UK Standards for Microbiology Investigations

Identification of Anaerobic Cocci

Issued by the Standards Unit, Microbiology Services PHE

Acknowledgments

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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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- SMIs provide clinicians with information about the standard of laboratory services they should expect for the investigation of infection in their patients and the documents provide information that aids the electronic ordering of appropriate tests from hospital wards
- SMIs also provide commissioners of healthcare services with the standards of microbiology investigations they should be seeking as part of the clinical and public health care package for their population

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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 essential laboratory methodologies which underpin quality, for example assay validation, quality assurance and understanding uncertainty of measurement.

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[#] UK Standards for Microbiology Investigations were formerly known as National Standard Methods.

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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@hpa.org.uk.

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

Amendment No/Date.	5/dd.mm.yy <tab+enter>
Issue no. discarded.	2.2
Insert Issue no.	xxx
Section(s) involved.	**Amendment.**

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

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 and

ID 10 - Identification of aerobic actinomycetes cover the identification of 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 which are known to cause infections in humans- *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 genera[1]. 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 genus[3]. 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 diplococcic 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 CO_2 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 nitrite[4].

They are found in the mouths and in the intestinal and respiratory tracts of man.

Megasphaera species

There are currently 5 species within this genus[5]. Cells are cocci, 0.4 - 2.0 µm 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 to 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, CO_2 , and some H_2[6].

On blood agar, the colonies are circular, convex, shiny, translucent with a smooth surface and approximately 0.5–1.0 mm 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[7].

*Acidaminococcus s*pecies

There are currently 2 species within this genus[8]. Cells are cocci, 0.6-1.0 um 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 to 0.2 mm 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 CO_2 are produced; propionic acid and hydrogen are not produced[9].

They have been isolated in the intestine of man as well as from other clinical samples [10].

Anaerobic Gram positive cocci

The medically important species are;

Peptococcus species

The genus *Peptococcus* now contains only one species, *Peptococcus niger*[11]. 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 plates[1].

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 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[12], of which only 2 species cause infections in humans - *P. anaerobius* and the recently identified *P. stomatis*. Cells are non-motile cocci and coccobacilli. They vary in size from 0.3 – 2.0 µm 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 48 h. Growth on enriched blood agar is more rapid than with other species of Gram positive anaerobic cocci (GPAC); most strains form distinctive colonies, 1 mm in diameter after 24 h, 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 culture[1].

Peptoniphilus species

The genus *Peptoniphilus* now contains 12 validly published species, of which 10 species have been isolated from human clinical specimens[13]. 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 - 3 mm 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*[14].

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 abscess[1].

Parvimonas species

The genus *Parvimonas* now contains only one species, *Parvimonas micra*[15]. 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 1 mm and are usually white in colour, domed, glistening and typically surrounded by a yellow-brown halo of discoloured agar up to 2 mm 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 alone[16].

They are isolated from dental plaque 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 infections[1].

Finegoldia species

The genus *Finegoldia* now contains only one species, *Finegoldia magna* [17]. Cells vary from 0.8 – 1.6 µm 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 1–2 mm 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 glucose [1]. Peptones and amino acids can be used as major energy sources. Coagulase, indole and urease are not formed.

It has been isolated from an abdominal wound [1].

Anaerococcus species

There are currently 7 validly published species[18] and they all affect humans. Cells are non-motile cocci that are in pairs, tetrads, irregular masses or chains. Individual cells vary in size from 0.6 – 0.9 µm in diameter. Colonies on blood agar plate at 5 days are grey, flat or low

convex, entire, circular, often matt, 1 to 2 mm 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 negative [14].

Members of the genus are typically isolated from the human vagina and various purulent secretions[14].

Other Gram positive cocci associated with human infection are;

Atopobium species

There are five species within this genus[19]. 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 microorganisms[20]. It has also been isolated from women with a tubo-ovarian abscess and from a healthy patient[21].

Coprococcus species

There are three species within this genus[22]. 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 to 1.3 µm 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 activity[23].

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 faeces[23].

The type species is *Coprococcus eutactus*.

Ruminococcus species

There are 18 species within this genus but 11 of which have been reclassified to the genera *Blautia* and *Trichococcus*[24]. 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 – 0.9 µm in diameter. They also produce yellow pigment on cellulose. They are catalase, indole and urease negative.

These species occurs in vast numbers in rumen of cattle and sheep, and probably also in that of other ruminants and in caecum and colon of herbivorous mammals[25]. It has also be isolated in human faeces[23].

The type species is *R. flavefaciens.*

Sarcina species

There are two species within this genus – *Sarcina maxima* and *Sarcina ventriculi*[26]. Cells are cocci and have a cuboidal cell arrangement. On blood agar, colonies are pale yellow, 2-4 mm 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 gastro-intestinal disorders and it has also been reported to have found in faeces from healthy adults[27].

