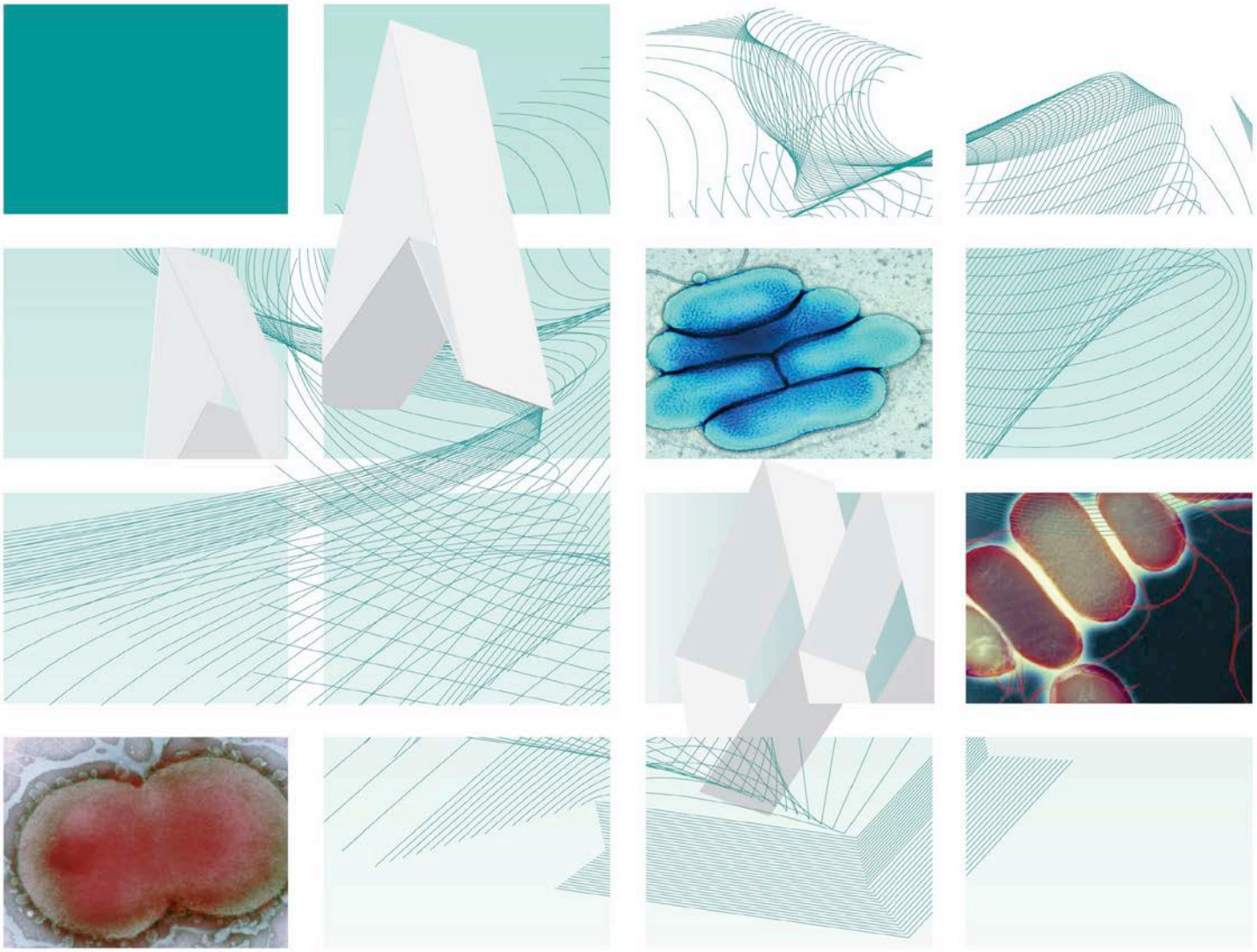
Public Health England

NHS

UK Standards for Microbiology Investigations

Introduction to the Preliminary Identification of Medically Important Bacteria



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Acknowledgments

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website http://www.hpa.org.uk/SMI/Partnerships. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see http://www.hpa.org.uk/SMI/WorkingGroups).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the Medical Editors for editing the medical content.

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UK Standards for Microbiology Investigations are produced in association with:

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@phe.gov.uk.

