New DNA-based PCR approaches for rapid real-time detection and prevention of group B streptococcal infections in newborns and pregnant women

Michel G. Bergeron and Danbing Ke

Group B streptococci (GBS) are an important cause of neonatal sepsis and meningitis, and maternal infection. Although the pathogenesis of GBS infection is not well understood, several virulence factors have been identified. Two prevention strategies have been proposed: chemoprophylaxis and immunoprophylaxis. Implementation of selective intrapartum chemoprophylaxis on the basis of either screening or risk assessment has led to a substantial decrease in the morbidity and mortality of GBS disease in both mothers and infants. Penicillin remains the antibiotic of choice with no reported resistant GBS so far, whereas resistance of 10–20% of GBS to erythromycin and clindamycin has been reported in North America. Chemoprophylaxis based on screening requires optimal detection methods for GBS, which involve selective broth culture of combined vaginal and anal samples. Other conventional methods are useful for rapid identification of heavily colonised women, but are unreliable for the detection of light GBS colonisation because of poor sensitivity. GBS-specific polymerase chain reaction (PCR) assays using real-time PCR coupled with fluorescence-labelling technology offer powerful tools for sensitive and specific, yet rapid (less than 1 h), detection of GBS directly from clinical specimens at the time of delivery. The application of these assays to the current prevention strategies will simplify the prevention practice and rationalise the use of antibiotics. Immunoprophylaxis relies on the development of new vaccines against GBS, and active research is being conducted in this area.

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Group B streptococci (GBS), *Streptococcus agalactiae*, are Gram-positive bacteria occurring in pairs and short chains (Ref. 1). *S. agalactiae* was originally investigated as a frequent cause of bovine mastitis (Ref. 2); before the widespread use of penicillin, it accounted for as much as 90% of all bovine mastitis. It was first isolated from parturient women in 1935 (Ref. 3). Since the 1970s, GBS have emerged as important human pathogens and have remained the major cause of bacterial diseases in newborns in the western world (Ref. 4). In 1990, there were approximately 7600 neonatal cases of invasive GBS disease in the USA (Ref. 5). Since then, intense intrapartum use of antibiotics has decreased the incidence of neonatal early-onset GBS disease, to 2200 cases in 1998 (Ref. 6). Babies can be exposed to GBS during labour and delivery, when they swallow or inhale the bacteria while passing through the birth canal of the colonised mothers. Babies can also be exposed in utero. GBS infection in infants causes sepsis and meningitis, which can result not only in illness and death but also in long-term disabilities such as hearing loss, impaired vision, developmental problems and cerebral palsy (Ref. 4). The direct costs of neonatal disease alone in the USA have been estimated at 300 million dollars annually (Ref. 7). GBS is also an important cause of maternal illness and a risk factor for preterm delivery (Refs 6, 8).

Clinical manifestation

GBS infection in infants can present as bacteraemia, septicaemia, pneumonia, skin infection and meningitis. Two distinct forms of disease are classified by the time of onset in infants. Most cases occur within the first week of life, and are termed early-onset disease. Cases that occur between 7 and 90 days of age are termed late-onset disease. Of the cases occurring in the first week of life, almost 80% occur in the first day (Ref. 3).

Although GBS colonisation does not cause disease in healthy women, GBS can cause infections in pregnant women. Most invasive maternal infections are bacteraemia. Other infections include urinary tract infections, chorioamnionitis (infection of the foetal membranes), endometritis (chronic inflammation of the endometrium), and wound infections (Refs 4, 6, 9).

Epidemiology of GBS

Incidence of GBS colonisation

Most early-onset GBS disease among newborns results from maternal-to-infant transmission during labour and delivery (Ref. 4). At any given time, 15–35% of healthy adult women are colonised by GBS in the genital and gastrointestinal tracts (Ref. 4). GBS colonisation is intermittent: approximately a third of women who had positive cultures at midpregnancy were no longer colonised at delivery, whereas 5–15% of women who had positive cultures at delivery were not colonised in midpregnancy (Ref. 10). Ethnicity is associated with the likelihood of GBS colonisation. Black women are colonised at a higher rate than are women in other ethnic groups (Refs 11, 12). A recent survey suggested that although there is some geographical variation in GBS colonisation in developing countries, the range is similar to that in the developed countries (Refs 13, 14). Previously observed lower colonisation rates in developing countries might therefore have resulted from inappropriate specimen collection and microbiological methodologies rather than an epidemiological factor.

