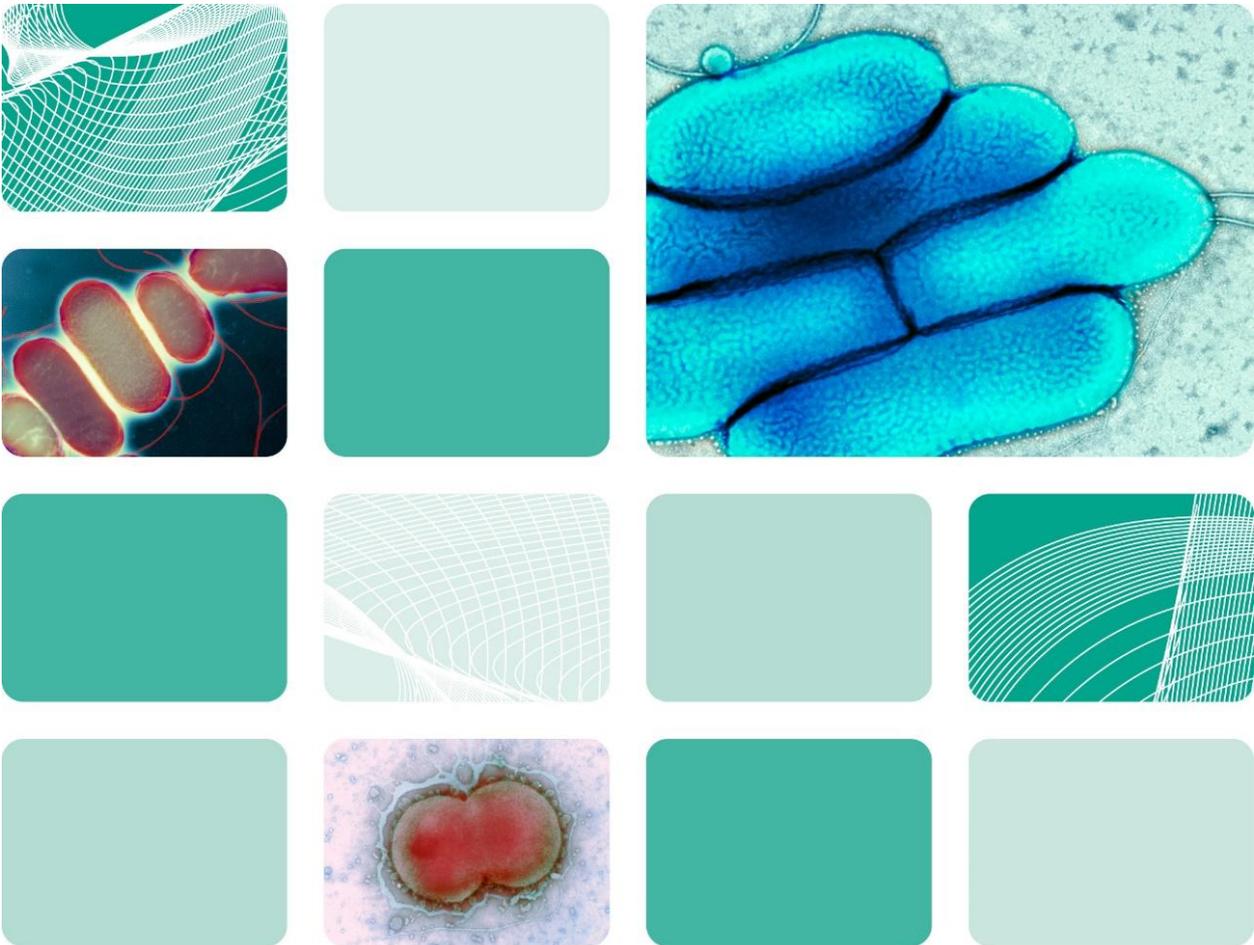




UK Health  
Security  
Agency

## UK Standards for Microbiology Investigations

Detection of carriage of group B streptococci  
(*Streptococcus agalactiae*)



## Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of UKHSA working in partnership with the partner organisations whose logos are displayed below and listed on [the UK SMI website](#). UK SMIs are developed, reviewed and revised by various working groups which are overseen by a [steering committee](#).