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

Amendment No/Date.	5/12.03.14
Issue no. discarded.	1.5
Insert Issue no.	1.6
Section(s) involved	**Amendment**
Whole document.	Document has been transferred to a new template to reflect the Health Protection Agency's transition to Public Health England. Front page has been redesigned. Status page has been renamed as Scope and Purpose and updated as appropriate. Professional body logos have been reviewed and updated. Standard safety and notification references have been reviewed and updated. Scientific content remains unchanged.

Amendment No/Date.	4/21.10.11
Issue no. discarded.	1.4
Insert Issue no.	1.5
Section(s) involved	**Amendment**
Whole document.	Document presented in a new format.
References.	Some references updated.

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

Users of SMIs

- SMIs are primarily intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK.
- SMIs provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests.
- SMIs provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages.

Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal Partnership Working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies.

The list of participating societies may be found at http://www.hpa.org.uk/SMI/Partnerships. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process.

[#]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.

SMIs are developed, reviewed and updated through a wide consultation process.

Quality Assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008.

SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development.

The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and Public Involvement

The SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information Governance and Equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions.

The development of SMIs are subject to PHE Equality objectives http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317133470313. The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal Statement

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next

review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

Suggested Citation for this Document

Public Health England. (2014). Introduction to the Preliminary Identification of Medically Important Bacteria. UK Standards for Microbiology Investigations. ID 1 Issue 1.6. http://www.hpa.org.uk/SMI/pdf.

Scope of Document

The aim of this document is to provide a guide to the preliminary identification of the common bacteria which may be encountered in clinical specimens. It is intended to lead the user to a more detailed identification method and is designed to be used for cultures of bacteria isolated on agar plates and not for identification of bacteria in direct smears.

This SMI should be used in conjunction with other SMIs. Of particular relevance are the SMIs on http://www.hpa.org.uk/SMI/pdf/Identification.

Introduction

Identification of bacteria by diagnostic laboratories is based on phenotypic characteristics, usually by direct comparison of unknown bacteria with those of type cultures[1]. Greater confidence in identification is in direct proportion to the number of similar characteristics. In medical microbiology, experience of the types of specimens, the infection and the bacteria associated with those sites of infection is useful as an aid in preliminary identification. When identifying bacteria it should be remembered that many of their characteristics might be variable. In addition, species within a genus may differ in some characteristics eg *Capnocytophaga canimorsus* is oxidase positive, whereas *Capnocytophaga ochracea* is oxidase negative. For this reason some genera may appear in more than one table or chart. Clinical information should also be taken into consideration during the identification process.

Taxonomy

N/A

Characteristics

When classifying microorganisms, all known characteristics are taken into consideration, but certain characteristics are selected and used for the purpose of identification. Primary identification usually involves a few simple tests such as morphology (usually shown by Gram stain), growth in the presence or absence of air, growth on various types of culture media, catalase and oxidase tests[1]. Using these few simple tests it is usually possible to place organisms, provisionally, in one of the main groups of medical importance.

Principles of Identification

There are three basic methods of identification. The first relies heavily on the experience of the investigator: a judgement is made on the probable identity of the organism based on clinical data, cultural and atmospheric characteristics. A limited range of tests are then used to confirm or disprove the hypothesis. This relies heavily on a stable pattern of phenotypic characteristics.

If identification is not made using the first principle, a different approach may be used subjecting the organism to a battery of tests, such as those found in commercial identification systems. The data is collated and compared to standard texts or used to create a numerical profile to obtain identification.

The final method follows a step-by-step approach to identification. Fundamental characteristics of the organism are determined by primary identification tests such as a Gram stain, oxidase or catalase. Results of these tests indicate secondary or even tertiary tests to confirm the identity of the subject. This is a systematic approach and does not rely on the expertise of the investigator. The disadvantage of this system involves the primary tests, incorrect results at this stage can lead the investigator down an incorrect path, which wastes both time and resources and may also lead to an erroneous result. It is also a time consuming process; further tests cannot be set up until results of the previous investigations are known.

Conditions under which tests are conducted should be defined as reactions may vary.

Microscopic Appearance

Microscopic study and staining reveals the shape (coccus or rod) and the characteristic grouping and arrangement of the cells, their size and the presence of intracellular inclusions eg spores. In addition to morphology, the Gram stained preparation (TP 39 - Staining Procedures) also divides bacteria in two categories - the Gram positive and the Gram negative bacteria[1,2]. For morphological appearance it is preferable to examine young cultures from growth on non-selective media.

