

NATIONAL STANDARD METHOD

IDENTIFICATION OF ANAEROBIC COCCI

BSOP ID 14

Issued by Standards Unit, Department for Evaluations, Standards and Training
Centre for Infections







IDENTIFICATION OF ANAEROBIC COCCI

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STATUS OF NATIONAL STANDARD METHODS

National Standard Methods, which include standard operating procedures (SOPs), algorithms and guidance notes, promote high quality practices and help to assure the comparability of diagnostic information obtained in different laboratories. This in turn facilitates standardisation of surveillance underpinned by research, development and audit and promotes public health and patient confidence in their healthcare services. The methods are well referenced and represent a good minimum standard for clinical and public health microbiology. However, in using National Standard Methods, laboratories should take account of local requirements and may need to undertake additional investigations. The methods also provide a reference point for method development.

National Standard Methods are developed, reviewed and updated through an open and wide consultation process where the views of all participants are considered and the resulting documents reflect the majority agreement of contributors.

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The performance of standard methods depends on the quality of reagents, equipment, commercial and in-house test procedures. Laboratories should ensure that these have been validated and shown to be fit for purpose. Internal and external quality assurance procedures should also be in place.

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The HPA is an independent organisation dedicated to protecting people's health. It brings together the expertise formerly in a number of official organisations. More information about the HPA can be found at www.hpa.org.uk.

The HPA aims to be a fully 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 conditions¹.

More details can be found on the website at www.evaluations-standards.org.uk. Contributions to the development of the documents can be made by contacting standards@hpa.org.uk.

The reader is informed that all taxonomy in this document was correct at time of issue.

Please note the references are now formatted using Reference Manager software. If you alter or delete text without Reference Manager installed on your computer, the references will not be updated automatically.

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AMENDMENT PROCEDURE

Controlled document reference	BSOP ID 14
Controlled document title	Identification Of Anaerobic Cocci

Each National Standard 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.

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
2/ 03.12.09	1.1	2	All	All	Document separated in to individual NSMs please see BSOP ID 25, and BSOP ID 15 as well.

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IDENTIFICATION OF ANAEROBIC COCCI

SCOPE OF DOCUMENT

This National Standard Method (NSM) describes the characterisation of anaerobic cocci bacteria.

Anaerobic sporing organisms are described in [BSOPID 8 - Identification of *Clostridium* species](#). Anaerobic Gram negative rods are described in [BSOPID 25 – Identification of anaerobic gram negative rods](#). [BSOP ID 15 – Identification Anaerobic *Actinomyces* species](#) and [BSOPID 10 - Identification of aerobic actinomycetes](#) cover the identification of actinomycetes. Anaerobic rods can be found in [BSOP ID 25 – Identification of anaerobic gram-negative rods](#).

INTRODUCTION

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.

Gram-positive cocci

The classification of the anaerobic Gram-positive cocci is continually changing with the addition of new species and renaming of old species². 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*³.

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% CO₂.

Anaerobic Gram-negative cocci

Veillonella species

Veillonella species are small asaccharolytic cocci, measuring approximately 0.5 µm 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 (365 nm), 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 to 1.3 µm 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

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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 48 hours incubation to produce visible growth. Identification tends to be undertaken only if clinically indicated. Further identification tests include fluorescence under long wave UV light (365 nm), 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. 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.

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1 SAFETY CONSIDERATIONS⁴⁻¹⁵

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

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 ORGANISMS³

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

Peptococcus assacharolyticus

Anaerococcus hydrogenalis

Peptoniphilus harei

Peptoniphilus ivorii

Peptoniphilus lacrimalis

Anaerococcus lactolyticus

Fingoldia magna

Parvimonas micra

Anaerococcus octavius

Anaerococcus prevotii

Anaerococcus tetradius

Anaerococcus vaginalis

Anaerococcus murdochii

Peptostreptococcus stomatis

Peptoniphilus gorbachii

Peptoniphilus olsenii

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

Ruminococcus hansenii

Ruminococcus productus

Sarcina albus

Sarcina pasteurii

Sarcina ventriculi

Coprococcus eutactus

Coprococcus comes

Coprococcus catus

Other species may be associated with human disease.

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3 IDENTIFICATION

3.1 MICROSCOPIC APPEARANCE

(See [BSOFTP 39 - Staining Procedures](#))

Gram's stain

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

3.4 TEST PROCEDURES

Metronidazole

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

Nitrate reduction

SPS disc

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

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3.5 FURTHER IDENTIFICATION

Commercial identification kit

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

Other more specialized 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.

