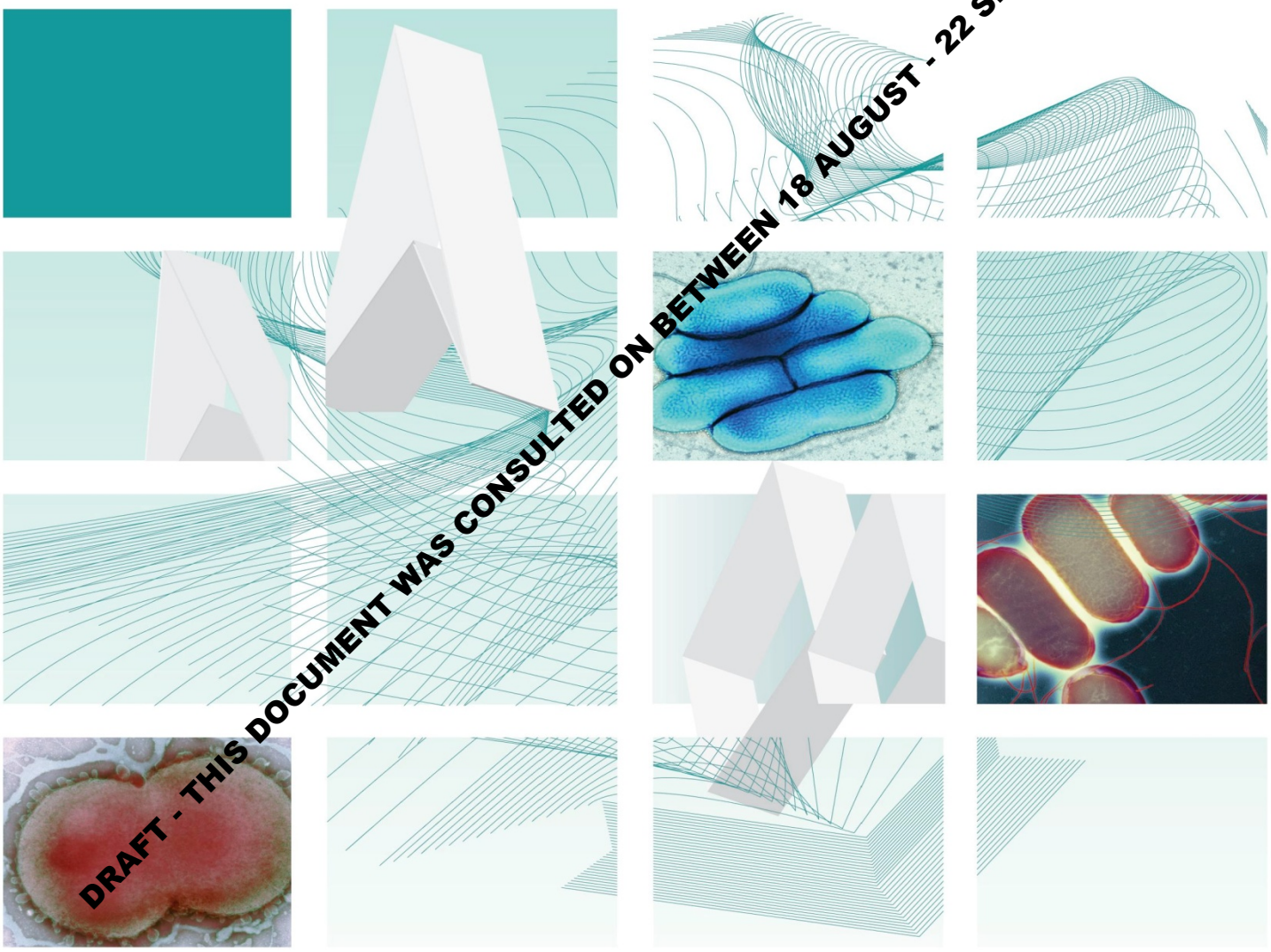
Public Health England

NHS

# UK Standards for Microbiology Investigations

## Detection method for Group B Streptococci





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# Acknowledgments

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website http://www.hpa.org.uk/SMI/Partnerships. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see http://www.hpa.org.uk/SMI/WorkingGroups).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the Medical Editors for editing the medical content.

