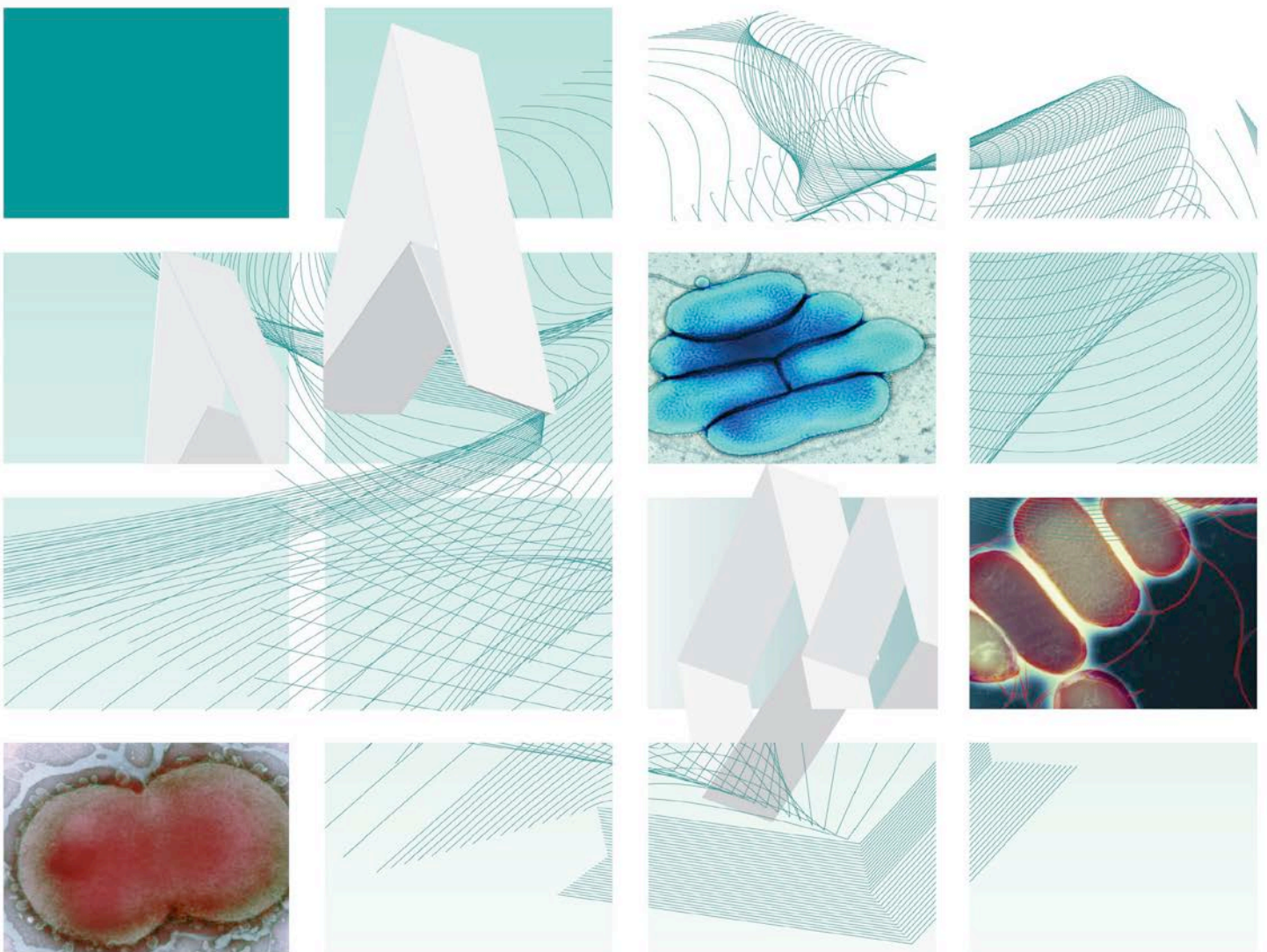




Protecting and improving the nation's health

UK Standards for Microbiology Investigations

Identification of *Neisseria* species



Acknowledgments

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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.	7/26.06.15
Issue no. discarded.	2.2
Insert Issue no.	3
Section(s) involved	Amendment
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Scope of document.	The scope has been updated to include all <i>Neisseria</i> species isolated from clinical material but with more emphasis on the two species most associated in infections of humans. A webpage link for ID 11 and ID 12 documents has been added.
Introduction.	The taxonomy of <i>Neisseria</i> species has been updated. More information has been added to the Characteristics section. The medically important species are mentioned and their characteristics described. Use of up-to-date references. Section on Principles of Identification has been updated to reflect the current name of the Reference Laboratories where presumptive <i>Neisseria</i> species are referred to.
Technical information/limitations.	Addition of information regarding oxidase test, agar media and commercial identification systems has been described and referenced.
Safety considerations.	This section has been updated on the laboratory acquired infections and its manipulation in the laboratory. The references have also been included.
Target organisms.	The section on the target organisms has been updated and presented clearly. References have

	been updated.
Identification.	Updates have been done on 3.1, 3.2 and 3.4 to reflect standards in practice. Subsection 3.5 has been updated to include the Rapid Molecular Methods.
Identification flowchart.	Modification of flowchart for identification of <i>Neisseria</i> species has been done for easy guidance.
Reporting.	Minor amendments were done in 5.1, and 5.2. Subsection 5.4 has been updated to reflect reporting practice.
Referral.	The address of the reference laboratories has been updated.
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.

