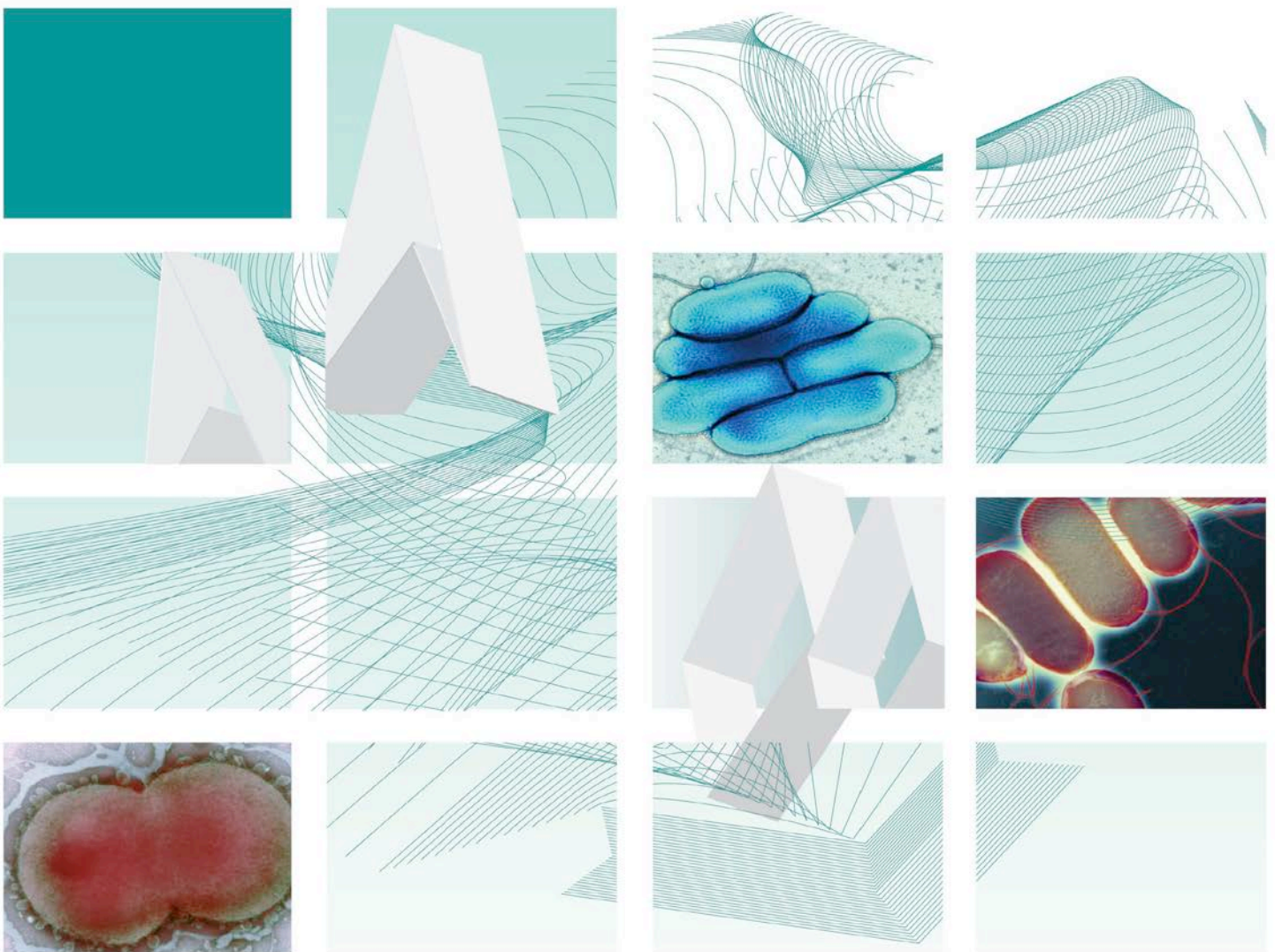




UK Standards for Microbiology Investigations

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 <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see <https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee>).

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UK Standards for Microbiology Investigations are produced in association with:



Logos correct at time of publishing.

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For full details on our accreditation visit: 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.

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

Amendment No/Date.	6/04.02.15
Issue no. discarded.	2.3
Insert Issue no.	3
Section(s) involved	Amendment
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Introduction.	The taxonomy of Anaerobic Gram negative cocci and Anaerobic Gram positive cocci have been updated. More information has been added to the Characteristics section. The medically important species are mentioned. Section on Principles of Identification has been updated to include the MALDI-TOF.
Technical Information/Limitations.	Addition of information regarding Agar Media, metronidazole susceptibility, commercial identification systems and MALDI-TOF MS.
Target Organisms.	The section on the Target organisms has been updated and presented clearly.
Identification.	Updates have been done on 3.2, 3.3 and 3.4 to reflect standards in practice. Section 3.4.3 and 3.4.4 have been updated to include MALDI-TOF MS and NAATs with references. Subsection 3.5 has been updated to include the Rapid Molecular Methods.
Identification Flowchart.	Modification of flowchart for identification of Anaerobic cocci has been done for easy guidance.
Reporting.	Subsections 5.3 have been updated to reflect the information required on reporting practice.