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UK Standards for Microbiology Investigations are produced in association with:

# Contents

# Amendment Table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

| | |
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| Amendment No/Date. | 5/dd.mm.yy <tab+enter> |
| Issue no. discarded. | 2.2 |
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| **Section(s) involved** | **Amendment** |
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| Amendment No/Date. | 4/02.08.12 |
| Issue no. discarded. | 2.1 |
| Insert Issue no. | 2.2 |
| **Section(s) involved** | **Amendment** |
| Whole document. | Document presented in a new format.<br>The term “CE marked leak proof container” is referenced to specific text in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) and to the Directive itself EC[1,2].<br>Edited for clarity.<br>Reorganisation of [some] text.<br>Minor textual changes. |
| Sections on specimen collection, transport, storage and processing. | Reorganised. Previous numbering changed. |
| References. | Some references updated. |

# UK SMI[#]: Scope and Purpose

## Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

## Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

## Equal Partnership Working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at http://www.hpa.org.uk/SMI/Partnerships. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations or the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. SMIs are developed, reviewed and updated through a wide consultation process.

## Quality Assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008. SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent



[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

## Patient and Public Involvement

The SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

## Information Governance and Equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIs are subject to PHE Equality objectives http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317133470313.

The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

## Legal Statement

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

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## Suggested Citation for this Document

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# Scope of Document

## Type of Specimen

Vaginal and rectal swabs

## Scope

The method describes the examination of specimens from pregnant women for Group B streptococci (GBS) and is made available to laboratories where there is a clinical indication or when the test is requested.

The recommendations within the SMI recognise the current policy from the Royal College of Obstetricians and Gynaecologists 2012[3], UK National Screening Committee 2012[4,5], National Institute for Health and Care Excellence[6] and Public Health England[7] stating that routine screening using bacteriological culture or near patient testing techniques should not be introduced in UK practice. This SMI does not seek to contradict these recommendations.

This SMI should be used in conjunction with other SMIs.



# Introduction

## Lancefield Group B streptococci

Lancefield Group B streptococci, or *Streptococcus agalactiae*, are oxidase negative, catalase negative Gram positive cocci occurring in chains. GBS are facultative anaerobes that are serologically classified on the basis of cell wall polysaccharide antigens. On blood agar, the species exhibit β-haemolysis, although a very small proportion of strains are non-haemolytic. This can be used as an early step in identifying clinical isolates. After 18-24 hours incubation at 35-37°C colonies tends to be slightly larger than other streptococci (approximately 1mm) and have a less distinct zone of β-haemolysis (see ID 7 – Identification of *Streptococcus* species, *Enterococcus* species and morphologically Similar Organisms).

## Colonisation

GBS normally colonises the vagina in many women and the intestines of men and women. Up to 20% of women in the UK carry GBS in the vagina or rectum without any associated symptoms[8,9]. The gastrointestinal tract is the likely human reservoir for GBS, and the likely source of vaginal colonisation.

## Infection

Although GBS colonisation is not associated with disease in healthy women, GBS can cause infection in pregnant women[10]. GBS may cause potentially devastating early onset disease primarily in newborns as well as infections in pregnant women and adults with underlying medical conditions (eg diabetes mellitus). In pregnancy this organism can infect the amniotic fluid (see B 26 – Investigation of Fluids from Normally Sterile Sites), which can lead to neonatal sepsis, pneumonia or meningitis[11].

In pregnant women, GBS infection is known to cause urinary tract infection, amnionitis, endometritis and wound infection. In addition stillbirths and premature delivery have also been attributed to GBS. In non-pregnant adults, skin or soft tissue

infection, bacteraemia, genitourinary infection, and pneumonia are the most common manifestations of disease[12].