Information governance and equality

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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

Public Health England. (2015). Identification of *Neisseria* species. UK Standards for Microbiology Investigations. ID 6 Issue 3. <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>

Scope of document

This SMI describes the identification of pathogenic *Neisseria* species isolated from clinical specimens and their differentiation from non-pathogenic *Neisseria* species and the related genera of *Moraxella* and *Kingella*. The identification of these two genera is covered in [ID 11 - Identification of *Moraxella* species and morphologically similar organisms](#) and [ID 12 – Identification of *Haemophilus* species and the HACEK group of organisms](#).

This SMI should be used in conjunction with other SMIs.

Introduction

Taxonomy

The genus *Neisseria* belongs to the family Neisseriaceae. There are currently 25 *Neisseria* species and 3 subspecies of which may be isolated from humans and animals¹. Four species have been reclassified^{1,2}. The clinically important *Neisseria* species (*Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Neisseria lactamica* and *Neisseria cinerea*) are relatively easy to identify from the non-pathogenic *Neisseria*. *N. gonorrhoeae* and *N. meningitidis* are the two main pathogens of the group. The other species of *Neisseria* such as *N. lactamica* and *N. cinerea* are generally considered commensals, but have been implicated as causes of infection in patients who are immunocompromised. More recent species to the genus *Neisseria* are *N. oralis*, *N. shayegani*, *N. wadsworthii*, *N. zoodegmatidis* and *N. animaloris* isolated from human clinical samples.

Characteristics

Neisseria species

Neisseria species are obligate human pathogens with no other natural host³. They are Gram negative cocci, 0.6 - 1.0µm in diameter, occurring singly but more often in pairs with adjacent sides flattened; except *Neisseria elongata*, *Neisseria weaveri* and *Neisseria bacilliformis* that consists of rods, 0.5µm wide, often arranged as diplococci or in short chains⁴⁻⁶. They are non-motile and flagella are absent. Some species produce a greenish-yellow carotenoid pigment and some may be nutritionally fastidious and haemolytic. Some species are saccharolytic. The optimum growth temperature is 35-37°C. *Neisseria* are oxidase positive and catalase positive (except *Neisseria elongata*). All except *Neisseria gonorrhoeae* and *Neisseria canis* reduce nitrite.

Pathogens

Neisseria gonorrhoeae

Cells are cocci and occur in pairs. They are non-haemolytic on blood agar and do not produce a yellowish pigment. *N. gonorrhoeae* form smooth, round, moist, uniform grey/brown colonies with a greenish colour underneath on primary isolation medium. *N. gonorrhoeae* may grow poorly on blood agar when the medium is very fresh or the number of bacteria present in the sample is especially high. They produce acid from utilising glucose and can also reduce potassium nitrite in low concentrations and not nitrates⁴.

Neisseria meningitidis

Cells are cocci occurring in pairs and they utilize glucose and maltose to produce acid. The serogroups A, D, and Y of *N. meningitidis* can reduce nitrites in low concentrations. They are non-haemolytic on blood agar plate and do not reduce nitrates. *N. meningitidis* like *N. gonorrhoeae* would form smooth, round, moist, uniform large grey/brown colonies with a glistening surface and entire edges². They do not produce yellowish pigment⁴. Due to autolysis with age, colonies may become more butyrous and rubbery to the touch of an inoculating needle.

Other *Neisseria* species that have been associated with human diseases***Neisseria lactamica***

Cells are cocci occurring in pairs and produce a yellowish pigment and some strains are haemolytic on horse blood agar. Colonies resemble that of *N. meningitidis* but may be less moist and smaller. They utilize glucose, maltose and lactose to produce acid. They differ from all other *Neisseria* species in their ability to produce acid from lactose. They reduce nitrites and also produce gas from it. They do not reduce nitrates⁴.

Neisseria cinerea

Cells are plump cocci occurring in pairs or more often in scattered clusters and are non-haemolytic on blood agar plate. Some strains produce a yellowish pigment. Colonies are small (1.0 - 1.5mm in diameter), greyish white with entire edges, and slightly granular. They do not utilise carbohydrates and can reduce nitrites and produce gas from it but not nitrates. This species are most likely misidentified as *N. gonorrhoeae* because they are phenotypically similar and fail to produce acid from glucose⁷.

Neisseria elongata

Cells are small slender rods that occur in chains and differs from the other cocci shaped members of the genus *Neisseria*. Unlike other *Neisseria*, they are catalase negative and are non-motile and not encapsulated. They elongate into filaments when exposed to sublethal concentrations of penicillin. On blood agar, they appear as greyish white, shiny opaque colonies, about 1 - 1.5mm in diameter, low-hemispherical with an entire edge. Colonies have a clay-like coherent consistency, are non-haemolytic and there is some pitting of the agar. Acid is not produced from carbohydrates and they do not reduce nitrates but nitrites. They also produce a weak yellowish pigment⁸.

There are currently 3 subspecies of *N. elongata* – *Neisseria elongata* subsp. *elongata*, *Neisseria elongata* subsp. *glycolytica* and *Neisseria elongata* subsp. *nitroreducens*. All subspecies have been reported to cause human diseases. Their classification is based on the biochemical differences between each subspecies⁹.

Neisseria elongata* subsp. *elongata

On blood agar, the colonies appear flat and non-haemolytic. They are oxidase positive but catalase negative, non-motile, and do not produce acid from glucose. They do not reduce nitrates but nitrites. *N. elongata* subsp. *elongata* differs from the other two subspecies due to its inability to produce acid from D-glucose¹⁰.

