laboratory analysis used for derivation of test results, blinding
- Index test: type of tests used for identification of GBS colonisation (real-time PCR, antenatal culture, or both), type of real-time PCR test, timing of tests, site of tests, laboratory transfer, culture medium, target gene, DNA extraction method,

threshold cycle, definition of positivity/negativity, other methods of laboratory analysis used for derivation of test results, blinding

- Diagnostic accuracy outcomes: number of true positives, false positives, true negatives and false negatives, and number of indeterminate/invalid test results or test failures for each test

Assessment of methodological quality

Two authors (FS, JC) will independently assess the methodological quality of each study using a modified and piloted QUADAS-2 instrument (Appendix 2) (Whiting 2011). We have tailored the tool to our review question, developing review-specific guidance on how to assess each signalling question and how to use this information to judge risk of bias and applicability (Appendix 2). We will assess each signalling question as 'yes', 'no', or 'unclear' (inadequate detail presented to allow a judgement to be made) risk of bias. Each domain will then be judged to be at 'low', 'high', or 'unclear' risk of bias, based on review-specific guidance that we have developed on the sources of bias that are most important for GBS screening tests (Appendix 2). We will resolve any disagreements through consensus or through discussion with a third author. We will summarise the methodological quality assessment of included studies in a table or summary graphs (or both).

Statistical analysis and data synthesis

Using Review Manager 5 (RevMan 2014), we will plot estimates of sensitivity and specificity for each test on forest plots and in receiver-operating characteristic (ROC) space, to explore differences in test performance between studies.

Antenatal culture tests differ from real-time PCR tests in that real-time PCR tests can have different thresholds (cycles of amplification within which test results are valid), whereas antenatal culture tests do not have different thresholds. Therefore, we will use hierarchical summary receiver-operating characteristic (HSROC) models for meta-analysis to enable estimation of summary curves where between-study variation could be explained by threshold variation (Rutter 2001). Given the relationship between bivariate and HSROC models (Harbord 2007), we will also use this model to estimate summary points where studies have used a common threshold. However, if studies typically report a common threshold for real-time PCR tests, we will use the bivariate model (Chu 2006; Reitsma 2005) instead of the HSROC model for all analyses. For the summary ROC curves, sensitivities will be deduced for a fixed specificity of 95%.

To compare test accuracy, we will perform indirect comparisons using all relevant studies, as well as direct comparisons restricted to studies that compared tests in the same study population. Hierarchical meta-regression models incorporating test type as a covariate will be used for the analyses. If the HSROC model is used, we will assess the effect of test type on the accuracy, threshold and/ or shape parameters. Alternatively, if the bivariate model is used we will assess association of test type with sensitivity specificity or

both. If there are enough studies to permit fitting of more complex models, we will also assess the effect of test type on the variance parameters of the hierarchical (HSROC or bivariate) models. All the analyses will be performed by comparing the accuracy of different commercially available brands of real-time PCR test with antenatal tests. As predictive values are useful to policy makers, we will use the approach described in Bossuyt 2013 to obtain predictive values and their confidence intervals using summary estimates of sensitivity and specificity and likelihood ratios derived from the meta-analyses, together with the median GBS prevalence across studies for each target condition.

We will fit HSROC models using the NLMIXED procedure in SAS version 9.4 (SAS 2017). Although bivariate models can also be fitted using NLMIXED, we will use the meqrlogit command in Stata 15 (Stata 2017) because in our experience model convergence problems occur less frequently when fitting the bivariate model in Stata.

Investigations of heterogeneity

To formally investigate potential sources of heterogeneity, we will perform meta-regression by incorporating each covariate in a hierarchical model. If sufficient studies are available, we will address the following.

- Reference standard

For GBS colonisation at labour, the primary reference standard is intrapartum culture. However, the following parameters can affect the accuracy of culture and therefore the accuracy of the different tests with intrapartum culture: culture medium (standard versus selective) and site of culture swab (vaginal versus vagino-rectal).