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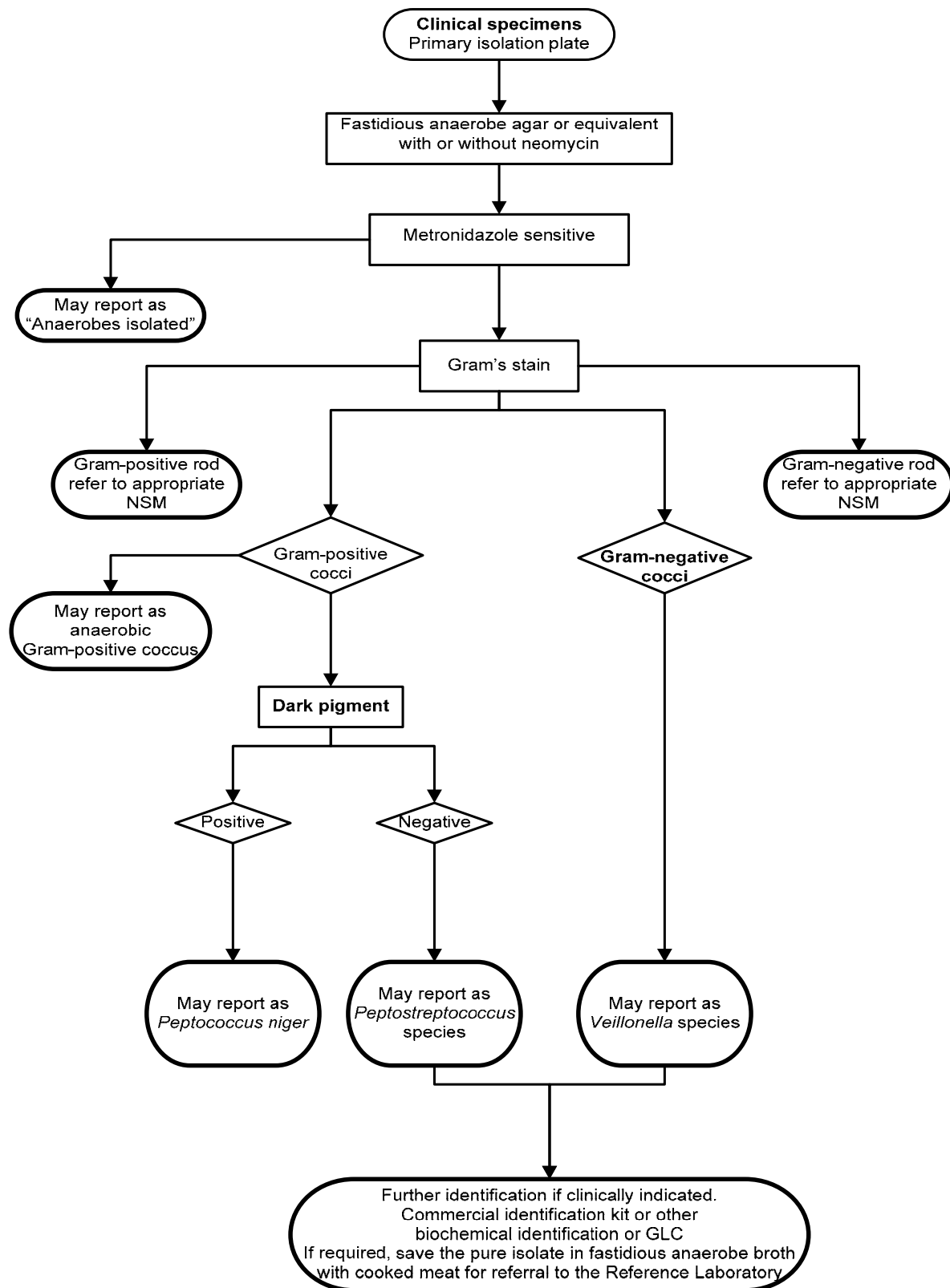
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4 PRESUMPTIVE IDENTIFICATION OF ANAEROBIC COCCI – FLOW CHART FOR GUIDANCE



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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 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 CENTRE FOR INFECTIONS¹⁶

Refer to current guidelines on CDSC and COSURV reporting.

5.6 INFECTION CONTROL STAFF

N/A

6 REFERRALS

6.1 REFERENCE LABORATORY

Anaerobe Reference Unit
NPHS
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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7 ACKNOWLEDGEMENTS AND CONTACTS

This National Standard Method has been developed, reviewed and revised by the National Standard Methods Working Group for Clinical Bacteriology (http://www.hpa-standardmethods.org.uk/wg_bacteriology.asp). The contributions of many individuals in clinical bacteriology laboratories and specialist organisations who have provided information and comment during the development of this document, and final editing by the Medical Editor are acknowledged.

The National Standard Methods are issued by Standards Unit, Department for Evaluations, Standards and Training, Centre for Infections, Health Protection Agency, London.

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