Neisseria elongata* subsp. *glycolytica

They are very small, short, slender rods with a marked tendency to occur in chains. They are non-motile and are similar to the cells of *N. elongata*. On blood agar plate,

the colonies are relatively large (2 - 3mm in diameter after about 20hr of incubation), grey, opaque, moderately raised with a flat top and smooth with a soft, homogenous consistency. The colonies are easily emulsified in saline and may be haemolytic or non-haemolytic but appear to have a slight yellow tinge after a few days. They produce acid from glucose but not from galactose, fructose, xylose, mannose, maltose, sucrose or lactose. They are strictly aerobic and are also positive for oxidase, catalase and nitrite reduction tests^{11,12}.

This subspecies differs from *N. elongata* subsp. *elongata* in producing acid from glucose and in giving a very strong catalase reaction and in the consistency of the colonies on agar.

They differed from *N. elongata* subsp. *nitroreducens* by the production of catalase and an inability to reduce nitrates¹³.

Neisseria elongata* subsp. *nitroreducens

They are rods and are similar to the cells of *N. elongata*. They are catalase negative, positive for nitrate and nitrite reduction without the production of gas, and exhibit a weak, variable D-glucose reaction or not at all. *N. elongata* subsp. *nitroreducens* are different from *N. elongata* subsp. *elongata* and *N. elongata* subsp. *glycolytica* in its ability to reduce nitrate⁹.

N. elongata subsp. *nitroreducens* appears to be a rarely occurring but often serious human pathogen. Its association with endocarditis and other systemic diseases differentiates it from the other *N. elongata* subspecies⁹.

Neisseria sicca

Neisseria sicca are cocci that occur in pairs and tetrads. Some strains produce a yellowish pigment and show haemolysis on blood agar. After 24hr of incubation, they appear as small round colonies, having a smooth surface and an entire edge developed on the blood agar plates but after 48hr, they increase in size and appear raised, rough, and black. The colonies are very firm and adherent to the medium, and are difficult to disintegrate and disperse¹⁴. They agglutinate spontaneously in saline².

They also utilize glucose, maltose, fructose and sucrose to produce acid and not lactose and mannose. They are oxidase and catalase positive and also reduce nitrites but not nitrates⁴.

Neisseria mucosa

Cells are cocci that occur in pairs. Some strains show no haemolysis on blood agar. Colonies are large, mucoid, and often adherent. Most strains are non-pigmented or greyish to buff yellow colonies. They also utilize glucose, maltose, fructose and sucrose to produce acid and not lactose and mannose. They are oxidase positive. They reduce both nitrites and nitrates which differentiates them from other *Neisseria* species⁴.

Neisseria canis

They are cocci that occur in pairs and rarely in tetrads. They do not produce a yellowish pigment and do not show haemolysis on blood agar. Colonies are smooth, butyrous, with a light yellow tinge. They do not utilize carbohydrates, but reduce nitrates and not nitrites⁴.

Neisseria flava

Neisseria flava are cocci that occur in pairs. On chocolate agar, they appear as discrete, opaque, pale yellow colonies, slightly flatter than those of the *Neisseria meningitidis*. The pigment was barely discernible, except when the organisms were grown on a light coloured medium, such as coagulated blood serum.

Strains of *N. flava* ferment glucose, maltose, and levulose to produce acid.

Neisseria flava differs from the *Neisseria gonorrhoeae* only in the possession of two additional enzyme systems, one of which permits the fermentation of disaccharides and the other the formation of pigment¹⁵.

N. subflava

Cells are cocci that occur in pairs and tetrads and have a tendency to resist Gram decolourization. They produce a yellowish pigment and show no haemolysis on blood agar. Colonies are smooth, transparent or opaque, often adherent. They often agglutinate spontaneously in saline. They also utilize glucose and maltose to produce acid and not lactose and mannose. Some strains will utilize fructose and sucrose to produce acid. They are oxidase positive and reduce nitrites but not nitrates^{2,4}.

Neisseria ovis

(Recently reclassified as *Moraxella ovis* under the family *Moraxellaceae*)²

Cells are cocci that occur in pairs. Aerobic incubation on bovine blood agar plates at 37°C produced grey, opaque, convex, β-haemolytic colonies. They are oxidase and catalase positive, non-motile and reduce nitrates but not nitrites. They do not produce acid from carbohydrates¹⁶.

Neisseria bacilliformis

They are the more recent bacillary *Neisseria* species that was isolated from human infections. They are shaped like small rods measuring 0.6µm by 1.3 - 3.0µm. This organism grows well on both chocolate agar and sheep blood agar with colony size measuring 0.5 - 1mm at 24hr. The colonies appear as round, smooth, glistening, light grey in colour. Biochemically, they are asaccharolytic and negative for indole production but positive for oxidase. Reactions in catalase and nitrate reduction tests vary according to the strain.

The morphology and asaccharolytic nature of *N. bacilliformis* may also lead to confusion with the identification of *Pasturella* species and *Moraxella* species, both of which are commensals of the upper airway. Presently, the single most reliable way to identify *N. bacilliformis* is through 16S rRNA gene sequencing⁵.

Neisseria weaveri

These are broad, plump, medium-to-large, straight rods of varying length when grown on slants and plates, with a tendency to grow in chains or longer rods in broth cultures. They are non-motile, aerobic, and non-salt requiring, and grow well between 25 and 35°C; most strains grow at 42°C. Colonies are grey-white with an entire border, flat, somewhat glistening, and smooth and variable in size. They are 1 - 2mm in diameter after 24hr of incubation at 35°C and 2 - 4mm after 48hr of incubation on sheep blood agar plate (SBAP). A zone of alpha-hemolysis is produced on SBAP in areas of heavy growth. The oxidase and catalase reactions are strongly positive. The bacterium does not utilize carbohydrates; it reduces nitrite but not nitrate and has a weakly positive phenylalanine deaminase reaction from culture grown on SBAP^{2,6}.