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) or systemic (septicaemia, meningitis). Presentation differs according to age at onset: early onset disease is more likely than late onset to present with sepsis[13].

The incidence of infection also 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, routine antenatal screening for GBS colonisation is currently not recommended[3-7]. However, according to local protocols, patients judged clinically to be at high risk for the development of Group B streptococcal infection may be investigated for carriage. The risk-based approach for identifying women for early onset GBS include pre term delivery (less than 37 weeks gestation), prolonged rupture of membranes, maternal fever in labour and known genital carriage[4].

## Method of Investigation

The isolation rate of GBS from clinical specimens depends on several factors. Studies have shown that detection of GBS colonisation can be improved by attention to the timing of cultures, the sites swabbed and the microbiological method used for culture of organisms. In 2002 updated guidelines were published by Centers for Disease Control and Prevention that recommended the universal screening approach by collection of swabs between 35 and 37 weeks gestation to improve the sensitivity and specificity for detection of colonisation at the time of delivery[14]. 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[15]. Recto-vaginal swabs are likely to isolate a diverse array of normal flora and use of selective enrichment broth is recommended to avoid overgrowth of other organisms[14].

After collection, swabs should be placed in a non-nutrient transport medium as Amies or Stuart. The use of a selective broth medium that inhibits the growth of competing organisms such as Gram negative enteric bacilli and other normal flora 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. The most widely used selective medium is Todd-Hewitt broth with nalidixic acid and colistin (eg Lim broth) or nalidixic acid and gentamicin further sub-cultured on blood agar plate. However, this

enrichment broth is not totally selective for GBS, and other Gram positive cocci may be enriched by this method, possibly hiding GBS and leading to false negative results.

For this reason, use of selective and differential media for subcultures can improve screening sensitivity as well as shortening the turnaround time, and is already recommended in some European countries for antenatal GBS-screening cultures. Several options are now available including selective and chromogenic agar. Compared with culture on blood agar, selective and chromogenic media have shown to increase the sensitivity of GBS isolation[10,16,17]. However, chromogenic media are not fully specific, so all presumptive colonies of GBS should be confirmed by a specific antigenic detection test.

### Rapid Methods

A variety of rapid identification methods are available to detect GBS colonisation in pregnant women directly from vagino-rectal swabs including immunological assays, DNA hybridisation and PCR methods. However, the assays generally have low sensitivity for direct identification compared to the culture method and the nucleic acid testing assays are found to either, take too long or require complicated procedures that are not applicable for direct identification from clinical specimens[10,18].

For presumptive isolates of GBS a variety of rapid methods are available with high sensitivity and specificity including PCR, Pulsed Field Gel Electrophoresis (PFGE), 16S rRNA gene sequencing, *atpA* Gene Sequence Analysis, Multilocus sequence typing (MLST) and Matrix Assisted Laser Desorption Ionisation Time-of-Flight (MALDI-TOF). Refer to ID 4 - Identification of Streptococcus species, Enterococcus species and Morphologically Similar Organisms for the identification of GBS.

## 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[1,2]

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".

# 1 Safety Considerations[1,2,19-33]

## 1.1 Specimen Collection, Transport and Storage[1,2,19-22]

Use aseptic technique.

Collect swabs into appropriate transport medium and transport in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

## 1.2 Specimen Processing[1,2,19-33]

Containment Level 2.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[25].

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

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

# 2 Specimen Collection

## 2.1 Type of Specimens

Vaginal and rectal swabs.

## 2.2 Optimal Time and Method of Collection[34]

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible[34].

Unless otherwise stated, swabs for bacterial and fungal culture should then be placed in appropriate transport medium[35-39].

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.