Terms Used for Stained Preparations[3]

Arrangement	singly, in pairs, in chains, in fours (tetrads), in groups, grape-like clusters, in cuboidal packets, in bundles, in Chinese letters (cuneiform)
Capsule	present or absent
Endospores	spherical, oval or ellipsoidal, equatorial, subterminal, terminal, cause bulging of rod
Ends	round, truncate, pointed
Irregular forms	variation in shape and size, clubs, filamentous, branched, navicular, citron, fusiform, giant swollen forms
Pleomorphism	variation in shape eg filamentous forms interspersed with coccobacillary forms
Shape	spheres, short rods (coccobacilli), long rods, filamentous, curved rods, spirals
Sides	parallel, bulging, concave or irregular
Size	length and breadth
Staining	even, irregular, unipolar, bipolar, beaded, barred

Cultural Appearance[1,2]

Bacterial colonies of a single species, when grown on specific media under controlled conditions, are described by their characteristic size, shape, consistency and sometimes pigment. When growth conditions are carefully controlled, colonial morphology is important for preliminary identification and for differentiating organisms[4].

The size of bacterial colonies, assuming favourable growth conditions, is generally uniform within a species. For example streptococci are small, usually 1mm in diameter, whilst those of staphylococci and Enterobacteriaceae are larger, and those of *Bacillus* species are still larger.

Colonial shape is determined by the edge and thickness of the colony. The edge may be smooth (entire) or irregular and serrated. If the colony is thicker in the centre than the edge, it is said to be raised, or it may be relatively uniform - appearing like a disc.

The texture of the colony is also important. It may vary from dry and friable (easily crumbled) to butyrous (buttery), to sticky, and the surface may be smooth, wet, dry or granular.

Some organisms produce a pigmented colony, which can aid in the identification process (eg *Pseudomonas aeruginosa*, *Serratia marcescens*), although non-pigmented strains within a species may occur[4].

Terms Used in Colonial Morphology[5,6]

Colour	by reflected or transmitted light: fluorescent, iridescent, opalescent
Consistency	butyrous, mucoid, friable, membranous
Edge	entire, undulate, lobate, crenated, erose, fimbriate, curled, effuse
Elevation	effuse, raised, low convex, convex or dome-shaped, umbonate, with or without bevelled margin
Emulsifiable	easy or difficult, forms homogeneous or granular suspension or remains membranous when mixed in a drop of water
Form	filiform, spreading, rhizoid
Opacity	transparent, translucent, opaque
Shape	circular, irregular, radiate, rhizoid
Size	diameter in millimetres
Structure	amorphous, granular, filamentous, curled
Surface	smooth, rough (fine, medium or coarsely granular), ringed, papillate, dull or glistening, heaped up, dry or moist

For individual colonial descriptions, see the relevant identification SMI.

Haemolysis

Some organisms produce haemolysins, which cause lysis of erythrocytes in blood-containing media[4]. This haemolysis may be β (clear zone around the colony), α (green halo surrounding the colony), α ‘(a small zone of intact red cells with a surrounding zone of haemolysis) or non (no haemolysis, no apparent change).

Growth Requirements

Atmosphere[1,2]

It is usual to divide organisms in five categories according to their atmospheric requirements:

- Strict aerobes grow only in the presence of oxygen
- Strict anaerobes grow only in the absence of oxygen
- Facultative organisms grow aerobically or anaerobically
- Microaerobic organisms grow best in an atmosphere with reduced oxygen concentration (addition of 5-10% CO_2 may enhance growth)
- Carboxyphilic (or capnophilic) organisms require additional CO_2 for growth

Temperature[1]

Organisms may also be divided according to their temperature requirement:

- Psychrophilic organisms grow at low temperatures 2-5°C (optimum 10-30°C)
- Mesophilic organisms grow at temperatures between 10-45°C (optimum 30-40°C)
- Thermophilic organisms grow very little at 37°C (optimum 50-60°C)
- Most clinically encountered organisms are mesophilic

Motility[7]

Many bacteria are observed to be motile and move from one position to another when suspended in fluid. True motility must not be confused with Brownian movement (vibration caused by molecular bombardment) or convection currents. Microscopic examination may indicate whether a motile organism has polar flagellae shown by a darting zigzag movement or peritrichate flagellae, which cause a less vigorous and more vibratory movement. Some bacteria may be motile at different temperatures eg motile at ambient temperature but not at 37°C, or vice versa (TP 21 - Motility Test).