Neisseria flavescens

Cells are cocci and occur in pairs and tetrads. Colonies are non-haemolytic, smooth and opaque with golden yellow pigment. They are positive for nitrite reduction tests and polysaccharide synthesis from sucrose. They do not produce acid from carbohydrates or reduce nitrates.

Neisseria oralis

Cells are 0.5µm in diameter, may be present in chains and are non-motile. Colonies are small, circular, entire, raised, moist, yellow, weakly α-haemolytic and 1 - 1.5mm in diameter after 48hr of growth at 37°C in 5% CO₂. They are facultative anaerobes and growth is observed at 28 and 42°C, with no growth at 10°C. No growth is observed on MacConkey agar after 5 days. They are positive for catalase, oxidase and nitrate reduction tests; and are negative for acid production from the oxidation-fermentation tests, utilization of Simmons' citrate, hydrolysis of aesculin, urea and gelatin, indole production, decarboxylation of arginine, lysine and ornithine using Moeller's decarboxylase medium and production of H₂S in triple sugar iron agar¹⁷.

They have been isolated from the healthy gingival plaque and other clinical samples³.

Neisseria shayegani

Cells are rod-shaped and 1.0 - 1.5µm wide x 2.5 - 5.5µm long. Growth is observed between 10 and 42°C. No growth is observed on MacConkey agar after 5 days. They are facultative anaerobes. Colonies are small, circular, entire, convex, moist, light yellow to grey and non-haemolytic. They are positive for catalase, cytochrome oxidase and nitrate reduction tests; and negative for acid production from carbohydrates, utilization of Simmons' citrate, hydrolysis of aesculin, urea and gelatin, indole production, decarboxylation of arginine, lysine and ornithine¹⁸.

They have been isolated from arm wounds and also from sputum in humans.

Neisseria wadsworthii

Cells are coccoid, 1.3 - 1.8µm in diameter, and may be present in pairs and chains. Growth is observed between 10 and 42°C. No growth is observed on MacConkey agar after 5 days. They are facultative anaerobes. Colonies are small, circular, entire, convex, moist, light yellow to orange and non-haemolytic. They are positive for catalase, cytochrome oxidase and nitrate reduction tests; and negative for acid production from carbohydrates, utilization of Simmons' citrate, hydrolysis of aesculin, urea and gelatin, indole production, decarboxylation of arginine, lysine and ornithine¹⁸.

They have been isolated from hand wounds and from peritoneal fluid of humans.

***Neisseria zoodegmatis* (was previously known as Centers for Disease Control (CDC) Group Eugonic Fermenter (EF)-4b).**

Cells are coccoid rods. Colonies are circular, convex, entire, opaque, shiny, smooth and haemolytic. They are positive for catalase production, cytochrome oxidase production, growth at 37°C and at room temperature (18 - 22°C) and growth on MacConkey agar. Most strains are positive for acid production (in peptone water medium) from glucose, fermentation in the Hugh and Leifson Oxidation-Fermentation test, gelatinase production, nitrate reduction and utilization of citrate. All strains are negative for acid production (in peptone water medium) from adonitol, arabinose, cellobiose, dulcitol, glycerol, inositol, lactose, maltose, mannitol, raffinose, rhamnose, salicin, sorbitol, starch, sucrose, trehalose and xylose. All strains are negative for

acetoin production, arginine dihydrolase production, casein digestion, gluconate oxidation, β -galactosidase production, motility, ornithine decarboxylase production, phenylalanine deamination, pigment production and utilization of citrate¹⁹.

They have been isolated from human wounds resulting from dog and cat bites²⁰.

***Neisseria animaloris* (was previously known as Centers for Disease Control (CDC) Group Eugonic Fermenter (EF)-4a).**

Cells are coccoid rods. Colonies are circular, convex, entire, opaque, shiny, smooth and haemolytic. They are positive for acid production (in peptone water medium) from glucose, arginine dihydrolase production, catalase production, cytochrome oxidase production, growth at 37°C and at room temperature (18–22°C), growth on MacConkey agar and nitrate reduction. Most strains are positive for fermentation in the Hugh and Leifson Oxidation–Fermentation test and gelatinase production. All strains are negative for acid production (in peptone water medium) from adonitol, arabinose, cellobiose, dulcitol, glycerol, inositol, lactose, maltose, mannitol, raffinose, rhamnose, salicin, sorbitol, starch, sucrose, trehalose and xylose. All strains are negative for acetoin production, motility, ornithine decarboxylase production, phenylalanine deamination, pigment production and utilization of citrate¹⁹.

They have been isolated from human wounds resulting from dog and cat bites²⁰.

Principles of identification

Isolates from primary culture are identified by Gram stain, oxidase and by at least two of the following identification principles: carbohydrate utilisation, detection of preformed enzymes or reactivity with immunological reagents.

If further identification is required, presumptive isolates of *N. gonorrhoeae* and other *Neisseria* species should be referred to the Sexually Transmitted Bacteria Reference Laboratory. If isolates are known to be *N. meningitidis*, they should be sent to the Meningococcal Reference Unit (MRU) Manchester.

Contact the laboratory or see the following website for details:

<https://www.gov.uk/stbru-reference-and-diagnostic-services>

Technical information/limitations

The social consequences to the patient and the organisation of an incorrect diagnosis of gonorrhoeal disease as a result of misidentification should not be underestimated.