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*[28].

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– 2 mm 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 H_2/CO_2 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 urease[29].

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

Murdochiella species

The genus *Murdochiella* now contains only one specie, *Murdochiella asaccharolytica*, which is the type species[30]. Cells are cocci and non-motile. Cells are 0.5–0.6 mm 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 2–3 mm.

They are Indole positive and 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 specimens[31].

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's stain reaction and are sensitive to metronidazole. Some species may require longer than 48hrs 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 Unit, 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

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 (e.g. *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 [32,33].

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 e.g. *Anaerococcus vaginalis* being misidentified as *Anaerococcus tetradius* or *Anaercoccus 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[21,34,35] .

1 Safety Considerations[36-42,42-44]

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 [4,7,10]

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 [1,32,45]

Peptococcus species reported to have caused human infection

P. niger

Peptoniphilus species reported to have caused human infection [45]

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 [20,21,23,27,29,31,32,46]

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 cocco-bacilli 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 48 hrs at 35-37°C[47].

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	
Finegoldia magna	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
Peptostreptococcus species	Colonies 1-2mm in diameter, grey with slightly raised off-white centres, sensitive to Sodium Polyanethol Sulfonate (SPS) disc
Anaerococcus species	Colonies 1-2mm in diameter, glistening, low convex and usually whitish to lemon-yellow
Parvimonas micra	Small colonies (<1.0mm), typically white (but sometimes grey), glistening and

	domed, sometimes surrounded by a yellow-brown halo up to 2mm wide
Peptococcus niger	Small colonies (<1.0mm), raised, grey, becoming dark brown/black
Peptoniphilus species	Colonies are grey, convex, circular, entire, opaque, 2 - 3 mm with a whiter central peak.
Atopobium species	Tiny pinhead non-haemolytic colonies (<1.0mm) are formed after 48 hrs incubation on agar.
Coprococcus species	Surface colonies are punctiform, circular, entire, convex, translucent, whitish, smooth, shiny, and without haemolytic activity.
Sarcina species	Colonies are pale yellow, 2-4 mm in diameter, and were usually surrounded by a yellow halo in the medium.
Ruminococcus species	Cells look almost spherical and 0.8 – 0.9 µm in diameter. They also produce yellow pigment on cellulose.
Blautia species	Colonies on blood agar are 1– 2 mm in diameter, grey with white centre, umbonate and opaque with entire edges.
Murdochiella asaccharolytica	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	
Veillonella species	Small colonies (<1.0mm) after 48hrs incubation. May fluoresce red under long wavelength UV light (365nm)
Megasphaera species	The colonies are circular, convex, shiny, translucent with a smooth surface and approximately 0.5–1.0 mm in diameter, non-pigmented and non-haemolytic. They are slightly rough and adherent to butyrous.
Acidaminococcus species	Colonies on blood agar are generally about 0.1 to 0.2 mm in diameter and are round, entire, slightly raised, and whitish grey or nearly transparent, non-pigmented and non-haemolytic.

3.4 Test Procedures

Metronidazole sensitivity

A zone of inhibition to metronidazole 5µg disc is considered susceptible. However, resistance has been reported for gram positive anaerobic cocci and such organisms may be overlooked by this approach (Hamit, 2006).

Carbohydrate Fermentation Tests

Urease Test (TP 36 - Urease Test)

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

Spot Indole Test (TP 19 - Indole test)

Commercial identification kits

Laboratories must follow manufacturer's instructions and rapid tests and kits and must 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.

Additional tests:

Catalase test (TP 08 – 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-consuming[48].

3.5 Further Identification

Rapid Methods

A variety of current rapid identification and sensitivity methods have been developed for isolates from clinical samples; these include molecular techniques such as Real-time Polymerase Chain reaction (PCR), PCR- restriction fragment length polymorphism (PCR-RFLP), 16S rRNA gene sequencing, Matrix Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) 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, these methods remain accessible to reference laboratories only and are difficult to implement for routine bacterial identification in a clinical laboratory.

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 use[49].

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 laboratories ([50].

MALDI-TOF MS has been used for the identification of phylogenetically heterogeneous groups of microorganisms such as Gram positive anaerobic cocci([51,52]} and for identifying Gram negative anaerobic cocci such as *Veillonella* species[53]. However, existing databases will need to be expanded and optimised to improve accuracy.

Real-time Polymerase Chain reaction (RT-PCR)

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 species 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 members[34]

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, e.g. *Peptostreptococcus anaerobius, Peptoniphilus harei, Finegoldia magna, Parvimonas micra, Atopobium* species etc. as well as Gram negative anaerobic cocci, e.g. *Megasphaera* species, *Acidaminococcus* species, etc.[32,35,54].

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, e.g. *Atopobium vaginae*[46], *Peptoniphilus gorbachii*, *Peptoniphilus olsenii*, and *Anaerococcus murdochii* isolated from human clinical specimens[45].