Morbidity and mortality of GBS infection

Acquisition of GBS from the colonised mother is a prerequisite for the development of neonatal early-onset disease. Approximately 25% of pregnant women are colonised by GBS. Half of them might pass GBS to their infants, and of all infants born to colonised mothers, approximately 1–2% will develop early-onset invasive disease (Ref. 14). The reported incidence of early-onset GBS disease is 1–4 per 1000 live births in the USA (Ref. 14). Similar morbidity and mortality rates have been reported from Australia, the UK and Finland (Refs 15, 16, 17, 18). A higher incidence of neonatal early-onset disease is also associated with black race and mothers younger than 20 years of age (Ref. 11).

Distribution of GBS serotypes

GBS are classified on the basis of type-specific capsular polysaccharides (CPSs). To date, nine serotypes have been identified: Ia, Ib, II, III, IV, V, VI, VII and VIII (Ref. 19). Some of these CPSs have identical structures to polysaccharides found in the host, so they tend not to be immunogenic (Ref. 20). Lack of antibodies to these type-specific CPS antigens is a crucial factor in the risk of development of GBS disease (Ref. 21). The presence of surface proteins, such as C protein, R protein and X protein, provides additional information for the classification of GBS strains (Ref. 22). The predominant serotypes have

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changed over time and vary in different geographic regions, and can be associated with different forms of the disease. For instance, a recent study demonstrated the decline of type III GBS infection and the emergence of type V GBS infection (Refs 23, 24). Several surveillance studies have reported a high prevalence of erythromycin- and clindamycin-resistant strains among serotype V GBS isolates in the USA and Canada (Refs 25, 26). It is speculated that the emergence of type V GBS in the USA might be due to a selective pressure created by frequent use of macrolide antibiotics such as erythromycin during pregnancy (Ref. 25). Many studies have shown a dominant distribution of serotypes Ia, Ib, II, III and V among infants and pregnant women in North America (Refs 25, 27), while other studies have revealed that GBS strains of serotypes VI and VIII are common vaginal isolates from pregnant women in Japan (Ref. 19). Type III GBS are more often identified from infants with late-onset disease and cause the majority of meningitis cases (Ref. 28).

Pathogenesis of GBS disease

Although little is known about the mechanisms by which GBS cause disease, several virulence factors have been identified. Among them, the type-specific CPSs have been well studied as components that inhibit opsonophagocytic killing of GBS by human hosts, leading to an increase in virulence (Ref. 29). In addition, most GBS strains carry C5a-ase, an enzyme of the serine esterase class that inactivates complement component C5a. The presence of this enzyme reduces the accumulation of potent chemoattractants for polymorphonuclear leukocytes, subsequently minimising acute focal inflammatory responses (Ref. 30). Finally, GBS β-haemolysin causes damage to lung microvascular endothelial cells both in vitro and in vivo, and might contribute to the pathogenesis of alveolar oedema and haemorrhage in early-onset GBS pneumonia (Ref. 31). Efforts are being made at the GBS genome level to provide more insight into the pathogenesis of GBS disease, both through complete genome sequence analysis of the 2 Mb *S. agalactiae* genome (Ref. 32) and by mutagenesis studies to identify genes required for growth and survival (Ref. 33). Winram et al. (Ref. 34) have demonstrated that GBS can invade chorion cells, but not amnion cells, in vitro, and are able to transcytose through intact chorion cell monolayers without disruption of intercellular junctions.

Different transmission routes are involved in different clinical syndromes of GBS infections. In most cases, neonatal GBS early-onset disease results from aspiration of contaminated amniotic fluid or acquisition of the bacteria during passage through the birth canal (Ref. 4). The same mechanism of maternal-to-infant transmission probably also causes late-onset disease (Ref. 4). The development of late-onset disease has also been linked to nosocomial infection (i.e. infection contracted in a healthcare institution) and breastfeeding (Refs 35, 36).

Maternal transfer of adequate concentrations of antibody against type-specific CPSs to newborns is important to protect against GBS infection (Ref. 37). The fact that women younger than 20 years of age are more likely to have low serum concentrations of IgG specific to CPS antigens might explain the increased risk of GBS disease in infants born to younger mothers (Ref. 27).

Methods available for GBS identification

Selective culture of GBS

GBS are components of the microbiologically complex normal flora in the human vagina and rectum; therefore, GBS-selective broth media supplemented with antibiotics that inhibit growth of non-GBS organisms have been developed to improve GBS recovery from clinical samples (Ref. 38). The US Food and Drug Administration (FDA) has issued a statement recommending their use as the standard means of identifying GBS from prenatal specimens (Ref. 39). Because the sensitivity of selective culture methods for detection of GBS colonisation depends on the timing and method of collection and on the processing of the specimens, the protocols recommended by the US Centers for Disease Control and Prevention (CDC) should be strictly followed (Ref. 40).