Oxidase test

Kingella species and *M. catarrhalis* are also oxidase positive and can be misidentified as *Neisseria*.

Media

If the *Neisseria gonorrhoeae* strain in question is sensitive to vancomycin it will fail to grow on selective medium.

Commercial identification system

The commercially available immunological reagents contain a mixture of monoclonal antibodies raised to specific epitopes on the major outer membrane protein, *Por*. Because the reagents contain a mixture of antibodies rather than a single antibody to

a cross-reactive epitope, false negative reactions do occur, although uncommonly. Because the mixtures themselves are different occasional isolates occur that give a false negative with one, but are positive with another reagent.

N. gonorrhoeae that have a mutation in the proline iminopeptidase gene and therefore appear negative for this enzyme are prevalent in England and Wales and kits that detect solely the production of aminopeptidases should not be used alone^{21,22}.

N. gonorrhoeae that are proline iminopeptidase negative will give anomalous results with carbohydrates and pre formed enzyme kits and should be confirmed with an immunological reagent.

Maltose negative strains of *N. meningitidis* have been described and may be differentiated from *N. gonorrhoeae* by their ability to produce gamma-glutamylaminotransferase. Glucose negative variants of *N. meningitidis* may also be observed.

Differentiation between *Neisseria* species

N. wadsworthii and *N. shayegani* are distinguished from most other species of *Neisseria* with validly published names by the absence of acid production from various sugars and/or the ability to reduce nitrate.

1 Safety considerations²³⁻³⁹

Although *N. meningitidis* is a Hazard group 2 organism, the processing of diagnostic samples can be carried out at Containment Level 2.

Due to the severity of the disease and the risks associated with generating aerosols, any manipulation of suspected isolates of *N. meningitidis* should always be undertaken in a microbiological safety cabinet until *N. meningitidis* has been ruled out (as must any laboratory procedure giving rise to infectious aerosols)³¹.

N. meningitidis causes severe and sometimes fatal disease. Laboratory acquired infections have been reported^{40,41}. The organism infects primarily by the respiratory route. An effective vaccine is available for some meningococcal groups. Vaccine is required for laboratory staff routinely working with the organism.

N. gonorrhoeae is also a Hazard group 2 organism which is responsible for the sexually transmitted infection, gonorrhoea and it has the potential to also cause threatening eye or throat infection - which is the most likely risk to laboratory workers through either vertical transmission or poor hygiene or inhalation of aerosols.

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

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

Compliance with postal and transport regulations is essential.

2 Target organisms^{1,5,9-11,13,15,17,19,42,43}

The main *Neisseria* species reported to have caused human infection -
N. gonorrhoeae, *N. meningitidis*, *N. lactamica*, *N. sicca*

Other *Neisseria* species that has been associated with human diseases -
N. flava, *N. subflava*, *N. cinerea*, *N. canis*, *N. elongata* subspecies *elongate*,
N. elongata subspecies *glycolytica*, *N. elongata* subspecies *nitroreducens*, *N. mucosa*,
N. bacilliformis, *N. weaveri*, *N. flavescens*, *N. oralis*, *N. shayegani*, *N. wadsworthii*,
N. zoodegmatidis (was previously known as Centers for Disease Control (CDC) Group Eugonic Fermenter (EF)-4b), *N. animaloris* (was previously known as Centers for Disease Control (CDC) Group Eugonic Fermenter (EF)-4a)

Asaccharolytic *Neisseria* species which may be misidentified as *N. gonorrhoeae* or *N. meningitidis* - *N. canis**, *N. caviae*, *N. cinerea**, *N. cuniculi*, *N. elongata**,
N. flavescens, *N. ovis** renamed as *Moraxella ovis*

Other organisms which may be misidentified as *Neisseria* species - *Moraxella catarrhalis**, *Kingella denitrificans**

* These have been reported to cause human infections.

3 Identification

3.1 Microscopic appearance

Gram stain (see [TP 39 - Staining procedures](#))

Neisseria species

Gram negative cocci arranged in pairs with long axes parallel

OR

Gram negative rods that are arranged in chains or as diplococci

3.2 Primary isolation media

GC selective agar incubated for up to 48 hours in 5-10% CO₂ at 35-37°C.

GC selective agar usually consists of GC agar base supplemented with lysed or chocolatised horse blood with or without the addition of VitoX or IsoVitaleX. Antibiotic cocktails used for selection contain vancomycin or lincomycin, colistin, trimethoprim, and nystatin or amphotericin⁴⁴.

Whole Blood agar/heated blood (chocolate) incubated for 18-48 hours in 5-10% CO₂ at 35-37°C. The media usually consist of Columbia agar base supplemented with 5% horse blood or chocolatised horse blood.

3.3 Colonial appearance

Neisseria species are usually pigmented and opaque. However, both *N. gonorrhoeae* and *N. meningitidis* form smooth, round, moist, uniform grey/brown colonies with a greenish colour underneath on primary isolation medium. *N. gonorrhoeae* grow less well on blood agar than *N. meningitidis*.

3.4 Test procedures

3.4.1 Oxidase test (see [TP 26 - Oxidase test](#))

Oxidase positive: *Neisseria* species

Note: *Kingella* species and *M. catarrhalis* are also oxidase positive and can be misidentified as *Neisseria*.