Referral.	The addresses for reference laboratories have been updated.
Whole document.	Document presented in a new format.
References.	Some references updated.

UK Standards for Microbiology Investigations[#]: Scope and Purpose

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 <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>.

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.

[#]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.

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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 <https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity>. 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.

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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

This SMI describes the characterisation of anaerobic cocci bacteria.

Anaerobic spore-forming 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](#)

[ID 10 - Identification of Aerobic Actinomycetes](#)

This SMI should be used in conjunction with other SMIs.

Introduction

Taxonomy

Anaerobic Gram negative cocci

There are four genera included in the anaerobic Gram negative cocci, but only three of these are known to cause infections in humans at the time of issue; *Acidaminococcus*, *Megasphaera* and *Veillonella*.

Anaerobic Gram positive cocci

The classification of the anaerobic Gram positive cocci is continually changing with the addition of new species and reclassifying species into new genera¹. There are currently six genera of anaerobic Gram positive cocci which may be isolated from humans. These include *Peptostreptococcus*, *Peptoniphilus*, *Parvimonas*, *Finegoldia*, *Anaerococcus* and *Peptococcus*. The majority of human isolates are *Peptostreptococcus*, *Peptoniphilus* and *Anaerococcus*².

Characteristics

Anaerobic Gram negative cocci

The medically important species are;

Veillonella species

There are currently 13 species and 7 subspecies of this genus³. A few of the subspecies have been reclassified within the genus. They are the only Gram negative anaerobic cocci which are mostly isolated from human clinical material and are rarely found in pure culture. *Veillonella* species are small asaccharolytic cocci occurring as diplococci and in short chains, measuring approximately 0.3-0.5µm in diameter. They are non-motile and are non-spore formers. Their nutritional requirements are complex but CO₂ is required for growth. Their optimum growth temperature is 30-37°C. These 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. They are oxidase negative and catalase negative, but some species produce an atypical catalase lacking porphyrin. They ferment pyruvate, lactate, malate, fumarate and oxaloacetate

but not carbohydrates and polyols. Indole is not produced and nitrate is reduced to nitrite⁴.

They are found in the mouth, in the intestinal and respiratory tracts of man. They have been implicated with rare cases of meningitis, osteomyelitis, pleuropulmonary infections, endocarditis and periodontal disease⁵.

***Megasphaera* species**

There are currently 5 species within this genus⁶. Cells are cocci, 0.4-2.0µm or more in diameter and occurring in pairs or occasionally in chains. They are strictly anaerobic and non-motile and are non-spore formers. Growth occurs at 25-40°C but generally not at 45°C. They are catalase and indole negative. Lactate and Glucose are fermented with the production of lower fatty acids, CO₂, and some H₂⁷.

On blood agar, the colonies are circular, convex, shiny, translucent with a smooth surface and approximately 0.5-1.0mm in diameter, non-pigmented and non-haemolytic. They are slightly rough and adherent to butyrous.

They are found in the faeces and intestine of man as well as other clinical specimens such as abscess⁸.

***Acidaminococcus* species**

There are currently 2 species within this genus⁹. Cells are cocci, 0.6-1.0µm in diameter, often occurring as oval or kidney-shaped diplococci. They are strictly anaerobic and there is no growth on the surface of agar media incubated in the air. Their optimum growth temperature is 30-37°C. Their nutritional requirements are complex. Colonies on blood agar are generally about 0.1-0.2mm in diameter and are round, entire, slightly raised, and whitish grey or nearly transparent, non-pigmented and non-haemolytic. They are also oxidase negative and catalase negative. Amino acids, of which glutamic acid is the most important, could serve as the sole energy source for growth. Acetic and butyric acids and CO₂ are produced; propionic acid and hydrogen are not produced¹⁰.

They have been isolated in the intestine of man as well as from other clinical samples¹¹.

Anaerobic Gram positive cocci

The medically important species are;

***Peptococcus* species**

The genus *Peptococcus* now contains only one species, *Peptococcus niger*¹². Typically, cells are 0.3-1.3µm in diameter arranged singly, in pairs or clumps, and it grows very slowly. They are non-motile. On blood agar, colonies appear like tiny black pearls, round, smooth and glistening, and non-haemolytic. Black pigment is produced after five days incubation, but is lost on subculture. However, in meat infusion-peptone agar deep, black colonies were formed by both fresh isolates and strains that would no longer form pigment on blood agar plates¹.