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UK SMIs are produced in association with:

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## Amendment table

Each UK SMI document has an individual record of amendments. The amendments are listed on this page. The amendment history is available from [standards@ukhsa.gov.uk](mailto:standards@ukhsa.gov.uk).

Any alterations to this document should be controlled in accordance with the local document control process.

Amendment number/date	8/06.02.26
Issue number discarded	3.1
Insert issue number	3.2
<b>Section(s) involved</b>	<b>Amendment</b>
Whole document.	<p><b>This is an administrative point change.</b></p> <p><b>The content of this UK SMI document has not changed.</b></p> <p><b>The last scientific and clinical review was conducted on 26/06/2018.</b></p> <p>Hyperlinks throughout document updated to Royal College of Pathologists website.</p> <p>Public Health England replaced with UK Health Security Agency throughout the document, including the updated Royal Coat of Arms</p> <p>Partner organisation logos updated.</p> <p>Broken links to devolved administrations replaced.</p> <p>References to NICE accreditation removed.</p> <p>Scope and Purpose replaced with General and Scientific information to align with current UK SMI template.</p> <p>'Public health responsibilities of diagnostic Laboratories' section added.</p>

Amendment No/Date.	7/26.06.18
Issue no. discarded.	3
Insert Issue no.	3.1
<b>Section(s) involved</b>	<b>Amendment</b>

Whole document.	<p>Title amendment to add “<i>Streptococcus agalactae</i>”.</p> <p>Content, references and hyperlinks updated to reflect current policy of the UK National Screening Committee and guidance from the National Institute for Health and Care Excellence and the Royal College of Obstetricians &amp; Gynaecologists which recommends the circumstances in which the screening may be helpful in determining the risk of developing early-onset neonatal GBS (EOGBS) or in examining cases of late-onset neonatal GBS.</p> <p>Specimen updated to be more specific to maternal samples.</p>
1.1 Specimen collection, transport and storage.	Amies or Stuart medium for transport added.
4.5.1 Culture media, conditions and organisms.	Gentamicin and nalidixic acid combination added as a LIM Broth option.
4.7 Antimicrobial susceptibility testing.	Antimicrobial susceptibility testing and reporting table added.
4.9 Referral to reference laboratories.	Updated sentence to: “Refer all GBS which are associated with an infection control or cluster investigation in addition to all invasive isolates.”
Appendix.	Updated.

Amendment No/Date.	6/19.06.15
Issue no. discarded.	2.3
Insert Issue no.	3
<b>Section(s) involved</b>	<b>Amendment</b>
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Whole document.	Emphasis on availability of this GBS detection method for laboratories to undertake when required and does not cut across the UK National Screening Committee recommendation that antenatal screening for GBS colonisation is not recommended.

Detection of carriage of group B streptococci (*Streptococcus agalactiae*)

	Any mention of screening pregnant women for colonisation of GBS has been removed to minimise ambiguity.
Title.	The title of the document has been changed from 'Processing swabs for Group B streptococcal carriage' to 'Detection of carriage of Group B streptococci'.
Scope.	Amended to make the scope and purpose of the document clear.
Introduction.	Re-structured to present the information clearly.
Colonisation.	Colonisation of GBS amended from up to 30% to up to 28% and referenced.
Infection.	Updated. Reference to a 1998 Working Group removed.
Method of investigation.	Updated. The use of selective agar for subculture from enrichment broth added. Collection of recto-vaginal swabs between 35 and 37 weeks gestation and USA guidelines has been removed.
Treatment.	The section is outside the scope of the document and has been removed.
Rapid methods.	Section added.
Specimen collection.	Updated for clarity. Type of manufactured swabs covered. Collection of specimens by qualified caregiver or patient added.
Culture and investigation.	Updated to provide information on culture examination.
4.5.1 Culture media, conditions and organisms.	Updated to include options for selective and chromogenic agar for subculture of enrichment broth.
Appendix.	Flowchart added.
References.	Updated.