3.5 Further identification

Neisseria have a typical Gram negative envelope, which consists of a cytoplasmic membrane, a thin layer of peptidoglycan and an outer membrane. Many of the major antigens of the cell envelope are shared between *N. gonorrhoeae* and *N. meningitidis*, with the exception of the capsule which is never expressed by *N. gonorrhoeae* but, when expressed by *N. meningitidis*, enhances survival in the blood.

Neisseria species can be differentiated from similar organisms by biochemical and other tests. At least two principles of identification should be used as there are very few taxonomic differences between members of the genus and therefore definitive identification can prove problematic.

3.5.1 *Neisseria gonorrhoeae*⁴⁵

N. gonorrhoeae is sexually transmitted, primarily causing infection of the anogenital tract and is always considered a pathogen. This contrasts with *N. meningitidis* which colonises the upper respiratory tract as a commensal and occasionally invades to cause systemic disease.

3.5.1.1 Testing of risk groups and other cases

High Risk: Patients attending for sexual health care such as GUM patients (high prevalence populations)

One, preferably two, additional tests are required for the confirmation of isolates from genital samples where the Gram stain and oxidase on the specimen has given a presumptive diagnosis of infection with *N. gonorrhoeae*. These should be either biochemical or immunological. Any isolates that give a negative result with an immunological test should be tested in addition with a biochemical test that detects carbohydrate utilisation with or without aminopeptidases to eliminate the possibility of an aminopeptidase negative *N. gonorrhoeae*.

Low Risk: Patients attending primary care (low prevalence populations)^{46,47}

It is recommended that for isolates from patients considered low risk (but without medico-legal implications) two additional tests should be used for confirmation following presumptive identification. These should be biochemical and immunological. Biochemical kits should not include those that detect aminopeptidases alone, but can be those kits that include both carbohydrates and aminopeptidases.

Medicolegal: Child or sexual abuse^{46,47}

Where results are likely to have medicolegal significance, specimens should be handled in accordance with Royal College of Pathologists' guidance^{48,49}.

Note: The guideline, "Guidelines for handling medicolegal specimens and preserving the chain of evidence" published by the Royal College of Pathologists has been withdrawn and is under review. Once published, this document (ID 6) will be updated accordingly.

3.5.1.2 Approaches to identification of *N. gonorrhoeae*

Identification should be achieved by a combination of test procedures which both identify the organism and exclude other *Neisseria* species. *N. gonorrhoeae* is usually isolated from high risk patients, where it is only necessary to perform presumptive identification followed by a single confirmatory test. However, in low risk patients and in child and sexual abuse (medicolegal) cases it is necessary to use more than one confirmatory test. Detection of *N. gonorrhoeae* can be achieved by NAATs or culture. A culture should be taken in all cases of *N. gonorrhoeae* diagnosed by NAATs⁵⁰.

Presumptive identification

There are four minimum criteria that all isolates of *N. gonorrhoeae* should meet⁴⁴:

1. Growth on media selective for pathogenic *Neisseria* species

Note: If the *N. gonorrhoeae* strain in question is sensitive to vancomycin it will fail to grow on this medium

2. Appropriate colonial morphology on such media
3. Typical Gram stain morphology (Gram negative diplococci)

4. Oxidase positive

Identification of *N. gonorrhoeae*

There are approaches that can be taken to confirm the identity of *N. gonorrhoeae* and eliminate other *Neisseria* species.

1. The use of gonococcal specific antibodies, which confirms *N. gonorrhoeae* alone
2. The use of carbohydrate utilization tests, with or without the detection of preformed enzymes such as the aminopeptidases and β -galactosidase, which will give the full speciation of the organism⁴⁶
3. The use of Matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF) confirms *N. gonorrhoeae*⁵¹
4. Molecular confirmation of the presence of *N. gonorrhoeae* specific DNA using Polymerase Chain Reaction (PCR)^{50,52}

3.5.1.3 Identification tests available:**Immunological methods**

Identification by immunological means can be achieved using antibodies linked to a staphylococcal protein A or latex. These commercially available reagents contain a mixture of monoclonal antibodies raised to specific epitopes on the major outer membrane protein, Por. Because the reagents contain a mixture of antibodies rather than a single antibody to a cross-reactive epitope, false negative reactions do occur, although uncommonly. Because the mixtures themselves are different occasional isolates occur that give a false negative with one, but are positive with another reagent.

Carbohydrate utilisation

Traditionally identity has been confirmed by detecting the acidification of glucose-containing media, but not those containing maltose, sucrose or lactose. This is an oxidative and not a fermentative process. It is important that the basal medium is carbohydrate-free (if serum sugars are used, the serum should be checked for maltase activity). The inoculated plates or bottles are incubated in 5-10% CO₂ for 24 hours with the caps loosened and are then allowed to stand on the bench for 30 min to allow any acidification due to dissolved CO₂ to dissipate. There are disadvantages to this method in that it is slow and requires a heavy, pure growth of gonococci. Some meningococci metabolise maltose slowly and may require at least two days for acidification of the conventional test system, and some gonococci can be slow to utilize glucose. Several commercial systems are available for the rapid detection of carbohydrate utilisation.

Preformed enzymes

Detection of aminopeptidases, gamma-glutamyl transferase and proline iminopeptidase together with β -galactosidase, with chromogenic substrates allows identification to species level. Reagents are available as commercial kits. This can be a useful alternative to the approaches above, but should only be used on strains isolated on selective media, as certain non-pathogenic *Neisseria* give similar reactions to those that are given by *N. gonorrhoeae*. However, *N. gonorrhoeae* that have a mutation in the proline iminopeptidase gene and therefore appear negative for this

enzyme are prevalent in England and Wales and kits that detect solely the production of aminopeptidases should not be used alone^{21,22}.