They are catalase positive and do not ferment carbohydrates. They are differentiated from *Peptostreptococcus anaerobius* by their inability to ferment carbohydrates and black pigmentation on blood agar.

It has been isolated from human clinical specimens – navel swab, rectal abscess and vaginal area swab.

***Peptostreptococcus* species**

There are currently 4 validly published species within this genus, of which only 2 species cause infections in humans - *P. anaerobius* and the recently identified *P. stomatis*¹³. Cells are non-motile cocci and coccobacilli. They vary in size from 0.3-2.0µm and are usually arranged in chains, pairs, tetrads or clumps; most species are present either as chains or clumps. Most species retain Gram stain well, but some present a characteristic decolorized appearance after incubation for 48hr. Growth on enriched blood agar is more rapid than with other species of Gram positive anaerobic cocci (GPAC); most strains form distinctive colonies, 1mm in diameter after 24hr, which are grey with slightly raised off-white centres and which usually give off a distinctive, sickly sweet odour. They are weakly saccharolytic, catalase and indole negative. Nitrate is not reduced to nitrite.

It has also been isolated from abscesses from a wide range of human clinical specimens including the brain, ear, jaw, pleural cavity, blood, spinal and joint fluid and pelvic, urogenital and abdominal regions. It is mostly associated with mixed infection sites but there have been some reports of isolation from pure culture¹.

***Peptoniphilus* species**

The genus *Peptoniphilus* now contains 12 validly published species, of which 10 species have been isolated from human clinical specimens¹⁴. Cells are non-motile cocci and they may occur in pairs, short chains, tetrads or small clusters. On blood agar, colonies are grey, convex, circular, entire, opaque, 2 - 3mm with a whiter central peak. Carbohydrates are not fermented. The major metabolic end-product from peptone/yeast extract/glucose (PYG) medium is butyric acid. The indole test results are strain-dependent. Species are coagulase negative except *Peptoniphilus indolicus*¹⁵.

These species are often isolated from various human clinical specimens such as vaginal discharges, ovarian and peritoneal abscesses. It has also been isolated from human sacral ulcer and from a human lachrymal gland abscess¹.

***Parvimonas* species**

The genus *Parvimonas* now contains only one species, *Parvimonas micra*¹⁶. Cells are non-motile cocci occurring in chains. They do not ferment carbohydrates and are indole, coagulase and urease negative. Their colonies have a diameter of 1mm and are usually white in colour, domed, glistening and typically surrounded by a yellow-brown halo of discoloured agar up to 2mm wide on enriched blood agar plates. This specie can have 2 colony types; a smooth-colony (S) morphology, which is recognizable by white, dome-shaped, mucous colonies; and a rough-colony (R) morphology, which produces dry white colonies with wrinkled edges. These two morphology types are serologically distinguishable; the S colony type represents serotype a, while the R colony type represents serotype b. Both types can be isolated from sub-gingival plaque samples; the R type is always isolated in association with the S type, whereas the S type can also be isolated alone¹⁷.

They are isolated from dental plaques in most periodontitis patients. It is often isolated from other oral infections, such as endodontic lesions and peritonsillar infections. This species is also commonly isolated from abscesses associated with mixed anaerobic infections throughout the human body; cases of polymicrobial pulmonary and cerebral abscesses, female genital tract infections, and endocarditis infections¹.

***Finegoldia* species**

The genus *Finegoldia* now contains only one species, *Finegoldia magna*¹⁸. Cells vary from 0.8 – 1.6µm in diameter and occur predominately in masses but occasionally in pairs or short chains. The growth rate in vitro is relatively slow. On enriched blood agar for 2–5 days, colonies range 1–2mm in diameter. The colour of the colonies is most frequently translucent, but can vary from white to grey and even yellow.

Acetic acid is the major fermentation product and most strains produce weak acid from fructose and only a few strains from glucose¹. Peptones and amino acids can be used as major energy sources. Coagulase, indole and urease are not formed.

It has been frequently isolated from human pathological specimens, particularly infections of skin, soft tissue, bone and joint¹⁹⁻²¹. It has also been isolated from an abdominal wound¹. This organism has been associated with multiple clinical syndromes - including cardiac and pulmonary infections, such as native and prosthetic valve endocarditis, pericarditis, mediastinitis, necrotizing pneumonia, empyema, skin, soft tissue and musculoskeletal infections including necrotizing fasciitis, septic arthritis, native and prosthetic joint infections and polymicrobial vaginosis²¹.