## 1 General information

[View general information](#) related to UK SMIs.

## 2 Scientific information

[View scientific information](#) related to UK SMIs.

## 3 Scope of document

### Type of specimen

Maternal low vaginal and rectal swabs

### Scope

The method describes the examination of specimens to detect carriage of group B streptococci (GBS). This method may also be of value to support research projects investigating GBS carriage.

The recommendations within the UK SMI recognise the current policy of the UK National Screening Committee (2017) and guidance from the National Institute for Health and Care Excellence and the Royal College of Obstetricians & Gynaecologists (2017) which states that routine universal antenatal screening using bacteriological culture or near patient testing techniques should not be introduced in UK practice<sup>1-3</sup>. However, current guidance recommends that in certain settings, screening may be helpful in determining the risk of developing early-onset neonatal GBS (EOGBS) or in examining cases of late-onset neonatal GBS<sup>4</sup>. For example, in women in whom GBS was detected in a previous pregnancy, administration of intrapartum antibiotic prophylaxis (IAP) provides a clear clinical benefit<sup>5</sup>. This UK SMI provides details on the test method in the event of undertaking the screening of an individual mother.

On rare occasions, clusters of invasive GBS infection occur. In such circumstances, non-invasive sampling of cases or screening of individuals at risk, contacts, or the environment may assist with outbreak management<sup>4</sup>.

This UK SMI does not include procedures for isolation and detection of GBS from invasive specimens such as blood and CSF; refer to the UK SMIs: [UK SMI S 12 - Sepsis and systemic or disseminated infections](#) and [UK SMI B 27 - Investigation of cerebrospinal fluid](#). However, during investigation of a maternal or neonatal invasive GBS infection or cluster, additional carriage and screening swabs may be taken from infants, mothers and the environment as part of the investigation.

The testing of environmental samples is outside of the scope of this UK SMI.

This UK SMI should be used in conjunction with other UK SMIs.

## 4 Introduction

### 4.1 Lancefield group B streptococci

Lancefield group B streptococci (GBS), or *Streptococcus agalactiae*, are facultatively anaerobic, oxidase-negative, catalase-negative, Gram-positive cocci occurring in chains. GBS are serologically classified on the basis of cell wall polysaccharide antigens and exhibit  $\beta$ -haemolysis on blood agar, although a very small proportion of strains are non-haemolytic. These characteristics can be used as an early step in identifying clinical isolates. After 18-24 hours incubation at 35-37°C colonies tend to be slightly larger than other streptococci (approximately 1mm) and have a less distinct zone of  $\beta$ -haemolysis (see [UK SMI ID 4 – Identification of Streptococcus species, Enterococcus species and morphologically similar organisms](#)).

### 4.2 Colonisation

GBS normally colonises the vagina in many women and the intestines of men and women. Up to 28% of women in the UK carry GBS in the vagina or rectum without any associated symptoms<sup>6-8</sup>. The gastrointestinal tract is the human reservoir for GBS and the likely source of vaginal colonisation<sup>9</sup>.

### 4.3 Infection

Although GBS colonisation is not normally associated with disease in non-pregnant women, GBS can cause infection including bacteraemia in pregnant women<sup>9</sup>. GBS may cause potentially devastating early onset disease, primarily in newborns and late onset disease in newborns as well as infections in pregnant women, children and adults. In pregnancy this organism can infect the amniotic fluid (see [UK SMI B 26 – Investigation of fluids from normally sterile sites](#)) which can lead to neonatal sepsis, pneumonia or meningitis<sup>10</sup>.

In pregnant women, GBS infection is known to cause urinary tract infection, amnionitis, endometritis and wound infection. In men and non-pregnant women, skin or soft tissue infection, bacteraemia, genitourinary infection, balanitis (in men) and pneumonia are the most common manifestations of disease<sup>11,12</sup>.