Carbohydrate and preformed enzymes combined

Many of the commercial kits that test for carbohydrate utilisation also include aminopeptidases. *N. gonorrhoeae* that are proline iminopeptidase negative will give anomalous results with these kits and should be confirmed with an immunological reagent.

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.

Although the problem of the *Neisseria* genus study is complex, MALDI-TOF has been developed and validated to determine the clinically important species of *Neisseria* — *N. gonorrhoeae* and *N. meningitidis*, both are relatively straightforward to identify, the differences between many of the non-pathogenic strains are small and the speciation of these strains within a diagnostic setting is not always possible⁵³. While the identification of non-pathogenic *Neisseria* to species level is generally not required, the misidentification of these strains as *N. gonorrhoeae* or *N. meningitidis* can have serious health, legal and social consequences⁵⁴.

Formal validation studies for MALDI-TOF MS of *N. gonorrhoeae* are limited⁵³. Therefore in sensitive or critical situations, confirmation of *Neisseria* species identification should be confirmed with phenotypic or molecular methods^{49,50}.

Molecular confirmation by polymerase chain reaction (PCR)

Molecular methods are currently the methods of choice for detection of *N. gonorrhoeae* but can also be used for confirmation of the identity of putative isolates. This can be performed using in-house assays and the pseudogene *porA* and *opa* gene real-time PCRs, which can be multiplexed, have been found to be useful or by commercially available assays. There are recent reports of *N. gonorrhoeae* that are missing the target for the pseudogene *porA* and so it is advisable to use these assays in combination with other approaches^{51,52,55}.

3.5.2 *Neisseria meningitidis*

For information on screening for meningococci see [B 51 - Screening for *Neisseria meningitidis*](#).

Once an isolate has been identified using the method outlined in section 3, confirmation of the isolate is made in the following way:

- biochemical testing kit. It is important to note that a number of glucose and maltose negative meningococci have been reported⁴⁶
- rapid biochemical commercial kit
- characterisation where it is required to serogroup level would normally involve a commercial latex kit or slide agglutination reagents. The latex agglutination kits

are designed for direct use on CSF or serum, but will also work for cultures. Slide agglutinating sera are for use on cultures only. Heated clinical samples or formalin treated suspensions of cultures should be processed within microbiological safety cabinets to reduce aerosols

- the use of MLST to characterise *N. meningitidis*

Multi-locus sequence typing (MLST)

MLST measures the DNA sequence variations in a set of housekeeping genes directly and characterizes strains by their unique allelic profiles. The principle of MLST is simple: the technique involves PCR amplification followed by DNA sequencing. Nucleotide differences between strains can be checked at a variable number of genes depending on the degree of discrimination desired. Due to the sequence conservation in housekeeping genes, MLST sometimes lacks the discriminatory power to differentiate bacterial strains, which limits its use in epidemiological investigations. Its advantages are that it is unambiguous and highly portable and sequence data can be compared readily between laboratories and data stored in a central database is easily accessible via the Internet.

This technique has been used by Maiden et al to characterize *N. meningitidis* using six loci⁵⁶. The application of MLST has clearly resolved the major meningococcal lineages known to be responsible for invasive disease. To improve the level of discriminatory power between the major invasive lineages, seven loci are now being used and have been accepted by many laboratories as the method of choice for characterizing meningococcal isolates.

Differentiation of *N. meningitidis* from similar phenotypes

N. meningitidis can be identified by acid production from glucose and maltose but not from lactose or sucrose, and by the production of gamma-glutamylaminotransferase. Maltose strains of *N. meningitidis* have been described and may be differentiated from *N. gonorrhoeae* by their ability to produce gamma-glutamylaminotransferase. Glucose negative variants of *N. meningitidis* may also be observed.

