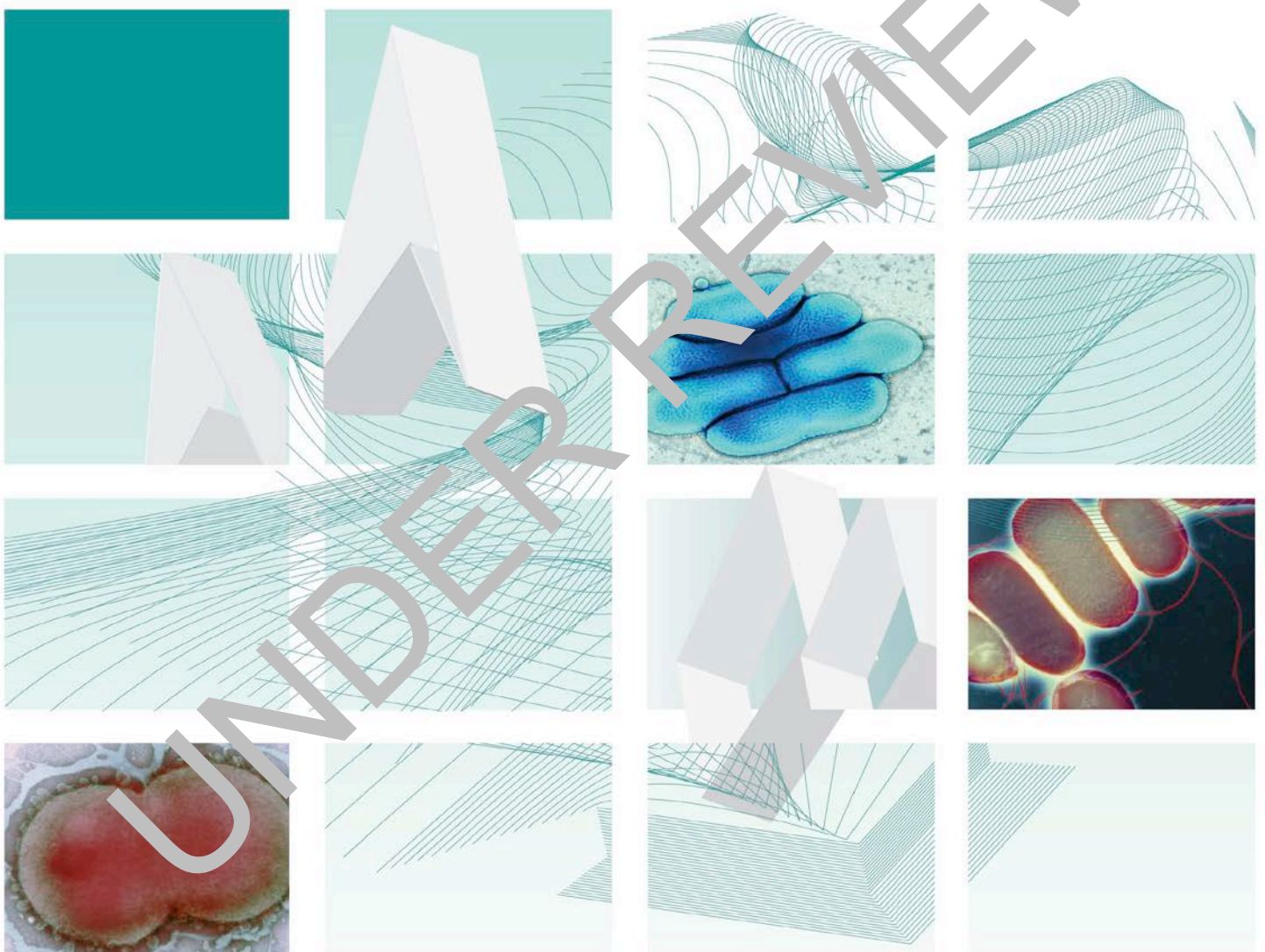




# UK Standards for Microbiology Investigations

## Identification of Anaerobic Cocci



## 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:



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NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at [www.nice.org.uk/accreditation](http://www.nice.org.uk/accreditation).

For full details on our accreditation visit: [www.nice.org.uk/accreditation](http://www.nice.org.uk/accreditation).

## 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](mailto:standards@phe.gov.uk).

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

Amendment No/Date.	5/11.03.14
Issue no. discarded.	2.1
Insert Issue no.	2.3
<b>Section(s) involved</b>	<b>Amendment</b>
Whole document.	<p>Document has been transferred to a new template to reflect the Health Protection Agency's transition to Public Health England.</p> <p>Front page has been redesigned.</p> <p>Status page has been renamed as Scope and Purpose and updated as appropriate.</p> <p>Professional body logos have been reviewed and updated.</p> <p>Standard safety and notification references have been reviewed and updated.</p> <p>Scientific content remains unchanged.</p>

Amendment No/Date.	4/02.07.12
Issue no. discarded.	2.1
Insert Issue no.	2.2
<b>Section(s) involved</b>	<b>Amendment</b>
Whole document.	Minor formatting amendments.
References.	Some references updated.