Nutrition[1]

Study of the nutritional requirements of an organism is useful in identification, eg the ability to grow on ordinary nutrient media, the effect of adding blood, serum or glucose, or the necessity for specific growth factors such as X factor (haemin) and V factor (NAD) for the growth of *Haemophilus* species.

Biochemical tests[2]

Numerous biochemical tests may be used for the identification of micro organisms (refer to individual identification SOPs). Some such as catalase and oxidase are rapid and easy to perform and may be used for preliminary differentiation purposes. The fermentation of glucose may also be used to distinguish between groups of organisms.

- **Catalase** (TP 8 - Catalase Test)

  Hydrogen peroxide is formed by some bacteria as an oxidative end product of the aerobic breakdown of sugars and, if allowed to accumulate, is highly toxic.

The catalase enzyme breaks down hydrogen peroxide to water and gaseous oxygen.

- **Oxidase** (TP 26 - Oxidase Test)

  The oxidase test is used to detect an intracellular cytochrome oxidase enzyme system. This system is usually present only in aerobic organisms, which are capable of utilising oxygen as the final hydrogen acceptor.

- **Fermentation of glucose** (TP 27 – Oxidation/Fermentation of Glucose Test)

  Some aerobic organisms metabolise glucose oxidatively (ie oxygen is the ultimate hydrogen acceptor). Other organisms ferment glucose and the hydrogen acceptor is then another element such as sulphur.

Technical Information/Limitations

N/A

1 Safety Considerations[8-18]

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.