***Anaerococcus* species**

There are currently 7 validly published species and they all affect humans²². Cells are non-motile cocci that are in pairs, tetrads, irregular masses or chains. Individual cells vary in size from 0.6-0.9µm in diameter. Colonies on blood agar plate at 5 days are grey, flat or low convex, entire, circular, often matt, 1-2mm in diameter with whiter centres. They metabolise peptones and amino acids and the major metabolic end-products are butyric acid, lactic acid and small amounts of propionic and succinic acids. Most species are able to ferment several carbohydrates, but most are weakly fermentative. Glucose, fructose, sucrose and lactose are major fermentative sugars. Most species do not produce indole and are also urease and coagulase negative¹⁵.

Members of the genus are typically isolated from the human vagina and various purulent secretions¹⁵.

Other Gram positive cocci associated with human infection are;***Atopobium* species**

There are five species within this genus²³. Gram stains revealed small Gram positive non-motile cocco-bacilli or elliptical found as single elements or in pairs or short chains. They are non-spore formers and grow only under anaerobic conditions (at 25-45°C) as tiny greyish non-haemolytic colonies. They are also known to produce large amounts of lactic acid from carbohydrates fermentation. They are indole, catalase and urease negative.

Atopobium species are members of the human commensal microbiota which have been reported only rarely in oral infections, abdominal wounds, blood, and pelvic abscesses, and in most instances, these bacteria were found associated with other microorganisms²⁴. It has also been isolated from women with a tubo-ovarian abscess and from a healthy patient²⁵.

***Coprococcus* species**

There are three species within this genus²⁶. Cells are non-motile, cocci which usually occur in pairs. Cells may decolorize easily, particularly in media containing a fermentable carbohydrate. Cells were usually round, and 0.7-1.3µm in diameter; they

could be slightly elongate in peptone-yeast extract (PY)-glucose cultures. On blood agar incubated for 2 days anaerobically, surface colonies are punctiform, circular, entire, convex, translucent, whitish, smooth, shiny, and without haemolytic activity²⁷.

They actively ferment carbohydrates, producing butyric and acetic acids with formic or propionic and/or lactic acids unlike ruminococci. Fermentable carbohydrates are either required or are highly stimulatory for growth and continued subculture unlike *Peptococcus* and *Peptostreptococcus* whereas peptones are used as a nitrogen source.

It can be isolated from human faeces²⁷.

The type species is *Coprococcus eutactus*.

Ruminococcus species

There are 18 species within this genus, 11 of which have been reclassified to the genera *Blautia* and *Trichococcus*²⁸. Cells are non-motile cocci occurring in chains or pairs and do not produce spores. They can ferment cellulose and other carbohydrates with the production of succinic acid. On agar, cells look almost spherical and 0.8-0.9µm in diameter. They also produce yellow pigment on cellulose. They are catalase, indole and urease negative.

These species occur in vast numbers in rumen of cattle and sheep, and probably also in that of other ruminants and in caecum and colon of herbivorous mammals²⁹. It has also been isolated in human faeces²⁷.

The type species is *R. flavefaciens*.

Sarcina species

There are two species within this genus – *Sarcina maxima* and *Sarcina ventriculi*³⁰. Cells are cocci and have a cuboidal cell arrangement. On blood agar, colonies are pale yellow, 2-4mm in diameter, and were usually surrounded by a yellow halo in the medium.

They ferment carbohydrates and reduce nitrates. The main difference between the two species are that *S. maxima* has no extracellular cellulose and produces butyric acid from glucose, whilst *S. ventriculi* has extracellular cellulose and produces ethanol and not butyric acid from glucose.

They have been isolated from gastric contents and faeces of patients with gastrointestinal disorders and it has also been reported to be found in faeces from healthy adults³¹.

Blautia species

There are currently ten species within the genus of which nine species have been isolated in human faeces (*Blautia coccoides*, *Blautia faecis*, *Blautia hansenii*, *Blautia hydrogenotrophica*, *Blautia luti*, *Blautia producta*, *Blautia schinkii*, *Blautia stercoris* and *Blautia wexlerae*)³².

Cells are non-motile coccoid or oval shaped, pointed ends are often observed. Spores are not normally observed, but may be produced by some strains. Colonies on blood agar are 1–2mm in diameter, grey with a white centre, umbonate and opaque with entire edges.

They are chemo-organotrophic and obligately anaerobic having a fermentative type of catabolism. Some species use H₂/CO₂ as major energy sources. The major end

products of glucose metabolism are acetate, ethanol, hydrogen, lactate and succinate. They are indole and catalase negative but are positive for urease³³.

The type species of the genus is *Blautia coccooides*.

Murdochiella species

The genus *Murdochiella* now contains only one specie, *Murdochiella asaccharolytica*, which is the type species³⁴. Cells are cocci and non-motile. Cells are 0.5–0.6mm in diameter and occur in pairs and short chains. They are obligately anaerobic. Colonies on blood agar plates at 5 days are grey, flat or low-convex, circular, entire, white and opaque with a diameter of 2–3mm.

They are Indole positive, catalase and urease negative. Nitrate is not reduced. Carbohydrates are not fermented. In broth, major amounts of lactic acid and moderate amounts of acetic, butyric and succinic acids are produced.