- Prevalence

Diagnostic accuracy may vary with disease prevalence (Leeflang 2009), whereby better performance is shown in populations with higher prevalence, because of clinical variability in the patients or artefactual variability due to imperfections in the study design. If we include a sufficient number of studies, we will categorise GBS prevalence to represent low-risk (below 10% GBS prevalence across studies), medium-risk (10% to 30% GBS prevalence across studies) and high-risk populations (above 30% GBS prevalence across studies), and use these categories as a covariate in the models. Otherwise we will dichotomise GBS prevalence to represent low-risk (below 20% GBS prevalence across studies) and high-risk populations (20% and above GBS prevalence across studies), and use these categories as covariates in the models.

- Antenatal culture

We will investigate the effect of different parameters that may affect the accuracy of culture, similar to those shown above for intrapartum culture (culture medium and site of swab), as well as timing of the test (e.g. less than 35 weeks, 35 to 37 weeks, more than 37 weeks).

- High risk of GBS

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As test accuracy can vary with spectrum of disease, we will compare test accuracy in populations with high predisposing risk of GBS, to the general population and unspecified population, as identified in the studies. We will define high risk as populations where pregnant women have either one of the following known maternal risk factors for GBS: premature delivery, previous baby with GBS, incidental finding of GBS in current pregnancy, maternal fever, and prolonged rupture of membranes (PROM). We will define the general population where it is stated as such in the study, and for studies where nothing is stated, we will define the population as unspecified. This analysis will only be conducted if there is a sufficient number of studies that report test accuracy in populations with these high predisposing risk factors for GBS.

Sensitivity analyses

We will carry out the following sensitivity analyses to explore the robustness of the result.

- We will only include studies which used selective culture medium and vagino-rectal swabs as a reference standard for intrapartum culture.
- We will exclude studies at high or unclear risk of bias according to the QUADAS-2 assessment (especially in terms of blinding of reference standard test results, and consecutive recruitment).

Assessment of reporting bias

We will not assess reporting bias because of current uncertainties about how to address the issue in test accuracy reviews (Macaskill 2010).

ACKNOWLEDGEMENTS

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* *Indicates the major publication for the study*

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APPENDICES

Appendix 1. Search strategies

Ovid EMBASE

	Searches
1	Streptococcus agalactiae/
2	exp group B streptococcal infection/
3	strep* adj2 agalact*.ti,ab,kw.
4	strep* adj2 b.ti,ab,kw.
5	1 or 2 or 3 or 4
6	exp pregnancy/
7	exp birth/
8	exp labor/
9	exp prenatal care/
10	prenatal period/
11	intrapartum care/
12	exp obstetric delivery/
13	exp prenatal diagnosis/
14	prenatal screening/
15	pregnant woman/
16	pregnan*.ti,ab,kw.
17	birth*.ti,ab,kw.
18	(labour* or labor*).ti,ab,kw.
19	antenatal*.ti,ab,kw.
20	(prenatal* or pre-natal*).ti,ab,kw.

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21	(intrapartum* or intra-partum*).ti,ab,kw.
22	(antepartum* or ante-partum*).ti,ab,kw.
23	deliver*.ti,ab,kw.
24	6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23
25	exp nucleic acid analysis/
26	cultur*.ti,ab,kw.
27	polymerase chain reaction.ti,ab,kw.
28	pcr.ti,ab,kw.
29	medi*.ti,ab,kw.
30	exp culture medium/
31	25 or 26 or 27 or 28 or 29 or 30
32	**5 and 24 and 31**

Ovid Medline

	Searches
1	Streptococcus agalactiae/
2	strep* adj2 b.ti,ab,kw.
3	strep* adj2 agalact*.ti,ab,kw.
4	1 or 2 or 3
5	exp Pregnancy/
6	exp Parturition/
7	exp Labor, Obstetric/
8	Prenatal Care/
9	exp Prenatal Diagnosis/

(Continued)