Neonatal infection refers to infection occurring during the first four weeks of life. Infection may be superficial and localised (eg conjunctivitis, pustules, skin infection), deep and localised (pneumonia, septic arthritis, meningitis) or systemic (septicaemia). Presentation differs according to age at onset: early onset disease is more likely than late onset to present with generalised sepsis<sup>13</sup>.

Since The British Paediatric Surveillance Unit (BPSU) study of 2000–2001 there has been a significant increase in the incidence of invasive GBS disease in all five British Isles countries. Results from a repeat of this study in 2014, showed the incidence for early-onset GBS disease was 0.54 cases per 1000 live births and a mortality rate of 4.7% compared to 0.48 cases per 1000 live births and a mortality rate of 9.7% in 2000<sup>13,14</sup>. Increases in erythromycin and clindamycin resistance have also been noted over this period, leading to a change in second line agent used for intrapartum prophylaxis<sup>15</sup>.

The incidence of infection increases with low birth weight or prematurity and may be divided into:

- Early onset (0-6 days) - this occurs in the first six days (usually within 48 hours) of life and is caused by infection ascending from the maternal genital tract or, very rarely, via the placenta. Only a small percentage of infants colonised with this organism develop early onset disease. Early infections tend to be associated with pneumonia and septicaemia and may be confused with respiratory distress syndrome
- Late onset (7-90 days) - this occurs after the first six days (7-90 days) and is associated with acquisition of the organism through vertical or nosocomial transmission or from the external (eg hospital) environment. GBS initially colonise the superficial sites and upper respiratory tract and progress to cause widespread sepsis. Late infection is more likely to be associated with meningitis

In the UK, universal antenatal screening for GBS colonisation is currently not recommended<sup>1-3,5,16</sup>. However, the Royal College of Obstetricians & Gynaecologists recommends screening in women in whom GBS was detected in a previous pregnancy at 35-37 weeks of gestation or 3-5 weeks prior to the anticipated delivery<sup>1</sup>. This screening would determine the carriage status close to delivery and provides information that helps to assess the risk of EOGBS. Based on the results of this screening, IAP can be offered and in the case of mothers with clinical risk factors they can choose to decline IAP if they test negative.

## 4.4 Method of investigation

The isolation rate of GBS from clinical specimens depends on several factors. Studies have shown that detection for GBS colonisation can be improved by attention to the timing of cultures, the sites swabbed and the microbiological method used for culture of microorganisms. The Centers for Disease Control and Prevention suggest that optimum yield will be achieved by selective enrichment procedures applied to swabs obtained from the vagina and the anorectum which increases the likelihood of GBS isolation compared with vaginal or cervical culture alone<sup>17</sup>. Recto-vaginal swabs are likely to isolate a diverse array of normal microflora and use of selective enrichment broth is recommended to avoid overgrowth of other microorganisms<sup>17</sup>.

The use of a selective enrichment broth that inhibits the growth of competing organisms such as Gram negative enteric bacilli and other normal microflora significantly increases the yield of GBS culture and is recommended since it has been found to be the most sensitive method to detect female colonisation<sup>17,18</sup>. The most widely used selective enrichment broth is Todd-Hewitt broth with nalidixic acid and colistin (eg Lim broth) or nalidixic acid and gentamicin, with further subculture on blood agar plate.

Subculture from the selective enrichment broth to a selective and chromogenic agar have demonstrated equivalent or superior GBS recovery compared to subculture to blood agar<sup>18-20</sup>.

Chromogenic media are not fully specific for GBS identification and presumptive colonies of GBS should be confirmed by a specific antigenic detection test or Matrix Assisted Laser Desorption Ionisation Time-of-Flight (MALDI-TOF)<sup>21-24</sup>.

## 4.5 Rapid methods

A variety of rapid identification methods are available to detect GBS presence in pregnant women directly from vagino-rectal swabs, of which some are FDA approved.

Assay sensitivity of some tests is reported to be higher than culture alone<sup>25</sup>. However, discordant results are noted and in some cases results are variable on repeat testing. Use of broth enrichment followed by subculture on most chromogenic media and PCR assays have comparable sensitivities and allow more rapid reporting of screening for GBS than conventional culture methods<sup>20</sup>.