PCR- restriction fragment length Polymorphism (PCR-RFLP)

This method requires only PCR and one or two enzymes and therefores 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 virulence[55].

It has also been used for the identification of *Peptostreptococcus* species in clinical microbiology laboratories[56].

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.

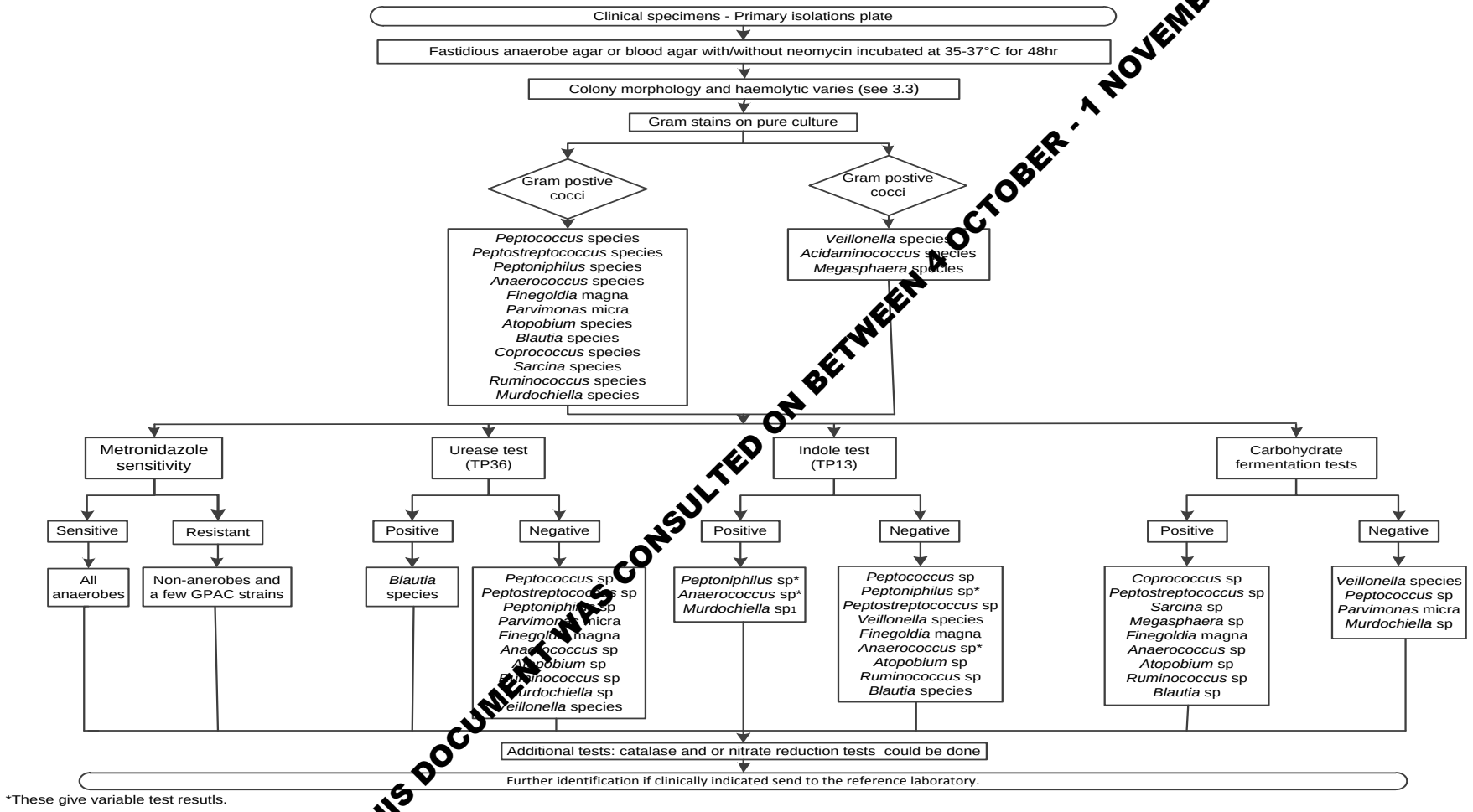
WGS was first used for the complete genome sequence of *Finegoldia magna* amongst other GPAC in detail and its nature as an opportunistic pathogen[57].This has been used to characterize the genomic structure of *Anaerococcus prevotii*[58].

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 periodontitis[59].

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 Flowchart



5 Reporting

5.1 Presumptive Identification

If appropriate growth characteristics, colonial appearance, Gram's 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 presumptive or confirmed anaerobes when the request card bears relevant information, e.g.:

- 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 England [60,61]

Refer to current guidelines on CDSC and COSURV reporting.

5.6 Infection Control Team

N/A

6 Referrals

6.1 Reference Laboratory

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory refer to:

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

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