# UK Standards for Microbiology Investigations<sup>#</sup>: Scope and Purpose

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## Users of SMIs

- SMIs are primarily intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK.
- SMIs 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.
- SMIs 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 nor 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.

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<sup>#</sup>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.

## 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 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/web/HPAwebFile/HPAweb\\_C/1317133470313](http://www.hpa.org.uk/web/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.

SMIs are Crown copyright which should be acknowledged where appropriate.

**Suggested Citation for this Document**

Public Health England. (2014). Identification of Anaerobic Cocci. UK Standards for Microbiology Investigations. ID 14 Issue 2.3. <http://www.hpa.org.uk/SMI/pdf>.

UNDER REVIEW

## Scope of Document

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This SMI describes the characterisation of anaerobic cocci bacteria.

Anaerobic spring organisms are described in:

[ID 8 - Identification of \*Clostridium\* species.](#)

Anaerobic Gram negative rods are described in:

[ID 25 – Identification of Anaerobic Gram Negative Rods.](#)

[ID 15 – Identification Anaerobic Actinomyces species](#) and

[ID 10 - Identification of Aerobic Actinomycetes](#) cover the identification of actinomycetes.

Anaerobic rods can be found in [ID 25 – Identification of Anaerobic Gram Negative Rods.](#)

This SMI should be used in conjunction with other SMIs.

## Introduction

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### Taxonomy

#### Gram negative cocci

Three genera are included in the anaerobic Gram negative cocci, but only one *Veillonella* is found in clinical material. There are seven species of *Veillonella*, of which *Veillonella parvula* is the most commonly isolated species from human specimens.

The classification of the anaerobic Gram positive cocci is continually changing with the addition of new species and re-naming of old species<sup>1</sup>. There are currently six genera of anaerobic Gram positive cocci which may be isolated from humans. These include *Peptostreptococcus*, *Peptoniphilus*, *Parvimonas*, *Fingoldia*, *Anaerococcus* and another group of uncertain taxonomy. The majority of human isolates are *Peptostreptococcus*, *Peptoniphilus* and *Anaerococcus*<sup>2</sup>.

### Characteristics

Anaerobic cocci give good growth on blood agar, under strict anaerobic conditions, and fail to grow on chocolate blood agar after up to seven days in air and 10% CO<sub>2</sub>.

#### Anaerobic Gram negative cocci

##### *Veillonella* species

*Veillonella* species are small asaccharolytic cocci, measuring approximately 0.5µm in diameter. They are the only Gram negative anaerobic cocci which are isolated from human clinical material and are rarely found in pure culture. *Veillonella* species fluoresce red on exposure to ultraviolet light (365nm), but this is medium dependent and may fade in a few minutes on exposure to oxygen. Some species produce catalase.



## Anaerobic Gram positive cocci

### *Peptococcus* species

The genus *Peptococcus* now contains only one species, *Peptococcus niger*. Typically, cells are 0.3-1.3µm in diameter, arranged singly, in pairs or clumps, and it grows very slowly. Black pigment is produced after five days incubation, but is lost on subculture.

## Other Gram positive cocci associated with human infection

*Atopobium parvula*

*Coprococcus* species

*Ruminococcus* species

*Sarcina* species

## Principles of Identification

Colonies are usually isolated on fastidious anaerobe agar (or equivalent) or blood agar incubated anaerobically. Colonies may be characterised by colonial morphology, Gram stain reaction and are sensitive to metronidazole. Some species may require longer than 48hr incubation to produce visible growth. Identification tends to be undertaken only if clinically indicated. Further identification tests include fluorescence under long wave UV light (365nm), pigment production, bile tolerance, glucose fermentation, and lecithinase and lipase activity on egg yolk agar. Classification of many anaerobes to species or even genus level requires additional biochemical tests or metabolic end product analysis by GLC. Identification may be undertaken, using of commercial kits. Identification of clinically significant or unusual organisms may be carried out by the Anaerobe Reference Laboratory, Cardiff. Some anaerobes are susceptible to neomycin; all samples from normally sterile sites should be cultured on neomycin selective agar and a non-selective agar.

## Technical Information/Limitations

Neomycin agar is used as a selective medium for anaerobes, but in certain instances because of the inhibitory aspects of the agar some anaerobes may not grow.

In the clinical diagnostic laboratory, susceptibility to metronidazole is frequently used as an indicator of an anaerobe being present in a clinical specimen. However, an increasing number of metronidazole resistant anaerobes are being recorded, and these organisms may be missed by such an approach. It is important to consider the possibility of involvement of anaerobes regardless of metronidazole susceptibility in certain clinical specimens or situations where anaerobes are suspected.