In addition to the use of selective broth medium, an optimal culturing technique includes culturing both the vaginal and the anal samples (combined) at 35–37 weeks of gestation. Either a single swab or two swabs can be cultivated in a single selective broth culture. Specimens should come from the lower third of the vagina, and the anal swab should pass through the anal sphincter. A good laboratory algorithm includes use of selective broth medium (Todd–Hewitt broth supplemented either with colistin and nalidixic acid or with gentamicin and nalidixic acid) and incubation for 18–24 h, followed by inoculation

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onto sheep-blood agar and incubation for an additional 18–24 h. If no GBS are identified, the plate should be re-incubated for an additional 24 h (Ref. 41). Culturing GBS to sufficient numbers to carry out subsequent identification can therefore take 2–3 days.

Presumptive identification of GBS

GBS are Gram-positive cocci and appear in pairs and chains on Gram-stained smears from cultured clinical specimens. On sheep-blood agar, GBS form a unique ring-like zone of β-haemolysis around the colony. However, a small proportion of GBS (3–11%) can be nonhaemolytic (Ref. 21). The CAMP test (named after Christie, Atkins and Munch-Petersen) is used for the presumptive identification of GBS, yielding positive results for up to 98% of GBS isolates (Ref. 21). It is based on the fact that GBS produce an extracellular protein called CAMP factor. This protein acts synergetically with *Staphylococcus aureus* β-toxin to hydrolyse red blood cells. However, a small percentage of group A streptococci and some strains of *Listeria monocytogenes* are also CAMP-positive (Ref. 21).

Two other methods have been used for the presumptive identification of GBS. First, a physiological characteristic of GBS is the ability to hydrolyse sodium hippurate. Approximately 96% of GBS are able to hydrolyse hippurate and this has been used as an identifying factor (Ref. 42). Second, a selective medium (known as Granada medium) has been used on the basis that GBS produce a red–orange carotenoid pigment when grown anaerobically on starch-containing medium (Ref. 43). Pigment is produced in 93.0–98.5% of GBS human isolates (Ref. 44).

Immunological methods

To date, all GBS strains identified have been found to possess group B antigen of the Lancefield classification. Many latex agglutination tests and immunoassay methods that test for this antigen have been developed and are commercially available for GBS identification (Ref. 45). The ability to identify GBS by these methods depends on the amount of the bacteria inoculated in the assay. Their sensitivities for detection of GBS directly from clinical specimens varied from 19% to 82% when selective broth media were used to recover GBS from clinical samples (Ref. 45). In general, these immunological tests are not sufficiently sensitive for the direct detection of GBS from clinical samples, and only women with heavy colonisation can be readily identified by these methods (Refs 46, 47).

Hybridisation-based methods

The most widely used hybridisation-based test to date is the Accuprobe Group B Streptococcus Identification Test (Gen-Probe, San Diego, CA, USA). The test is intended to identify GBS from cultures. Although this test can be performed in about 45 min (Ref. 48), a pre-incubation period of 18–24 h in selective broth media to allow growth amplification is needed to achieve a satisfactory test sensitivity (Ref. 49). Compared with the standard culture described above, the sensitivity of this test is 94.7–98.2%, and the specificity is 98.4–100%. When shorter pre-incubation periods were used, the sensitivity of this test was 44% for a 2.5 h incubation (94% specificity) and 71% for a 3.5 h incubation (90% specificity) (Ref. 48). Bourbeau et al. (Ref. 49) demonstrated significant potential labour savings utilising this product compared with the traditional culture methods.

Another test based on DNA hybridisation is the Affirm GBS Microbial Identification System (MicroProbe, Bothell, WA, USA) (Ref. 50). Although it is highly specific and can be performed in about 50 min, this test had only 8.3% sensitivity in the direct identification of colonised women. After a 16–24 h pre-incubation, the sensitivity of the test increased to only 81%. Such a test does not offer any advantages over culture methods (Ref. 50) and has never been commercialised.

Conventional PCR-based methods

Polymerase chain reaction (PCR)-based assays offer promising tools for sensitive, specific and rapid detection of GBS directly from clinical specimens at the time of delivery, bypassing the need for lengthy culture of GBS. PCR assays for identification of GBS have been developed by targeting a variety of genetic targets, including genes encoding C protein (Ref. 51), the 16S rRNA (Refs 52, 53), and the 16S–23S spacer region (Ref. 54). We have developed a GBS-specific PCR assay that targets the *cfb* gene, which encodes the CAMP factor (Fig. 1) (Refs 55, 56). This PCR assay allows the detection of as little as one genome copy of GBS. In addition to its excellent performance with purified genomic DNA samples, the PCR assay achieves almost the same sensitivity as standard culture methods when vaginal and/or anal specimens from pregnant women are tested for GBS colonisation (Table 1) (Ref. 56).