For presumptive isolates of GBS a variety of rapid identification methods are available with high sensitivity and specificity including PCR and Matrix Assisted Laser Desorption Ionisation Time-of-Flight (MALDI-TOF MS)<sup>21,22</sup>. Refer to [UK SMI ID 4 - Identification of Streptococcus species, Enterococcus species and morphologically similar organisms](#) for the identification of GBS.

## 5 Technical information/limitations

### Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (eg sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

### Selective media in screening procedures

Selective media which does not support the growth of all circulating strains of organisms may be recommended based on the evidence available. A balance therefore must be sought between available evidence, and available resources required if more than one media plate is used.

### Specimen containers<sup>26,27</sup>

UK SMIs use the term “CE marked leak proof container” to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: “The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes”.

## 6 Safety considerations<sup>26-42</sup>

### 6.1 Specimen collection, transport and storage<sup>26-31</sup>

Use aseptic technique.

Collect swabs into appropriate transport medium eg Amies or Stuart, and transport in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

### 6.2 Specimen processing<sup>26-42</sup>

Containment Level 2.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet<sup>34</sup>.

Refer to current guidance on the safe handling of all organisms documented in this UK SMI.

The above guidance should be supplemented with local COSHH and risk assessments.

## 7 Specimen collection

### 7.1 Type of specimens

Maternal low vaginal and anorectal swabs. Maternal high vaginal swabs should not be collected as these have a lower sensitivity.

### 7.2 Optimal time and method of collection<sup>43</sup>

For safety considerations refer to Section 6.1.

Collect specimens before antimicrobial therapy where possible<sup>43</sup>.

It is essential to specify “Detection of GBS carriage” in the specimen request.

Unless otherwise stated, swabs for GBS culture should then be placed in appropriate transport medium<sup>44-48</sup>.

Rayon or Dacron, Fibre or Flocked swabs, with non-nutritive transport media (eg Amies or Stuart’s), preserve the viability of the organism by providing moisture, and buffering to maintain the pH.

Specimen(s) for culture may be collected either by the physician or other qualified caregiver (or may be self-collected by the patient, with appropriate instruction). This involves swabbing the distal vagina (vaginal introitus), followed by the rectum.

A single swab for both sites of collection is rational, but two different swabs can be used. Because lower vaginal as opposed to cervical cultures are recommended, cultures should not be collected by speculum examination.

## 7.3 Adequate quantity and appropriate number of specimens<sup>43</sup>

One combined maternal vaginal/rectal swab or two separate swabs processed as one. Numbers and frequency of specimen collection are dependent on clinical condition of patient.

## 8 Specimen transport and storage and retention<sup>26,27</sup>

### 8.1 Optimal transport and storage conditions

For safety considerations refer to Section 6.

Specimens should be transported and processed as soon as possible<sup>43</sup>.

If processing is delayed, refrigeration is preferable to storage at ambient temperature<sup>43</sup>.

GBS isolates can remain viable in transport media for several days at room temperature. However, the recovery of isolates declines over 1-4 days, especially at elevated temperatures, which can lead to false-negative results. Specimens should be refrigerated before processing<sup>17</sup>.

## 9 Specimen processing/procedure<sup>26,27</sup>

### 9.1 Test selection

N/A

### 9.2 Appearance

N/A

### 9.3 Sample preparation

For safety considerations refer to Section 6.2.

### 9.4 Microscopy

N/A

### 9.5 Culture and investigation

#### Selective enrichment culture

Remove the cap aseptically from the container and place the swab(s) in the LIM broth, break off (or cut) the swab stick(s) and replace the cap. Caps should be kept loose during incubation.

## Culture

After an overnight incubation at 35-37°C, 5% CO<sub>2</sub>, subculture with a sterile loop and inoculate appropriate media (see table 4.5.1).

Optimum detection of GBS may require the use of more than one culture medium.

For the isolation of individual colonies, spread inoculum with a sterile loop onto blood agar, selective or chromogenic agar.