10	exp Delivery, Obstetric/
11	Pregnancy Complications, Infectious/
12	Obstetric Labor Complications/
13	Mass Screening/
14	pregnan*.ab,kw,ti.
15	(labour or labor).ab,kw,ti.
16	antenatal*.ab,kw,ti.
17	(prenatal* or pre-natal*).ab,kw,ti.
18	(intrapartum* or intra-partum*).ab,kw,ti.
19	(antepartum* or ante-partum*).ab,kw,ti.
20	deliver*.ab,kw,ti.
21	birth*.ab,kw,ti.
22	unborn*.ab,kw,ti.
23	5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22
24	exp Nucleic Acid Amplification Techniques/
25	cultur*.ab,kw,ti.
26	exp Culture Media/
27	polymerase chain reaction.ab,kw,ti.
28	pcr.ab,kw,ti.
29	medi*.ab,kw,ti.
30	24 or 25 or 26 or 27 or 28 or 29
31	**4 and 23 and 30**

Ovid Medline In-Process & Other Non-Indexed Citations

	Searches
1	strep* adj2 b.ti,ab,kw.
2	strep* adj2 agalact*.ti,ab,kw.
3	Streptococcus agalactiae/
4	1 or 2 or 3
5	exp Pregnancy/
6	exp Parturition/
7	exp Prenatal Diagnosis/
8	pregnan*.ab,kw,ti.
9	birth*.ab,kw,ti.
10	(labour* or labor*).ab,kw,ti.
11	antenatal*.ab,kw,ti.
12	(prenatal* or pre-natal*).ab,kw,ti.
13	(intrapartum* or intra-partum*).ab,kw,ti.
14	(antepartum* or ante-partum*).ab,kw,ti.
15	deliver*.ab,kw,ti.
16	unborn*.ab,kw,ti.
17	Mass Screening/
18	exp Labor, Obstetric/
19	Prenatal Care/
20	exp Delivery, Obstetric/
21	Pregnancy Complications, Infectious/
22	Obstetric Labor Complications/
23	5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22

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24	cultur*.ab,kw,ti.
25	polymerase chain reaction.ab,kw,ti.
26	pcr.ab,kw,ti.
27	exp Nucleic Acid Amplification Techniques/
28	medi*.ab,kw,ti.
29	exp Culture Media/
30	24 or 25 or 26 or 27 or 28 or 29
31	**4 and 23 and 30**

Wiley Cochrane Database of Systematic Reviews, CENTRAL, DARE, HTA

ID Search

#1 MeSH descriptor: [Streptococcus agalactiae] this term only
#2 strep* adj2 b
#3 strep* adj2 agalact*
#4 #1 or #2 or #3
#5 MeSH descriptor: [Pregnancy] explode all trees
#6 MeSH descriptor: [Parturition] explode all trees
#7 MeSH descriptor: [Labor, Obstetric] explode all trees
#8 MeSH descriptor: [Prenatal Care] this term only
#9 MeSH descriptor: [Prenatal Diagnosis] explode all trees
#10 MeSH descriptor: [Delivery, Obstetric] explode all trees
#11 MeSH descriptor: [Pregnancy Complications, Infectious] this term only
#12 MeSH descriptor: [Obstetric Labor Complications] this term only
#13 MeSH descriptor: [Mass Screening] this term only
#14 pregnan*
#15 labour or labor
#16 antenatal*
#17 prenatal* or pre-natal*
#18 intrapartum* or intra-partum*
#19 antepartum* or ante-partum*
#20 deliver*
#21 birth*
#22 unborn*
#23 #5 or #6 or #7 or #8 or #9 or #10 or #11 or #12 or #13 or #14 or #15 or #16 or #17 or #18 or #19 or #20 or #21 or #22
#24 MeSH descriptor: [Nucleic Acid Amplification Techniques] explode all trees
#25 cultur*
#26 MeSH descriptor: [Culture Media] explode all trees
#27 polymerase chain reaction
#28 pcr
#29 medi*
#30 #24 or #25 or #26 or #27 or #28 or #29
#31 #4 and #23 and #30