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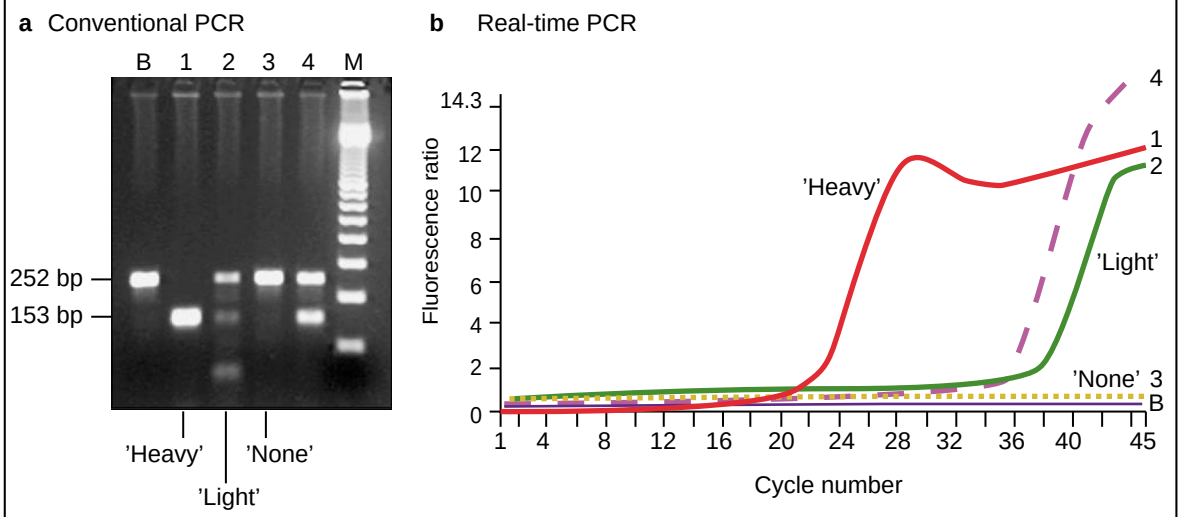

Figure 1. A new polymerase chain reaction-based assay for detecting group B streptococci. The figure compares results of (a) a conventional PCR assay and (b) a new, real-time PCR assay, for the detection of group B streptococci (GBS) in combined vaginal and anal specimens from pregnant women. In each panel, sample 1 was obtained from a woman heavily colonised with GBS ('heavy'); sample 2 was obtained from a lightly colonised woman ('light'); sample 3 was obtained from a woman with no colonisation ('none'); sample 4 was a positive control, to which 10 fg of purified GBS genomic DNA had been added; and sample B was a negative control, to which no target DNA had been added. (a) In the conventional PCR assay, the product of GBS-specific amplification (targeting the *cfb* gene) is 153 bp, whereas the 252 bp product represents the internal-control amplicon (exogenous DNA template to monitor the efficiency of PCR amplification; this amplicon is not detected in sample 1 because of competitive inhibition as a result of the high level of GBS in the sample). Lane M shows a 100 bp ladder molecular-size standard. (b) In the new, real-time PCR assay, the extent of GBS-specific amplification is measured in terms of increased fluorescence signal generated by the hybridised adjacent probe during the amplification process. Adapted, with permission, from Ref. 56. **(fig001mbl)**.

Although 2% of GBS isolates were phenotypically negative for the CAMP test (Ref. 21), molecular characterisation of the *cfb* gene showed that this gene was present in virtually every isolate (Ref. 57). Thus, the PCR assay targeting this gene provides the basis for a specific and sensitive GBS test for further development. Two developments of this methodology are described below.

Real-time PCR-based methods

Advances in fluorescence measurements [light- emitting diode (LED) technology] have allowed the development of reasonable-cost instrumentation coupled with PCR technology for real-time detection of specific PCR products (Refs 58, 59). This real-time PCR technology offers several advantages over conventional PCR methods, such as a lower risk of PCR contamination, shorter turnaround time, and quantitative PCR analysis (Ref. 60). Some of the most popular commercially available systems coupling PCR technology with real-time detection of PCR products include the ABI PRISM 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA, USA), the LightCycler™ (Idaho Technologies, Idaho Fall, ID, USA; and Roche Diagnostics, Indianapolis, IN, USA), and the Smart Cycler® (Cepheid, Sunnyvale, CA, USA). GBS-specific PCR assays have been developed using the latter two platforms.