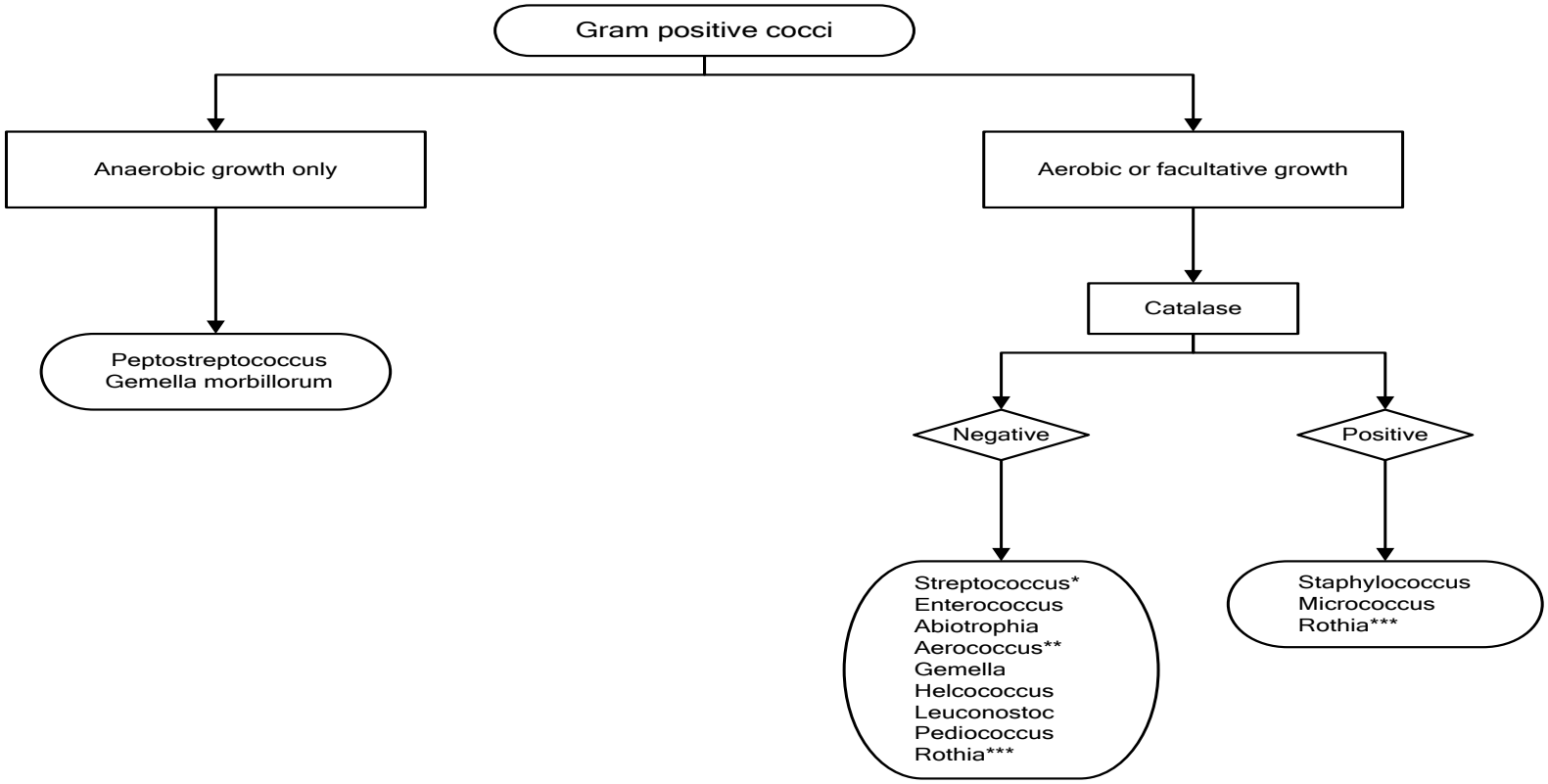
2 Target Organisms

N/A

3 Identification

N/A

4 Characteristics of Gram Positive Cocci[6,19,20]



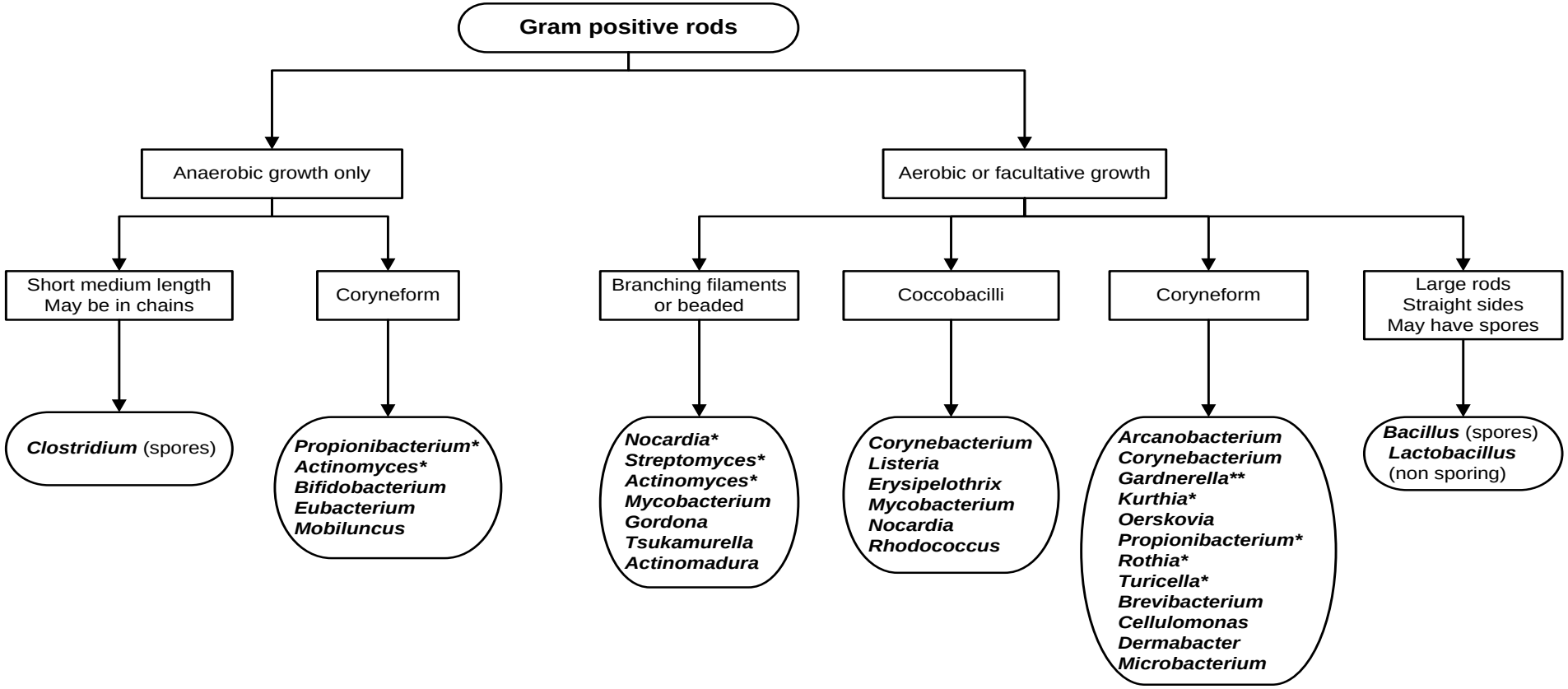
***** Some species may be anaerobic

****** May be weak catalase positive

******* This organism is pleomorphic and catalase variable, catalase test may not be helpful for differentiation

The flowchart is for guidance only.