At 35-37 weeks gestation of specimen(s) for culture may be done either by physician or other qualified caregiver (or self-collected by the patient, with appropriate instruction). This involves swabbing the distal vagina (vaginal introitus), followed by the rectum (ie through the anal sphincter).

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.

## 2.3 Adequate Quantity and Appropriate Number of Specimens[34]

One combined vaginal/rectal swab or two separate swabs processed as one.

Numbers and frequency of specimen collection are dependent on clinical condition of patient.

# 3 Specimen Transport and Storage[1,2]

## 3.1 Optimal Transport and Storage Conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible[34].

If processing is delayed, refrigeration is preferable to storage at ambient temperature[34].

# 4 Specimen Processing/Procedure[1,2]

## 4.1 Test Selection

N/A

## 4.2 Appearance

N/A

## 4.3 Sample Preparation

For safety considerations refer to Section 1.2.

## 4.4 Microscopy

N/A

## 4.5 Culture and Investigation

**Enrichment Culture**

Remove the cap aseptically from the container and place the swab(s) in the LIMs 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% $CO_2$, sub-culture with a sterile loop and inoculate appropriate media (see table 4.5.1).

Subculture the broth after 18 to 24hr of incubation on to a blood agar and/or selective or chromogenic agar plates. 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.

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.

### 4.5.1 Culture media, conditions and organisms

| Clinical details/ Conditions | Specimen | Standard media | Incubation | | | Cultures read | Target organism(s) |
|---|---|---|---|---|---|---|---|
| | | | Temp °C | Atmos | Time | | |
| Enrichment Culture | | LIM Broth (5mL): Todd-Hewitt broth supplemented with 10µg/mL colistin and 15µg/mL nalidixic acid) | 35-37 | 5% $CO_2$ | 18-24hr | N/A | |
| Selective Culture | | Then subculture to: | | | | | |
| | | Blood agar | 35-37 | 5% CO2 | 24-48hr | 18-24hr and 48hr | Group B streptococci |
| | | and/or | | | | | |
| | | Selective agar | 35-37 | Ambient | 24-48hr | 18-24hr | |
| | | and/or | | | | | |
| | | Chromogenic agar | 35-37 | Ambient | 24-48hr | 18-24hr | |
| †The bottle should contain a volume of broth sufficient to cover the swabs | | | | | | | |



## 4.6 Identification

Refer to [IP 4 - Identification of Streptococcus species, Enterococcus species and Morphologically Similar Organisms] for the identification of GBS.

### 4.6.1 Minimum level of identification in the laboratory

| | |
|---|---|
| *Streptococcus agalactiae* | species level |

Organisms may be further identified if this is clinically or epidemiologically indicated.

## 4.7 Antimicrobial Susceptibility Testing

Refer to British Society for Antimicrobial Chemotherapy (BSAC) and/or EUCAST guidelines. Prudent use of antimicrobials according to local and national protocols is recommended.

## 4.8 Referral for Outbreak Investigations

N/A

## 4.9 Referral to Reference Laboratories

For information on the tests offered, turn around times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

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, turn around times, transport procedure and any other requirements for sample submission:

England and Wales
http://www.hpa.org.uk/webw/HPAweb&Page&HPAwebAutoListName/Page/1158313434370?p=1158313434370

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.belfasttrust.hscni.net/Laboratory-MortuaryServices.htm



# 5 Reporting Procedure

## 5.1 Microscopy

N/A

## 5.2 Culture

### Report:

Negatives

“Group B streptococci not isolated”

Positives

“Group B streptococci isolated”

### 5.2.1 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.

## 5.3 Antimicrobial Susceptibility Testing

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

# 6 Notification to PHE[40,41] or Equivalent in the Devolved Administrations[42-45]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) 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.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE 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.