They have been isolated from human wound specimens³⁵.

Principles of Identification

Colonies are usually isolated on fastidious anaerobe agar with or without neomycin or blood agar incubated anaerobically. Colonies may be characterised by colonial morphology, Gram stain reaction and sensitivity to metronidazole. Some species may require longer than 48hr incubation to produce visible growth. Some anaerobes are susceptible to neomycin; all samples from normally sterile sites should be cultured on neomycin selective agar and a non-selective agar. Identification tends to be undertaken only if clinically indicated.

Classification of many anaerobes to species or even genus level requires additional biochemical tests such as fluorescence under long wave UV light (365nm), pigment production, carbohydrate fermentation tests or metabolic end product analysis by GLC. Further identification may be undertaken, using commercial kits. Full molecular identification using for example, MALDI-TOF MS can be used to identify anaerobic cocci isolates to species level.

Identification of clinically significant or unusual organisms may be carried out by the Anaerobe Reference Laboratory, Cardiff.

Technical Information/Limitations

Agar Media

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.

Metronidazole susceptibility

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 (eg *Peptostreptococcus* species, *Anaerococcus* species, *Atopobium vaginae*) 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^{36,37}.

Commercial Identification Kits

Databases accompanying commercial kits are often incomplete or inaccurate, and with a rapid increase in the number of newly described anaerobic cocci species, this will become more of a problem. In addition, the interpretation of test results involves substantial subjective judgement eg *Anaerococcus vaginalis* being misidentified as *Anaerococcus tetradius* or *Anaerococcus prevotii*, as well as *Atopobium vaginae* which are not readily identified by commercial diagnostic kits and so results are interpreted with caution and in conjunction with other test results^{25,38,39}.

MALDI-TOF MS

MALDI-TOF method has special importance in routine identification of pathogens that require long incubation times for isolation and are biochemically inactive, such as anaerobic bacteria. However, its ability to identify anaerobic species currently is not as robust as it is for the routine species-level identification of other groups of bacteria; therefore, the use of additional confirmatory testing will likely be necessary for some time to come. There is also a need for existing databases to be expanded and optimised to improve accuracy^{40,41}.

1 Safety Considerations⁴²⁻⁵⁸

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^{4,8,11}

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

V. parvula, *V. atypical*, *V. dispar*, *V. montpellierensis*, *V. rogosae*, *V. tobetsuensis*

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

A. fermentans, *A. intestini*

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

M. elsdenii, *M. micronuciformis*

Anaerobic Gram Positive Cocci^{1,36,59}

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

P. niger

***Peptoniphilus* species reported to have caused human infection⁵⁹**

P. assacharolyticus, *P. harei*, *P. ivorii*, *P. lacrimalis*, *P. gorbachii*, *P. olsenii*, *P. coxii*, *P. duerdenii*, *P. koenoeneniae*, *P. tyrrelliae*,

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

P. anaerobius, *P. stomatis*

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

A. prevotii, *A. octavius*, *A. hydrogenalis*, *A. tetradius*, *A. vaginalis*, *A. murdochii*, *A. lactolyticus*

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

F. magna

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

P. micra

Other Genera of Anaerobic Gram Positive Cocci Reported to have Caused Human Infection^{24,25,27,31,33,35,36,60}

Atopobium parvulum, *Atopobium minutum*, *Atopobium rimae*, *Atopobium vaginae*, *Coprococcus eutactus*, *Coprococcus comes*, *Coprococcus catus*, *Sarcina ventriculi*, *Ruminococcus champanellensis*, *Ruminococcus faecis*, *Ruminococcus gauvreauii*, *Blautia hansenii*, *Blautia producta*, *Blautia hydrogenotrophica*, *Blautia luti*, *Murdochiella asaccharolytica*

Other species may be associated with human disease.

3 Identification

3.1 Microscopic Appearance

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

Anaerobic Gram positive cocci

Peptostreptococcus, *Peptococcus* and *Peptoniphilus* species are cocci arranged in chains, pairs, tetrads or clumps.

Parvimonas species are cocci occurring in chains.

Finegoldia species vary in size and occur predominately in masses but occasionally in pairs or short chains.

Anaerococcus species are cocci that occur in pairs, tetrads, irregular masses or chains.

Other Anaerobic Gram positive cocci

Atopobium species are small Gram positive coccobacilli or elliptical found as single elements or in pairs or short chains.

Coprococcus species are cocci; occasionally ovoid, usually occur in pairs.

Ruminococcus and *Murdochiella* species are cocci occurring in pairs or chains.

Sarcina species are cocci and they have a cuboidal cell arrangement.

Blautia species are coccoid or oval shaped, pointed ends are often observed.

Anaerobic Gram negative cocci

Veillonella are small cocci arranged in clumps.