GBS-specific real-time PCR with the LightCycler™

The LightCycler™ is an air thermocycler with a built-in fluorimeter for real-time fluorescence

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Table 1. Evaluation of GBS-specific PCR assays for direct detection of GBS colonisation from vaginal/anal swabs in 112 parturient women by comparison with standard culture[a] (tab001mbl)

PCR method	Sensitivity[b]	Specificity[c]	Positive predictive value[d]	Negative predictive value[e]
Conventional	97% (32/33)	100% (79/79)	100% (32/32)	98.8% (79/80)
Real-time	97% (32/33)	100% (79/79)	100% (32/32)	98.8% (79/80)

[a] The standard culture method for group B streptococci (GBS) was performed as follows: a vaginal/anal swab was first cultivated in a broth medium containing the antibiotic gentamicin to allow for selective growth of GBS after 18–24 h of incubation. The culture was then passaged onto sheep-blood agar to isolate bacterial colonies after 18–24 h of incubation. Suspected colonies were then verified by the latex agglutination test, which specifically detects the presence of group B antigen. Conventional PCR used the PTC-200 thermocycler, with detection by agarose gel electrophoresis. Real-time PCR was performed using the LightCycler™. Sensitivity, specificity, and positive and negative predictive values are used to illustrate the correlation between the culture- and the PCR-based methods. This table is reproduced, with permission, from Ref. 56. Copyright © 2000 Massachusetts Medical Society. All rights reserved.
[b] Sensitivity represents number of both culture- and PCR-positive results divided by number of culture-positive results.
[c] Specificity represents number of both culture- and PCR-negative results divided by number of culture-negative results.
[d] Positive predictive value represents number of both culture- and PCR-positive results divided by number of PCR-positive results.
[e] Negative predictive value represents number of both culture- and PCR-negative results divided by number of PCR-negative results.

detection. The commercialised instrument was first described by Wittwer et al. (Ref. 58) from Idaho Technologies and is now manufactured by Roche Diagnostics. An important advantage of the LightCycler™ is its rapid temperature transition rate of up to 20°C per second, allowing completion of 45 cycles of amplification in 20–30 min (Ref. 61).

The GBS-specific real-time PCR assay was based on an adjacent hybridisation probe system. Adjacent probes are pairs of fluorescently labelled probes separated by a single nucleotide, which allow fluorescence resonance energy transfer to generate an increased fluorescence signal when both probes are hybridised to their target sequences. Fluorescence detection at each cycle enables detection of GBS-specific PCR products (Fig. 1). This real-time PCR assay developed in our laboratory allowed rapid (in under 45 min) identification of GBS directly from vaginal/anal swabs (Refs 55, 56). To achieve such speed, an extremely rapid sample-processing and DNA-extraction protocol (10 min) from Infectio Diagnostics (I.D.I.) Inc. (Sainte-Foy, Quebec, Canada) was used (Refs 55, 56). This PCR assay was able to identify pregnant women colonised by GBS in 30–45 min (Fig. 2), with a sensitivity of 97% and a specificity of 100% as compared with the standard culture method (Table 1) (Ref. 56).

GBS-specific real-time PCR with the Smart Cycler®

The Smart Cycler® is a miniature analytical thermal cycling instrument designed for real-time PCR; it was first described by Northrup et al. (Ref. 59) and is manufactured by Cepheid. The system is designed to allow low-power operation, high-temperature transition speed and thermal uniformity (Ref. 62).

We have also adapted the conventional GBS-specific PCR assay described above to a real-time PCR format using the Smart Cycler® [Bergeron, M.G. et al. (2000) Rapid PCR detection of group B streptococci in pregnant women from vaginal/anal specimens using the Smart Cycler®, American Society for Microbiology (ASM) 100th General Meeting, Abstract #C-19]. Detection of the GBS-specific amplicon was based on the molecular beacon probe technology, which was first described by Tyagi and Kramer (Ref. 63). In this prospective study, the Smart Cycler® PCR assay identified all 32 GBS carriers among 106 pregnant women studied, with a sensitivity of 100% and a specificity of 97.3% as compared with