Incubate the plate(s) at 35 to 37°C in the appropriate atmosphere for 24-48hr.

## Culture examination

After an overnight incubation, observe plates for suggestive GBS colonies and identify them. If negative after overnight incubation, re-incubate an additional 24 hours before reporting a negative result.

On blood agar, suggestive colonies of GBS are grey, translucent, with a surrounding zone of beta-hemolysis (or no hemolysis: very rare).

Refer to manufacturer's instructions for GBS detection on selective and chromogenic agar.

Serotyping of isolates is available by latex agglutination or on referral to the reference laboratory if from invasive or associated to invasive cases.

### 8.5.1 Culture media, conditions and organisms

Clinical details/ Conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Carriage of Group B streptococci	Maternal low vaginal and anorectal swabs	LIM Broth (5mL) <sup>†</sup> : Todd-Hewitt broth supplemented with 10µg/mL colistin - or 8µg/mL gentamicin and 15µg/mL nalidixic acid	35-37	5% CO <sub>2</sub>	18-24hr	N/A	
		Then subculture to: Blood agar or	35-37	5% CO <sub>2</sub>	24-48hr	18-24hr and 48hr	Group B streptococci
		Selective agar or	35-37	Ambient	24-48hr	18-24hr	
		Chromogenic agar	35-37	Ambient	24-48hr	18-24hr	

<sup>†</sup>The bottle should contain a volume of broth sufficient to cover the swabs

## 8.6 Identification

Refer to [UK SMI ID 4 - Identification of Streptococcus species, Enterococcus species and morphologically similar organisms](#) for the identification of GBS.

### 8.6.1 Minimum level of identification in the laboratory

<a href="#">Streptococcus agalactiae</a>	species level
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Organisms may be further identified if this is clinically or epidemiologically indicated.

## 8.7 Antimicrobial susceptibility testing

Refer to [EUCAST](#) guidelines. Prudent use of antimicrobials according to local and national protocols is recommended.

This UK SMI recommends selective and restrictive reporting of susceptibilities to antimicrobials. Any deviation must be subject to consultation that should include local antimicrobial stewardship groups.

### 8.7.1 Antimicrobial Susceptibility Testing and Reporting Table

It is recommended that the antimicrobials in bold in the table below are reported. Those antimicrobials not in bold may be reported based on local decisions.

Bacteria	Examples of agents to be included within primary test panel (recommended agents to be reported are in bold depending on clinical presentation)	Examples of agents to be considered for supplementary testing (recommended agents to be reported are in bold depending on clinical presentation)	Notes
GBS	<b>Penicillin</b> Clindamycin <b>Cefotaxime</b> <b>Vancomycin</b>	Clarithromycin <b>Teicoplanin</b>	After consultation with users, laboratories may report only Penicillin routinely and report the other agents routinely only if shown to be resistant.

## 8.8 Referral for outbreak investigations

See section 8.9.

## 8.9 Referral to reference laboratories

Refer all GBS which are associated with an infection control or cluster investigation in addition to all invasive isolates.

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory [click here for user manuals and request forms \(England and Wales\)](#).

Organisms with unusual or unexpected resistance, and whenever there is a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

[England](#)

[Wales](#)

[Scotland](#)

[Northern Ireland](#)

## 9 Reporting procedure

### 9.1 Microscopy

N/A

### 9.2 Culture

Report:

#### Negatives

“Group B streptococci not isolated”

#### Positives

“Group B streptococci isolated”

#### 9.2.1 Culture reporting time

Clinically urgent results: to be telephoned or sent electronically.

Written report: 16 – 72hr stating, if appropriate, that a further report will be issued.

### 9.3 Antimicrobial susceptibility testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

## 10 Notification to UKHSA<sup>49,50</sup> or equivalent in the devolved administrations<sup>51-54</sup>

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify UK Health Security Agency (UKHSA) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days. GBS is a notifiable disease in Northern Ireland but not in England, Wales and Scotland.