Web of Science Social Science Citation Index and Conference Proceedings

#19	#3 AND #13 AND #18 *DocType=All document types; Language=All languages;*
#18	#17 OR #16 OR #15 OR #14 *DocType=All document types; Language=All languages;*
#17	TS=(medi*) OR TI=(medi*) *DocType=All document types; Language=All languages;*
#16	**TOPIC:** (pcr) *OR* **TITLE:** (pcr) *DocType=All document types; Language=All languages;*
#15	**TOPIC:** (polymerase chain reaction) *OR* **TITLE:** (polymerase chain reaction) *DocType=All document types; Language=All languages;*
#14	**TOPIC:** (cultur*) *OR* **TITLE:** (cultur*) *DocType=All document types; Language=All languages;*
#13	#12 OR #11 OR #10 OR #9 OR #8 OR #7 OR #6 OR #5 OR #4 *DocType=All document types; Language=All languages;*
#12	**TOPIC:** (unborn* or unborn*) *OR* **TITLE:** (unborn* or unborn*) *DocType=All document types; Language=All languages*
#11	**TOPIC:** (deliver*) *OR* **TITLE:** (deliver*) *DocType=All document types; Language=All languages;*
#10	**TOPIC:** (antepartum* or ante-partum*) *OR* **TITLE:** (antepartum* or ante-partum*) *DocType=All document types; Language=All languages;*
#9	**TOPIC:** (intrapartum* or intra-partum*) *OR* **TITLE:** (intrapartum* or intra-partum*) *DocType=All document types; Language=All languages;*
#8	**TOPIC:** (prenatal* or pre-natal*) *OR* **TITLE:** (prenatal* or pre-natal*) *DocType=All document types; Language=All languages;*
#7	**TOPIC:** (antenatal*) *OR* **TITLE:** (antenatal*) *DocType=All document types; Language=All languages;*
#6	**TOPIC:** (labour* or labor*) *OR* **TITLE:** (labour* or labor*) *DocType=All document types; Language=All languages;*
#5	**TOPIC:** (birth*) *OR* **TITLE:** (birth*) *DocType=All document types; Language=All languages;*
#4	**TOPIC:** (pregnan*) *OR* **TITLE:** (pregnan*) *DocType=All document types; Language=All languages;*

#3	#2 OR #1 *DocType=All document types; Language=All languages;*
#2	**TOPIC:** (strep* adj2 b) *OR* **TITLE:** (strep* adj2 b) *DocType=All document types; Language=All languages;*
#1	**TOPIC:** (strep* adj2 agalact*) *OR* **TITLE:** (strep* adj2 agalact*) *DocType=All document types; Language=All languages;*

CINAHL Ebscohost

Search Terms	Search Options
S33	S3 AND S25 AND S32
S32	S26 OR S27 OR S28 OR S29 OR S30 or S31
S31	TI medi* OR AB medi*
S30	TI pcr OR AB pcr
S29	TI polymerase chain reaction OR AB polymerase chain reaction
S28	TI cultur* OR AB cultur*
S27	(MH "Nucleic Acid Amplification Techniques+")
S26	(MH "Culture Media")
S25	S4 OR S5 OR S6 OR S7 OR S8 OR S9 OR S10 OR S11 OR S12 OR S13 OR S14 OR S15 OR S16 OR S17 OR S18 OR S19 OR S20 OR S21 OR S22 OR S23 OR 24
S24	TI unborn* OR AB unborn*
S23	TI birth* OR AB birth*
S22	TI deliver* OR AB deliver*
S21	TI antepartum OR AB antepartum
S20	TI intrapartum OR AB intrapartum
S19	(MH "Intrapartum Care+")
S18	TI pre-natal OR AB pre-natal

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S17	TI prenatal OR AB prenatal
S16	TI antenatal OR AB antenatal
S15	TI labor OR AB labor
S14	TI labour OR AB labour
S13	TI pregnan* OR AB pregnan*
S12	(MH "Health Screening")
S11	(MH "Labor Complications")
S10	(MH "Pregnancy Complications, Infectious")
S9	(MH "Delivery, Obstetric+")
S8	(MH "Prenatal Diagnosis+")
S7	(MH "Prenatal Care")
S6	(MH "Labor+")
S5	(MH "Childbirth+")
S4	(MH "Pregnancy+")
S3	S1 OR S2
S2	TI strep* adj2 agalact* OR AB strep* adj2 agalact*
S1	TI strep* adj2 b OR AB strep* adj2 b

Appendix 2. Application of QUADAS-2 for methodological quality assessment of included studies

QUADAS-2 is structured checklist consisting of 4 domains: patient selection, index test, reference standard, and flow and timing. Each domain is rated in terms of risk of bias and three of the domains are also rated in terms of concern regarding applicability to the review question. Each key domain has a set of signalling questions to help reach the judgments regarding bias and applicability. The table below outlines how QUADAS-2 will be applied in this review.