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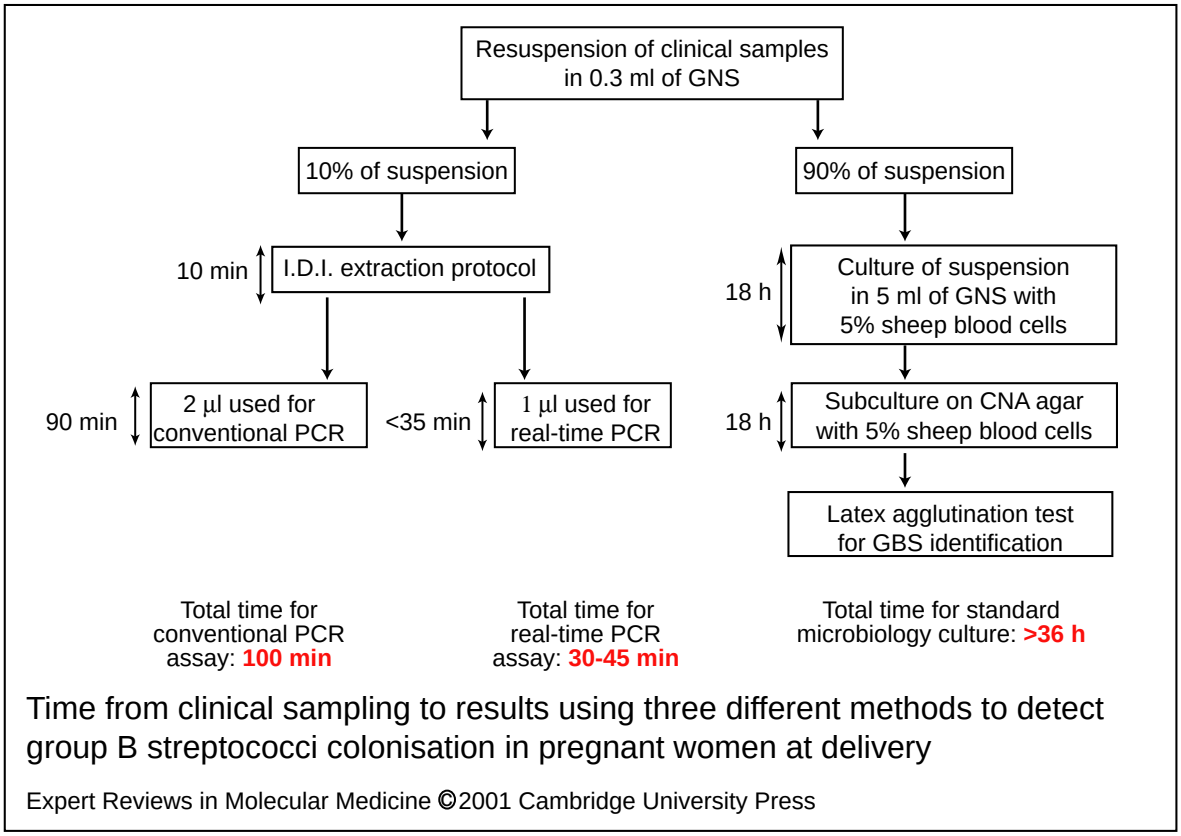

Figure 2. Time from clinical sampling to results using three different methods to detect group B streptococci colonisation in pregnant women at delivery. Culture methods, conventional PCR and real-time PCR for the analysis of anal and/or vaginal swabs obtained at delivery are compared in the figure. For the identification of group B streptococci (GBS) by standard microbiology culture, the specimens were incubated in GNS broth medium [Todd–Hewitt broth supplemented with gentamycin (8 µg/ml) and nalidixic acid (15 µg/ml)] and then on CNA (Columbia colistin nalidixic acid) agar, and a minimum of 36 h was required for GBS to be identified. The PCR protocols involve DNA extraction and sample preparation using the Infectio Diagnostics (I.D.I.) protocol and reagents. Results were obtained in 100 min for conventional PCR, and in 30–45 min for real-time PCR **(fig002mbl)**.

the standard culture method. The assay gave results in under 45 min, again using the rapid sample-processing and DNA-extraction protocol from Infectio Diagnostics. Compared with the assay using the LightCycler™, the Smart Cycler® assay offers more operation flexibility because it has 16 independently controlled sites that can accommodate different assays performed simultaneously or sequentially, thus allowing random access.

Prevention of GBS infection

Since the impact of GBS disease in infants and pregnant women was first recognised, great efforts have been made to treat and control this infection (Ref. 4). Two approaches have been proposed to prevent infection with GBS: chemoprophylaxis to minimise colonisation of the mother and so prevent colonisation of newborns; and immunoprophylaxis to induce protective immunity in mothers and newborns (Ref. 41).