Acidaminococcus species are cocci often occurring as oval or kidney-shaped diplococci.

Megasphaera species are cocci arranged in pairs or occasionally occurring in chains.

3.2 Primary Isolation Media

Fastidious anaerobe agar or blood agar with/without neomycin (some anaerobic organisms may be inhibited by neomycin) incubated anaerobically for 48hr at 35-37°C⁶¹.

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
Gram positive anaerobic cocci	
<i>Finegoldia magna</i>	Small colonies (<1.0mm), often with variation in size and colour. Colonies may be both convex and whitish and flatter and translucent on the same plate.
<i>Peptostreptococcus</i> species	Colonies 1-2mm in diameter, grey with slightly raised off-white centres, sensitive to Sodium Polyanethol Sulfonate (SPS) disc.
<i>Anaerococcus</i> species	Colonies 1-2mm in diameter, glistening, low convex and usually whitish to lemon-yellow.
<i>Parvimonas micra</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 niger</i>	Small colonies (<1.0mm), raised, grey, becoming dark brown/black.
<i>Peptoniphilus</i> species	Colonies are grey, convex, circular, entire, opaque, 2-3mm with a whiter central peak.
<i>Atopobium</i> species	Tiny pinhead non-haemolytic colonies (<1.0mm) are formed after 48hr incubation on agar.
<i>Coprococcus</i> species	Surface colonies are punctiform, circular, entire, convex, translucent, whitish, smooth, shiny, and without haemolytic activity.
<i>Sarcina</i> species	Colonies are pale yellow, 2-4mm in diameter, and were usually surrounded by a yellow halo in the medium.
<i>Ruminococcus</i> species	Cells look almost spherical and 0.8-0.9µm in diameter. They also produce yellow pigment on cellulose.
<i>Blautia</i> species	Colonies on blood agar are 1-2mm in diameter, grey with a white centre, umbonate and opaque with entire edges.
<i>Murdochiella asaccharolytica</i>	Colonies on blood agar plates at 5 days are grey, flat or low-convex, circular, entire, white and opaque with a diameter of 2-3mm.
Gram negative anaerobic cocci	
<i>Veillonella</i> species	Small colonies (<1.0mm) after 48hr incubation. May fluoresce red under long wavelength UV light (365nm).
<i>Megasphaera</i> species	The colonies are circular, convex, shiny, translucent with a smooth surface and approximately 0.5-1.0mm in diameter, non-pigmented and non-haemolytic. They are slightly rough and adherent to butyrous.
<i>Acidaminococcus</i> species	Colonies on blood agar are generally about 0.1-0.2mm in diameter and are round, entire, slightly raised, and whitish grey or nearly transparent, non-pigmented and non-haemolytic.

3.4 Test Procedures

3.4.1 Biochemical tests

Metronidazole sensitivity

A zone of inhibition to metronidazole 5µg disc is considered susceptible. However, resistance has been reported for Gram positive anaerobic cocci – such as *Peptococcus niger* and several species within the genus *Peptostreptococcus* (many of which have been reclassified to other genera). These organisms may be overlooked by this approach⁶².

Carbohydrate Fermentation Tests

Urease Test ([TP 36 - Urease Test](#))

This is used to aid in species differentiation eg between *Peptostreptococcus* species.

Spot Indole Test ([TP 19 - Indole Test](#))

Additional tests:

Catalase test ([TP 8 – Catalase Test](#))

Nitrate reduction Tests

Sodium Polyanethol Sulphonate (SPS) Identification discs

Specialized tests:

Gas Liquid Chromatography (GLC)

This is also known as “Gas Chromatography”. This is a separation technique in which the substances to be separated are moved by an inert gas along a tube filled with a finely divided inert solid coated with a non-volatile oil; each component migrates at a rate determined by its solubility in oil and its vapour pressure.

This has been successfully used to classify Gram positive anaerobic cocci into group based on the major end products of metabolism. Its limitations are that many laboratories do not have ready access to GLC equipment and because the protocol is not only laborious but time-consuming⁶³.

3.4.2 Commercial identification systems

Laboratories should follow manufacturer’s instructions and rapid tests and kits and should be validated and shown to be fit for purpose prior to use. Results should be interpreted with caution and in conjunction with other test results.

3.4.3 Matrix-Assisted Laser Desorption/Ionisation - Time of Flight (MALDI-TOF)

Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS), which can be used to analyse the protein composition of a bacterial cell, has emerged as a new technology for species identification. This has been shown to be a rapid and powerful tool because of its reproducibility, speed and sensitivity of analysis. The advantage of MALDI-TOF as compared with other identification methods is that the results of the analysis are available within a few hours rather than several days. The speed and the simplicity of sample preparation and result acquisition associated with minimal consumable costs make this method well suited for routine and high-throughput use⁶⁴.