## 1 Safety Considerations<sup>3-19</sup>

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

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

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

Compliance with postal and transport regulations is essential.

## 2 Target Organisms

### Anaerobic Gram negative cocci

***Veillonella* species reported to have caused human infection**

*V. parvula*

*V. atypica*

*V. dispar*

### Anaerobic Gram positive cocci

**Species reported to have caused human infection**

*Peptostreptococcus anaerobius*

*Anaerococcus octavius*

*Peptococcus assacharolyticus*

*Anaerococcus prevotii*

*Anaerococcus hydrogenalis*

*Anaerococcus tetradius*

*Peptoniphilus hareii*

*Anaerococcus vaginalis*

*Peptoniphilus ivorii*

*Anaerococcus murdochii*

*Peptoniphilus leuconalis*

*Peptostreptococcus stomatis*

*Anaerococcus lactolyticus*

*Peptoniphilus gorbachii*

*Finnegoldia magna*

*Peptoniphilus olsenii*

*Parvimonas micra*

***Peptococcus* species reported to have caused human infection**

*P. niger*

### Other Genera of Anaerobic Gram positive cocci Reported to Have Caused Human Infection

*Atopobium parvulum*

*Sarcina ventriculi*

*Ruminococcus hansenii*

*Coprococcus eutactus*

*Ruminococcus productus*

*Coprococcus comes*

*Sarcina albus*

*Coprococcus catus*

*Sarcina pasteurii*

Other species may be associated with human disease.

## 3 Identification

### 3.1 Microscopic Appearance

#### Gram stain ([TP 39 - Staining Procedures](#))

*Peptostreptococcus* and *Peptococcus* are Gram positive cocci arranged in chains, pairs, tetrads or clumps.

*Veillonella* are small Gram negative cocci arranged in clumps.

### 3.2 Primary Isolation Media

Fastidious anaerobe agar or equivalent, with or without neomycin (some anaerobic organisms may be inhibited by neomycin) 40–48hr incubation anaerobically at 35–37°C.

**Note:** Some species may require longer incubation.

### 3.3 Colonial Appearance

Genus	Characteristics of growth on fastidious anaerobe agar after incubation anaerobically at 35–37°C
<i>Finegoldia magna</i>	Small colonies (<1.0mm), often with variation in size and colour. Colonies may be both convex and whitish, or flatter and translucent on the same plate
<i>Peptostreptococcus anaerobius</i>	Colonies 1–2mm in diameter, grey with slightly raised off-white centres, sensitive to sodium Polyanethol Sulfonate (SPS) disc
<i>Anaerococcus</i>	Colonies 1–2mm in diameter, glistening, low convex and usually whitish to lemon-yellow
<i>Peptostreptococcus micros</i>	Small colonies (<1.0mm), typically white (but sometimes grey), glistening and domed, sometimes surrounded by a yellow-brown halo up to 2mm wide
<i>Peptococcus</i>	Small colonies (<1.0mm), raised, grey, becoming dark brown/black
<i>Veillonella</i>	Small colonies (<1.0mm) after 48hr incubation. May fluoresce red under long wavelength UV light (365nm)

### 3.4 Test Procedures

#### Metronidazole

Isolate shows a zone of inhibition to metronidazole 5µg disc.

#### Nitrate reduction

#### SPS disc

Spot indole ([TP 19 - Indole test](#))

### 3.5 Further Identification

**Commercial identification kit:**

Results should be interpreted with caution and in conjunction with other test results.