Current recommendations for prevention of GBS infection: chemoprophylaxis

In an effort to prevent perinatal GBS disease, consensus guidelines have been issued by the American Academy of Pediatrics (Ref. 64), the American College of Obstetrics and Gynecology (Ref. 65), and the CDC (Ref. 41). These guidelines recommend selective intrapartum chemoprophylaxis based on either positive GBS identification from late prenatal screening cultures or the presence of intrapartum risk factors alone. Such risk factors include preterm

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delivery, rupture of membranes longer than 18 h before onset of labour, intrapartum fever of >38°C, having a previous infant with GBS disease, or GBS bacteriuria during the pregnancy (Ref. 41). The screening-based approach recommends that all pregnant women should be screened at 35–37 weeks of gestation for vaginal/anal GBS colonisation. All women with a positive culture result should be offered intrapartum antibiotics as early in labour as possible. For women with a negative culture result, no prophylactic antibiotics are recommended. In the risk-factor approach, screening for GBS is not done, but intrapartum antibiotics are given if any of the risk factors are identified.

Compared with earlier approaches, the guidelines use prenatal screening optimally. By choosing late prenatal screening, there should be fewer false-negative results (women with negative prenatal cultures who then become colonised by the time they deliver). In addition, there should be less pressure for clinicians to treat carriers with oral antibiotics in the antepartum. The strategy offers antibiotics during labour to all carriers, which was expressly preferred by many consumers and clinicians. It also allows prophylaxis to start earlier (before complications develop, such as fever or prolonged membrane rupture), which might lead to higher efficacy, or fewer antibiotic failures (Ref. 41).

The risk-based approach also has several advantages. Because it avoids routine screening, the risk-based approach is easier and potentially less expensive. It is also particularly useful in settings in which women might receive little prenatal care and might not be screened for GBS (Ref. 41).

Although the screening-based approach is more effective than the risk-based approach, concerns about the complexity of the screening-based approach are a barrier to its wider application. One of these concerns is the speed and accuracy of prenatal culture methods, which have to be carried out 3–5 weeks before delivery. Even with optimal methods, it is still impossible to identify all GBS carriers during labour and a small portion of colonised women are untreated due to intermittent colonisation of GBS in pregnant women. The GBS-specific real-time PCR assay provides a rapid and sensitive alternative for GBS detection directly from clinical specimens. The PCR assay is rapid enough (it can be performed in under 45 min) to allow the detection of GBS colonisation during labour and a better management of colonised mothers and their infants. A targeted antibiotic treatment will then be limited to GBS carriers, reducing antibiotic-related adverse reactions to the minimum.

The current recommendation for intrapartum prophylaxis is intravenous administration of penicillin G (Ref. 41). Ampicillin is an acceptable alternative because it crosses the placenta more rapidly and achieves higher concentrations in the foetal compartment. However, because of its broader spectrum against various bacteria, resistant strains are likely to emerge. For individuals allergic to penicillin, clindamycin or erythromycin may be used (Ref. 41).

Implementation of selective intrapartum chemoprophylaxis has been associated with a falling incidence of early-onset GBS disease in the USA and Australia (Refs 6, 16). However, excessive use of antibiotics might lead to increased numbers of serious adverse effects, the emergence of perinatal infections caused by antimicrobial-resistant pathogens, and a complicated evaluation and management of newborns whose mothers were exposed to intrapartum antibiotics (Ref. 66). Towers et al. (Ref. 67) reported that the increased incidence of early-onset neonatal disease with non-GBS organisms that are resistant to ampicillin might be attributable to the increased administration of ampicillin to pregnant women. Although no emerging resistance to penicillin was noted, the increase of resistance to erythromycin and clindamycin in GBS isolates from neonates has been widely observed (Refs 25, 26).

Immunoprophylaxis

A practical alternative to chemoprophylaxis might be immunisation (Ref. 37). In theory, a GBS vaccine could offer great protection against both early-onset and late-onset GBS disease in infants, as well as against maternal GBS infection (Ref. 68). Such a vaccine would need to elicit a systemic immune response with a sufficient level of antibodies specific for the several type-specific CPSs underlying GBS infections. If produced in sufficient amounts, these antibodies could be transferred through the placenta from immunised mothers to their infants, preventing both mothers and infants from developing GBS disease.

Human vaccine trials with CPSs of types Ia, II and III have shown low and variable levels of CPS-specific antibodies following immunisation of healthy adults (Ref. 68). To enhance their

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immunogenicity, different types of CPSs have been coupled to tetanus toxoid (TT), a common protein carrier for conjugate vaccines (Ref. 37). These CPS–TT conjugate vaccines can induce higher immune responses than uncoupled CPS vaccines in healthy women (Refs 69, 70, 71). Phase I (safety and immunogenecity) and Phase II (dosing) trials with polysaccharide–conjugate vaccines of serotypes Ia, Ib, II and III have been successfully completed (Refs 69, 70, 71). Since protection by CPS vaccination is serotype-specific and accumulation of TT might decrease the efficiency of vaccination, the use of a GBS multivalent vaccine against major CPS serotypes would be necessary for effective protection of pregnant women against GBS infections (Ref. 72). Such a multivalent vaccine has demonstrated protective efficacy against GBS infection in a mouse model based on maternal immunisation and neonatal challenge, and might prove to be an effective maternal vaccine to protect against GBS infection in humans (Ref. 73). Because the serotype distribution of GBS infection varies with different geographic regions and time, the multivalent formulation might need to be varied to provide better immunisation against potential GBS infection (Ref. 68).