MALDI-TOF MS has become the new gold standard for the routine identification of clinical anaerobes and will over time replace other identification techniques in the clinical microbiology laboratories⁶⁵.

MALDI-TOF MS has been used for the identification of phylogenetically heterogeneous groups of microorganisms such as Gram positive anaerobic cocci and for identifying Gram negative anaerobic cocci such as *Veillonella* species^{41,66,67}. However, existing databases will need to be expanded and optimised to improve accuracy⁴⁰.

3.4.4 Nucleic Acid Amplification Tests (NAATs)

PCR is usually considered to be a good method for bacterial detection as it is simple, rapid, sensitive and specific. The basis for PCR diagnostic applications in microbiology is the detection of infectious agents and the discrimination of non-pathogenic from pathogenic strains by virtue of specific genes. However, it does have limitations. Although the 16S rRNA gene is generally targeted for the design of species-specific PCR primers for identification, designing primers is difficult when the sequences of the homologous genes have high similarity.

This has been used for the rapid identification of Gram positive anaerobic cocci and will therefore permit a more accurate assessment of the role of various GPAC species in infection and of the degree of antimicrobial resistance in each of the group members³⁸.

3.5 Further Identification

Rapid Methods

A variety of current typing methods have been developed for isolates from clinical samples; these include molecular techniques such as PCR- restriction fragment length polymorphism (PCR-RFLP), 16S rRNA gene sequencing and even whole-genome sequencing (WGS). All of these approaches enable subtyping of strains, but do so with different accuracy, discriminatory power, and reproducibility.

However, some of these methods remain accessible to reference laboratories only and are difficult to implement for routine bacterial identification in a clinical laboratory.

16S rRNA gene sequencing analysis

A genotypic identification method, 16S rRNA gene sequencing is used for phylogenetic studies and has subsequently been found to be capable of re-classifying bacteria into completely new species, or even genera. It has also been used to describe new species that have never been successfully cultured.

This has been used to accurately identify Gram positive anaerobic cocci, eg *Peptostreptococcus anaerobius*, *Peptoniphilus harei*, *Finegoldia magna*, *Parvimonas micra*, *Atopobium* species etc. as well as Gram negative anaerobic cocci, eg *Megasphaera* species, *Acidaminococcus* species, etc^{36,39,68}.

This technique has also been used to reclassify organisms to other genera (for example, the genus *Peptostreptococcus* is very heterogeneous and so *Peptostreptococcus magnus* and *Peptostreptococcus micros* were transferred to two new genera, *Finegoldia* and *Parvimonas*, respectively) as well as to describe and characterise new species, eg *Atopobium vaginae*, *Peptoniphilus gorbachii*, *Peptoniphilus olseni*, and *Anaerococcus murdochii* isolated from human clinical specimens^{59,60}.

PCR- restriction fragment length Polymorphism (PCR-RFLP)

This method requires only PCR and one or two enzymes and therefore is technically less demanding than the majority of other molecular approaches. It is easier to use, less expensive and less equipment dependent than sequencing. Due to the limited number of stable features that can be used for species discrimination, many taxa remain difficult to distinguish from one another and are misidentified by phenotypic tests.

PCR protocols based on 16S rRNA gene sequences has been developed and used for the identification of *Parvimonas micra* by using specie specific primers followed by RFLP analysis. This has proved to be an adequate tool for the correct identification, irrespective of their phenotypic characterization but further studies needs to be done to confirm the copy number of rRNA operons in *P. micra* and to correlate the different genotypes with phenotypic traits and virulence⁶⁹.

It has also been used for the identification of *Peptostreptococcus* species in clinical microbiology laboratories⁷⁰.

Whole Genome Sequencing (WGS)

This is also full genome sequencing, complete genome sequencing, or entire genome sequencing. It is a laboratory process that determines the complete DNA sequence of an organism's genome at a single time. There are several high-throughput techniques that are available and used to sequence an entire genome such as Pyrosequencing, nanopore technology, Illumina sequencing, Ion Torrent sequencing, etc. This sequencing method holds great promise for rapid, accurate, and comprehensive identification of bacterial transmission pathways in hospital and community settings, with concomitant reductions in infections, morbidity, and costs.