## Detection of carriage of group B streptococci (*Streptococcus agalactiae*)

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local UKHSA Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to UKHSA. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to UKHSA and many UKHSA Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

**Note:** The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAs) and Creutzfeldt–Jakob disease (CJD) under ‘Notification Duties of Registered Medical Practitioners’: it is not noted under ‘Notification Duties of Diagnostic Laboratories’.

<https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010>

Other arrangements exist in [Scotland](#)<sup>51,52</sup>, [Wales](#)<sup>53</sup> and [Northern Ireland](#)<sup>54</sup>.

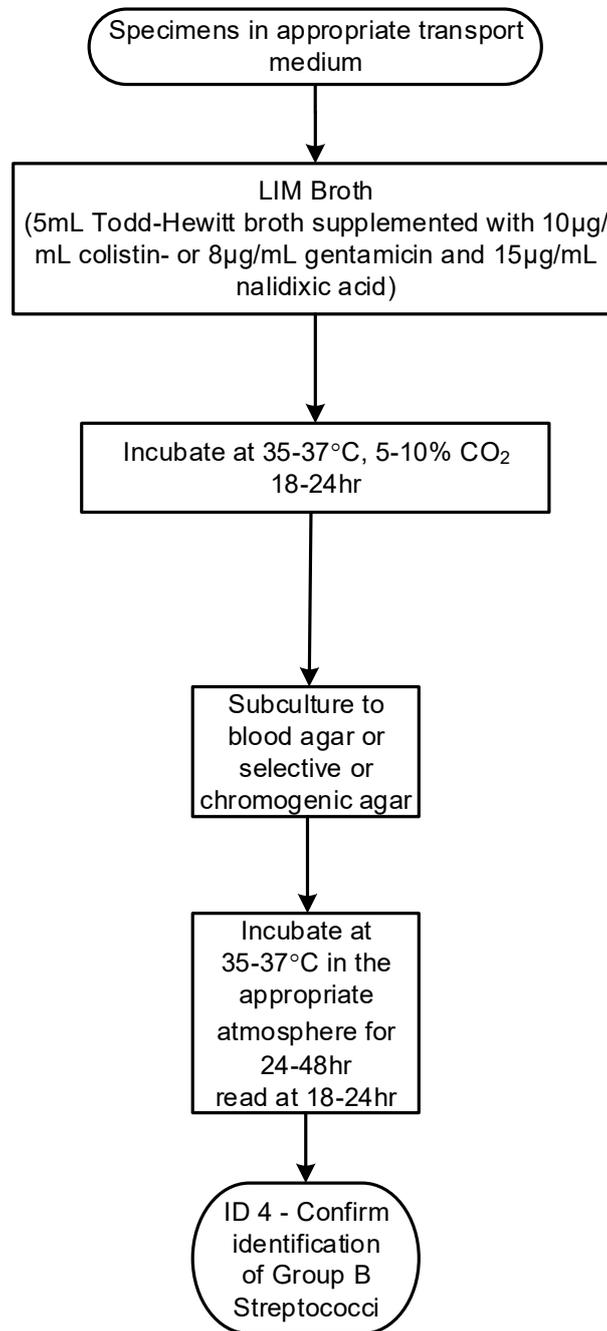
## 11 Public health responsibilities of diagnostic laboratories

Diagnostic laboratories have public health responsibility as part of their duties. Amongst these are additional local testing, or referral to further characterise the organism as required, primarily for public health purposes e.g. routine cryptosporidium detection; serotyping or microbial subtyping; and a duty to refer appropriate specimens and isolates of public health importance to a reference laboratory.

Diagnostic laboratory outputs inform public health intervention, and surveillance data is required to develop policy and guidance forming an essential component of healthcare. It is recognised that additional testing and referral of samples may entail some costs that has to be borne by the laboratory but in certain jurisdictions these costs are covered centrally.

Diagnostic laboratories should be mindful of the impact of laboratory investigations on public health and consider requests from the reference laboratories for specimen referral or enhanced information.

## Algorithm: Detection of carriage of group B streptococci (*Streptococcus agalactiae*)



## References

An explanation of the reference assessment used is available in the [scientific information section on the UK SMI website](#).

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