3.5.3 Other *Neisseria* species

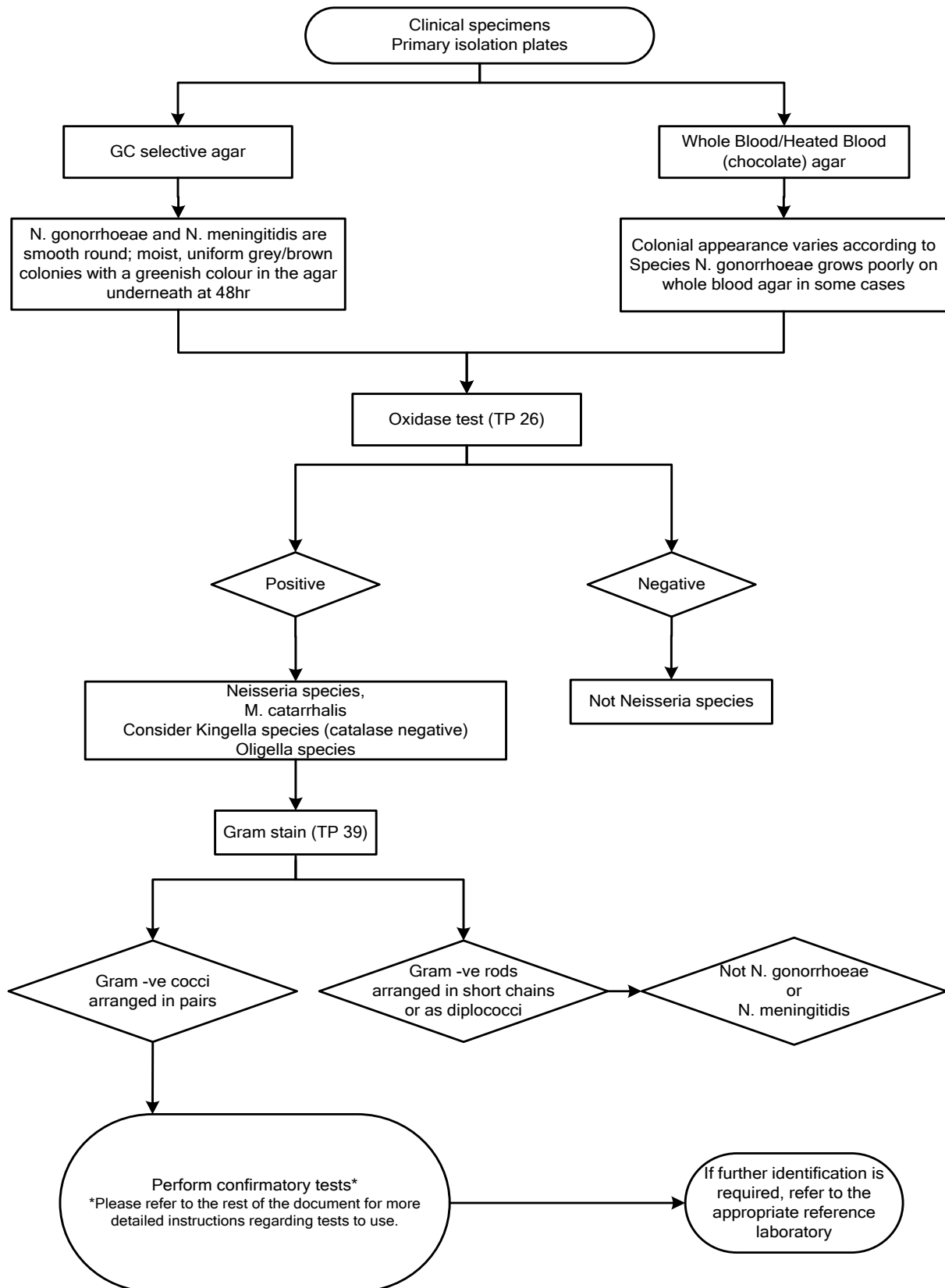
These can be identified by use of commercially available kits that have been validated. The accuracy of these kits has not been fully determined for species other than *N. gonorrhoeae* and *N. meningitidis* and therefore all results obtained should be treated with caution.

3.6 Storage and referral

Short term storage – isolates should be kept in a viable state on heated blood (chocolate) agar slopes.

Long term storage – isolates should be frozen at -20°C to -80°C.

4 Identification of *Neisseria* species



The flowchart is only for guidance only.

5 Reporting

5.1 Presumptive identification

N. gonorrhoeae

If appropriate growth characteristics, colonial appearance, Gram stain of the culture and oxidase.

N. meningitidis

If appropriate growth characteristics, colonial appearance, Gram stain of the culture, oxidase and serology results are demonstrated.

There are 4 minimum criteria that all isolates of *Neisseria* should meet:

1. growth on media selective for pathogenic *Neisseria* species

Note: If the *Neisseria gonorrhoeae* strain in question is sensitive to vancomycin it will fail to grow on this medium

2. appropriate colonial morphology on such media
3. exhibit typical Gram stain morphology (Gram negative diplococci)
4. oxidase positive

5.2 Confirmation of identification

Using biochemical/immunological/molecular results following identification processes as outlined in this document (using 2 or 3 confirmatory tests) and/or Reference Laboratory report.

5.3 Medical microbiologist

Inform the medical microbiologist of all presumptive and confirmed *N. meningitidis* isolates, and of all *Neisseria* species isolated from normally sterile sites, or in cases of invasive infection.

The medical microbiologist should also be informed if the request bears relevant information eg:

- cases of meningitis, septicaemia (especially with purpuric rash)
- investigation of *N. meningitidis* outbreak, or of the carrier state

Inform the medical microbiologist of all presumptive and confirmed *N. gonorrhoeae* isolates, and of all *Neisseria* species from:

- minors
- cases of sexual assault, rape or abuse
- all persons not known to be attending a Genitourinary Medicine clinic
- extragenital sites (eg throat, anorectum because special care is indicated with identification procedures)

Follow local protocols for reporting to clinician.

5.4 CCDC

Refer to local Memorandum of Understanding.

It is a legal responsibility of the clinician to inform the laboratory that will be receiving samples and likewise for all diagnostic laboratories, to notify of all clinically significant isolates to ensure urgent initiation of proper procedures.

5.5 Public Health England⁴⁷

Refer to current guidelines on CIDSC and COSURV reporting.

5.6 Infection prevention and control team

Inform the infection prevention and control team of presumptive and confirmed isolates of *N. meningitidis*.

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:

Sexually Transmitted Bacteria Reference Laboratory
 Microbiology Services
 Public Health England
 61 Colindale Avenue
 London
 NW9 5EQ
 Tel.+44 (0) 20 8327 6464
<https://www.gov.uk/stbru-reference-and-diagnostic-services>

Meningococcal Reference Unit (MRU)
 Manchester Medical Microbiology Partnership
 PO Box 209
 Clinical Sciences Building 2
 Manchester Royal Infirmary
 Oxford Road
 MANCHESTER
 M13 9WZ
 Tel.+44 (0) 0161 276 6757

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^{47,57} or equivalent in the devolved administrations⁵⁸⁻⁶¹

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](#)^{58,59}, [Wales](#)⁶⁰ and [Northern Ireland](#)⁶¹.

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