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DOMAIN 1: PATIENT SELECTION

A. Risk of Bias

Describe methods of patient selection:	
+ Was a consecutive or random sample of patients enrolled?	Yes/No/ Unclear
· Yes: if all eligible patients were enrolled; or if the authors reported that the patients were either a consecutive series or randomly selected; · No: if the authors reported that the selection was based on clinical judgement of health workers, or participation of randomly selected people in the study was low; · Unclear: if there is discrepancy between the numbers of eligible people and the number of included people, but no reasons given for that, or the selection procedure is not clearly described	
+ Did the study avoid inappropriate exclusions?	Yes/No/ Unclear
· Yes: if no patients were excluded before enrolment, OR explanation was given for excluded participants and the reason is deemed appropriate e.g. participant had an elective caesarean section, participant had intrapartum antibiotic prophylaxis before reference standard · No: if patients were excluded before enrolment and no explanation was given or the explanation is unacceptable, e.g. exclusions based on age, ethnicity, multiple pregnancies · Unclear: if insufficient information is provided to make a judgement, for example, it is unclear if or why patients were excluded	
Could the selection of patients have introduced bias?	**RISK: LOW/ HIGH/ UN- CLEAR**

B. Concerns regarding applicability

Describe included patients (prior testing, presentation, intended use of index test and setting):	
Is there concern that the included patients do not match the review question?	**CON- CERN: LOW/ HIGH/ UN- CLEAR**
· High concern: if the study population does not resemble a population that would be considered for a GBS screening programme in practice; · Low concern: if the study population does resemble a population that would be considered for a GBS screening programme in practice; · Unclear: if not reported or insufficient information is provided to decide	

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(Continued)

DOMAIN 2: INDEX TEST(S)
If more than one index test was evaluated in a study, the domain will be completed for each test.
A. Risk of Bias

Describe the index test and how it was conducted and interpreted:	

+ Were the index test results interpreted without knowledge of the results of the reference standard?	Yes/No/Unclear

· Yes: if the screening test was interpreted without knowing the reference standard intrapartum or newborn GBS culture result
· No: if the screening test was interpreted with knowledge of the reference standard intrapartum or newborn GBS culture result
· Unclear: if insufficient information is provided to decide. For example, if it was unclear whether the interpreter was blinded to the results of the reference test or if they were 'usually' blinded

+If a threshold was used, was it pre-specified?	Yes/No/Unclear

· Yes: if the methods or referenced manual state this threshold
· No: if the methods or referenced manual do not state this threshold
· Unclear: if insufficient information is provided to decide

Could the conduct or interpretation of the index test have introduced bias?	**RISK: LOW/HIGH/UNCLEAR**

B. Concerns regarding applicability

Describe who processed the real-time PCR test and interpreted the result

Is there concern that the index test, its conduct, or interpretation differs from the review question?	**CONCERN: LOW/HIGH/UNCLEAR**

Who processes the real-time PCR test and who interprets the result has important implications on its clinical applicability to labour wards
· High concern: if test can only be processed in the laboratory due to equipment required or test can be processed in labour wards but was processed in the laboratory by laboratory staff in the study
· Low concern: if midwives on labour wards processed the test and interpreted the result
· Unclear concern: if not reported or insufficient information is provided to decide

DOMAIN 3: REFERENCE STANDARD(S)
A. Risk of Bias

Describe the reference standard and how it was conducted and interpreted:

+ Were reference standard results interpreted without knowledge of the results of the index test?	Yes/No/Unclear

· Yes: if the screening test results were not known to the people interpreting the intrapartum or newborn culture reference standard results;
· No: if the screening test results were known to the people interpreting the intrapartum or newborn culture reference standard results;
· Unclear: if insufficient information is provided to decide