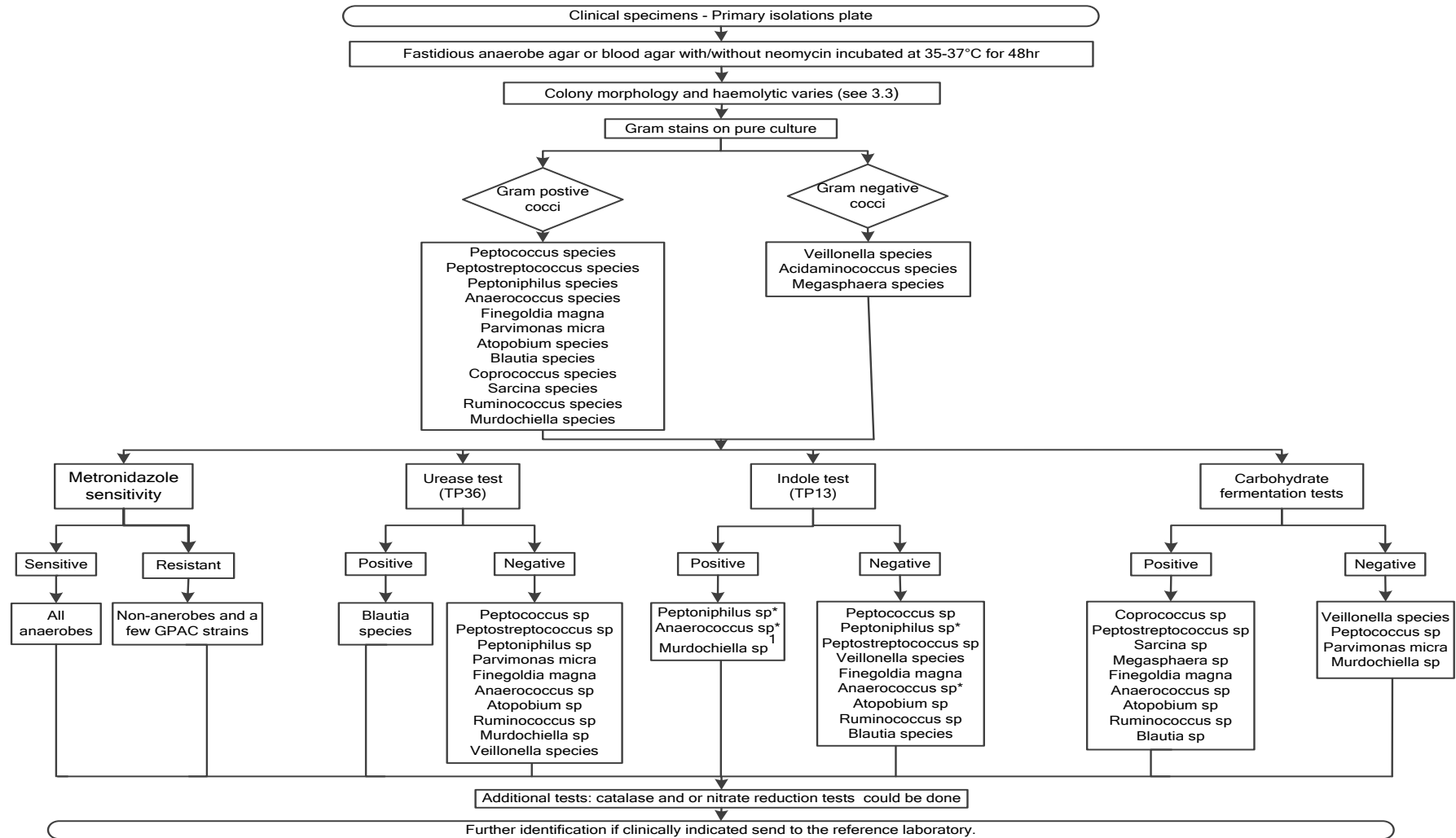
WGS was first used for the complete genome sequence of *Finnegoldia magna* amongst other GPAC in detail and its nature as an opportunistic pathogen⁷¹. This has been used to characterize the genomic structure of *Anaerococcus prevotii*⁷².

This rapid method has also been used successfully to explore the phylogeny of human oral pathogen, *Atopobium parvulum* that has been found to be associated with halitosis (oral malodour) but not with periodontitis⁷³.

3.6 Storage and Referral

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

4 Presumptive Identification of Anaerobic Cocci



*These give variable test results.

5 Reporting

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

According to local protocols, inform the medical microbiologist of presumptive or confirmed anaerobes when the request 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 anginal sepsis, often with jugular suppurative endophlebitis and haematogenous pulmonary abscesses)

Follow local protocols for reporting to clinician.

5.4 CCDC

Refer to local Memorandum of Understanding.

5.5 Public Health England⁷⁴

Refer to current guidelines on CIDSC and COSURV reporting.

5.6 Infection Prevention and Control Team

N/A

6 Referrals

6.1 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:

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

<https://www.gov.uk/government/collections/anaerobe-reference-unit-arucardiff>

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

England and Wales

<https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services>

Scotland

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

Northern Ireland

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

7 Notification to PHE^{74,75} or Equivalent in the Devolved Administrations⁷⁶⁻⁷⁹

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'.

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

Other arrangements exist in [Scotland](#)^{76,77}, [Wales](#)⁷⁸ and [Northern Ireland](#)⁷⁹.

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