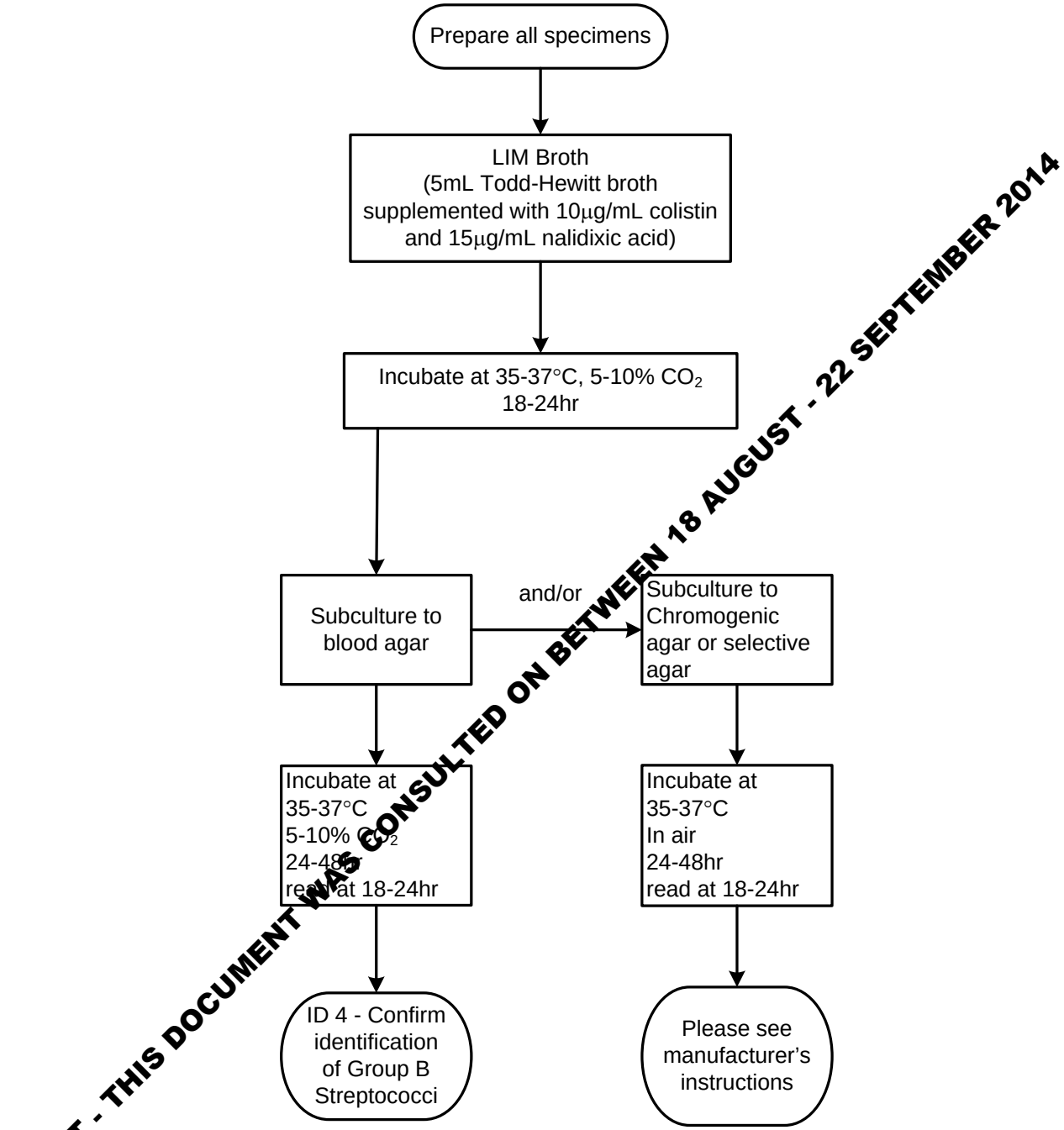
Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local 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 (HCAIs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/HealthProtectionRegulations/

Other arrangements exist in Scotland[42,43], Wales[44] and Northern Ireland[45].

# Appendix: Detection method for Group B Streptococci



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