(Continued)

+ Is the reference standard likely to correctly identify GBS colonisation/disease?	Yes/No/Unclear

· Yes: if the reference standard was intrapartum selective enrichment culture from a vaginal and rectal swab (combined vagino-rectal or separate vaginal and rectal swabs processed together) for GBS maternal colonisation; newborn selective enrichment culture from any surface swab for neonatal GBS colonisation; newborn culture from blood, CSF, or another sterile site for GBS neonatal disease
· No: if the reference standard was intrapartum culture from a vaginal or rectal swab alone or culture without selective enrichment for GBS maternal colonisation; newborn culture was without selective enrichment for neonatal GBS colonisation; and newborn culture for GBS neonatal disease was not from a sterile site
· Unclear: if insufficient information is provided to decide

Could the reference standard, its conduct, or its interpretation have introduced bias?	**RISK: LOW/HIGH/UNCLEAR**

B. Concerns regarding applicability

Is there concern that the target condition as defined by the reference standard does not match the review question?	**CONCERN: LOW/HIGH/UNCLEAR**

DOMAIN 4: FLOW AND TIMING
A. Risk of Bias

Describe any patients who did not receive the index test(s) and/or reference standard or who were excluded from the 2x2 tables:
Describe the time interval and any interventions between index test(s) and reference standard:

+ Did all women or newborns receive the same reference standard?	Yes/No/Unclear

· Yes: if all enrolled patients in the 2x2 tables received the same reference standard
· No: if all patients in the 2x2 tables did not receive the same reference standard
· Unclear: if insufficient information is provided to decide

+ Were all enrolled patients included in the analysis?	Yes/No/Unclear

· Yes: if all enrolled participants were included in the 2x2 tables and no patients withdrew (all received the index and reference test) and data was available for all, OR reasons for withdrawal (not receiving both index and/or reference test) /missing data (e.g. invalid result or missing from records) were explained. 'Missing data' is not acceptable; needs to explain why
· No: if enrolled participants were excluded from 2x2 table but their reasons for withdrawal (not receiving both index and/or reference test) or missing data (e.g. invalid result or missing from records) are not explained. Nothing is said about potential withdrawals or if it appears that some participants who entered the study did not complete the study, and these participants are not accounted for
· Unclear: if it is unclear how many participants entered the study and thus whether there were withdrawals

Could the pa-	**RISK: LOW/HIGH/UNCLEAR**

(Continued)

tient flow have introduced bias?	

Guidance for when to score a domain as high, low, or unclear risk of bias:

Domain 1, Patient selection: Any one 'No' means that the domain is at high risk of bias, as the way participants were recruited, and who may have been excluded, are important sources of bias. Any one 'Unclear' means that the domain is at unclear risk of bias. Otherwise the domain is at low risk of bias.

Domain 2, Index test: If the question regarding threshold is scored 'No', the domain will be at high risk of bias. If the question regarding threshold is scored 'Unclear', the domain will be at unclear risk of bias. Otherwise the domain is at low risk of bias. The question on blinding is not a high concern as reference standard results cannot physically be available when interpreting the index tests.

Domain 3, Reference standard: If the question regarding blinding is scored 'No', the domain will be at high risk of bias. If the question regarding blinding is scored 'Unclear, the domain will be at unclear risk of bias. Otherwise the domain is at low risk of bias. The question on correct identification is not a high concern because there is uncertainty whether selective enrichment is required, and the impact that the different testing methods may have on accuracy will be assessed in the investigations of heterogeneity and sensitivity analyses.

Domain 4, Flow and timing: Any one 'No' means the domain is at high risk of bias, as both sources of bias are important, e.g. if some participants had an ear swab while others had a neck swab OR missing data and withdrawals were not explained. Otherwise any one 'Unclear' means that the domain is at unclear risk of bias. Otherwise the domain is at low risk of bias.

CONTRIBUTIONS OF AUTHORS

Farah Seedat drafted the first version of the protocol for this review, and all review authors made comments, edited and contributed to the final draft.

DECLARATIONS OF INTEREST

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SOURCES OF SUPPORT