Gravekamp et al. (Ref. 72) have used the alpha C protein as a carrier protein coupled with type III CPS to form a conjugate vaccine, which could be protective against most GBS infections since the alpha C protein is present in approximately 70% non-type-III GBS strains. This vaccine might be an alternative to the multivalent CPS–TT conjugate vaccines for GBS.

Concluding remarks and research in progress

An increased awareness of the impact of GBS early-onset disease has led to great efforts in the treatment and prevention of GBS infection. Recognition of the nature of maternal-to-infant transmission, the efficacy of intrapartum maternal chemoprophylaxis and the optimised detection methods for identification of GBS carriers allow for an efficient way to manage infections associated with GBS. Implementation of consensus guidelines by the CDC, the American College of Obstetrics and Gynecology and the American Academy of Pediatrics is likely to be associated with a substantial decline in the incidence of GBS disease in newborns. In addition, the commercialisation of GBS-specific real-time PCR assays will speed up early detection of infections and will likely reduce the burden of early-onset disease. The real-time PCR assays are simple enough for even inexperienced technicians to perform. Up to now, only biochemistry and haematology laboratories could give results that were available to clinicians within 1 h. In the future, as these real-time PCR tests become available, microbiology laboratories will deliver precise microbial identification in 30–45 min instead of 2–3 days, enabling clinicians to manage their patients appropriately and immediately. This approach will revolutionise the practice of not only obstetrics in the case of GBS, but also medicine as a whole as most microbes and their antimicrobial-resistance patterns will soon be detectable in real-time.

As mentioned in the CDC recommendation, chemoprophylaxis for prevention of GBS disease has some limitations, including the emergence of antibiotic-resistant bacteria and the occurrence of side effects associated with the administered antibiotics (Ref. 41). The continued development of strategies based on active maternal immunisation would seem to offer a better solution for prevention of GBS disease. A GBS vaccine could protect infants from not only early-onset but also late-onset GBS disease, as well as protect colonised mothers from maternal complications associated with GBS infection (Ref. 68). In addition, immunoprophylaxis is generally more cost-effective than chemoprophylaxis (Ref. 7), although it can also be difficult to implement during pregnancy because of fear of adverse outcomes from manufacturers, obstetricians and pregnant women themselves. The GBS genome sequencing project is currently in the phase of shotgun sequencing (Ref. 32). Once the genome sequence is available, new clues about GBS pathogenesis might emerge and new genetic targets for molecular diagnostics might be identified. In addition, the functional study and comparative genomic analysis of GBS will identify new candidate targets for vaccine development.

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Further reading, resources and contacts

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Electronic resources

The website of the US Centers for Disease Control and Prevention covers many health topics, including this page describing group B streptococcal disease. It provides news, and links to related resources.

http://www.cdc.gov/ncidod/dbmd/gbs

The website of MEDLINEplus Health Information, a service of the US National Library of Medicine, provides many links to information on streptococcal infections regarding diagnosis, research and clinical trials.

http://www.nlm.nih.gov/medlineplus/streptococcalinfections.html

Several websites describe principles and applications of systems used in GBS detection (Molecular Beacon technology, Taqman™ detection system of Applied Biosystems, Smart Cycler® technology, and LightCycler™ technology).

http://www.molecularbeacons.com

http://www.appliedbiosystems.com/products/productdetail.cfm?id=42

http://www.smartcycler.com

http://biochem.roche.com/lightCycler

Infectio Diagnostics (I.D.I.) Inc. is developing the GBS rapid detection kit.

http://www.infectio.com

Associations/societies

The Group B Strep Association was founded by parents of children who died of GBS disease. The association aims to provide information and to create increased public awareness about the problems associated with GBS infection during pregnancy.

http://www.groupbstrep.org

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Features associated with this article

Figures

Figure 1. A new polymerase chain reaction-based assay for detecting group B streptococci (fig001mbl).
Figure 2. Time from clinical sampling to results using three different methods to detect group B streptococci colonisation in pregnant women at delivery (fig002mbl).

Table

Table 1. Evaluation of GBS-specific PCR assays for direct detection of GBS colonisation from vaginal/anal swabs in 112 parturient women by comparison with standard culture (tab001mbl).

Citation details for this article

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