**Other more specialised tests:**

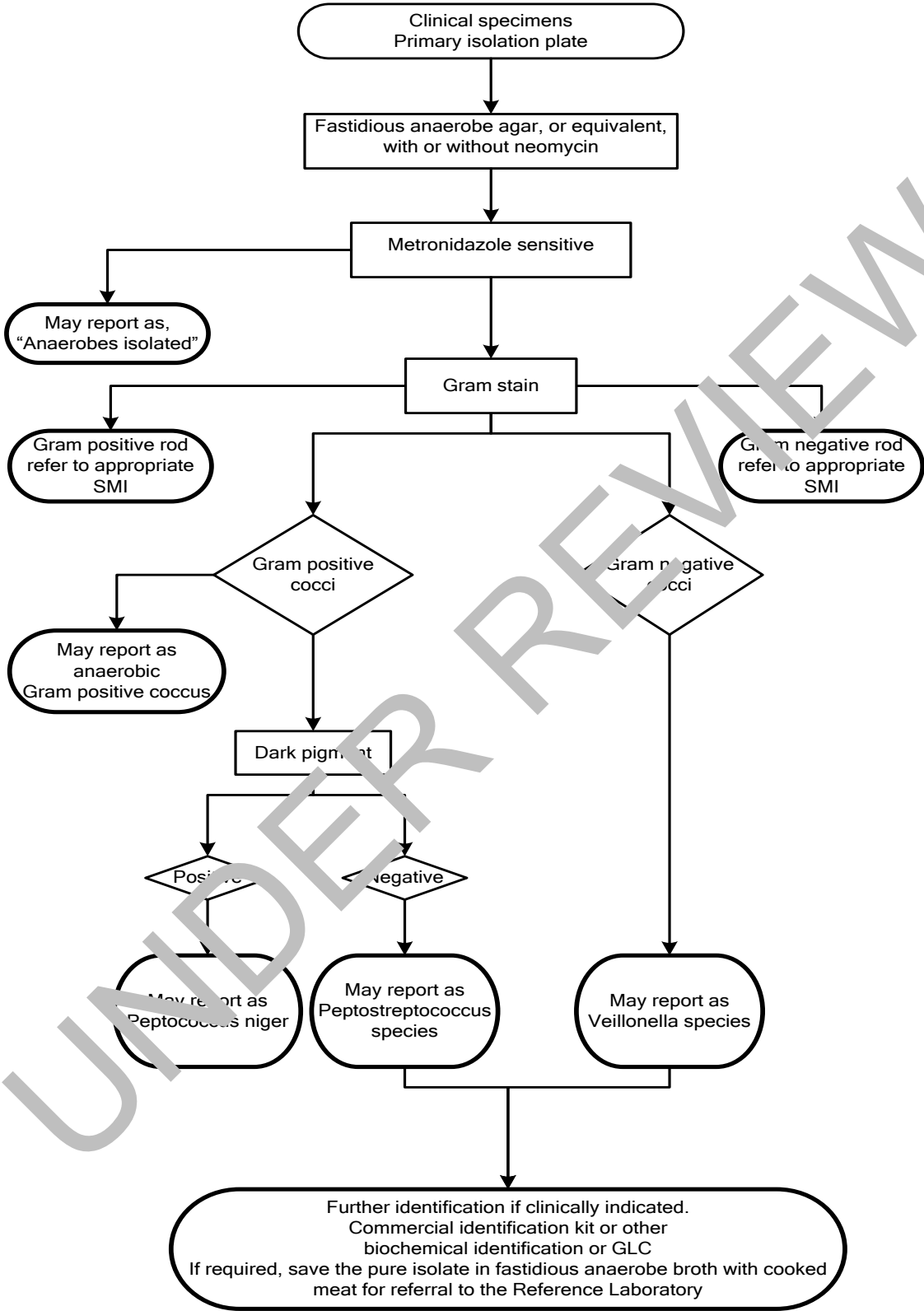
Gas Liquid Chromatography, PCR.

### 3.6 Storage and Referral

If required, save the pure isolate in fastidious anaerobe broth with cooked meat for referral to the Reference Laboratory.

UNDER REVIEW

# 4 Presumptive Identification of Anaerobic Cocci



## 5 Reporting

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### 5.1 Presumptive Identification

If appropriate growth characteristics, colonial appearance, Gram stain and metronidazole susceptibility is demonstrated

### 5.2 Confirmation of Identification

Following commercial identification kit results and/or the Reference Laboratory report.

### 5.3 Medical Microbiologist

Inform the medical microbiologist of presumed or confirmed anaerobe when the request card bears relevant information, eg:

- Septicaemia.
- Empyema, surgical wound infection, abscess formation (especially cerebral, intraperitoneal, lung, liver or spleen).
- Puerperal sepsis.
- (Necrotising) myofasciitis.
- Suspicion of Lemierre's Syndrome (post dental sepsis, often with jugular suppurative endophlebitis and haematogenous pulmonary abscesses).

Follow local protocols for reporting to clinicians.

### 5.4 CCDC

Refer to local Memorandum of Understanding.

### 5.5 Public Health England<sup>20</sup>

Refer to current guidelines on CDSC and COSURV reporting.

### 5.6 Infection Control Team

N/A

## 6 Referrals

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### 6.1 Reference Laboratory

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

Anaerobe Reference Laboratory  
 Public Health Wales Microbiology Cardiff  
 University Hospital of Wales  
 Heath Park  
 Cardiff  
 CF14 4XW  
 Telephone +44 (0) 29 2074 2171 or 2378

<http://www.hpa.org.uk/cfi/arl/default.htm>

Contact PHE's main switchboard: Tel. +44 (0) 20 8200 4400

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>

## 7 Notification to PHE<sup>20,21</sup> or Equivalent in the Devolved Administrations<sup>22-25</sup>

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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 (HCAs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

Other arrangements exist in Scotland<sup>22,23</sup>, Wales<sup>24</sup> and Northern Ireland<sup>25</sup>.

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