5 Characteristics of Gram Positive Rods[6,19,20]



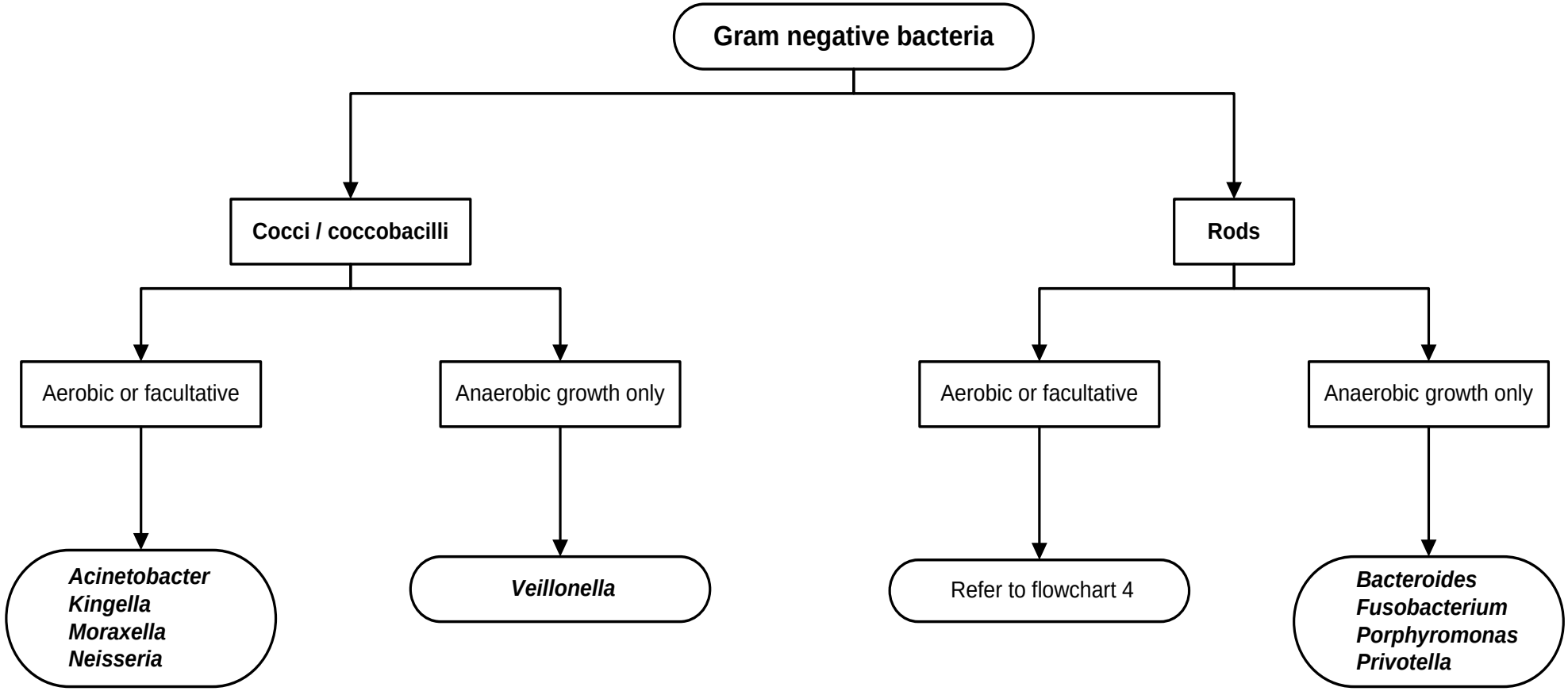
*This organism is pleomorphic

***Gardnerella vaginalis* is a Gram variable rod and may usually be differentiated by its microscopic appearance

Mycobacterium species should be referred to the Reference Laboratory for full identification.

The flowchart is for guidance only.

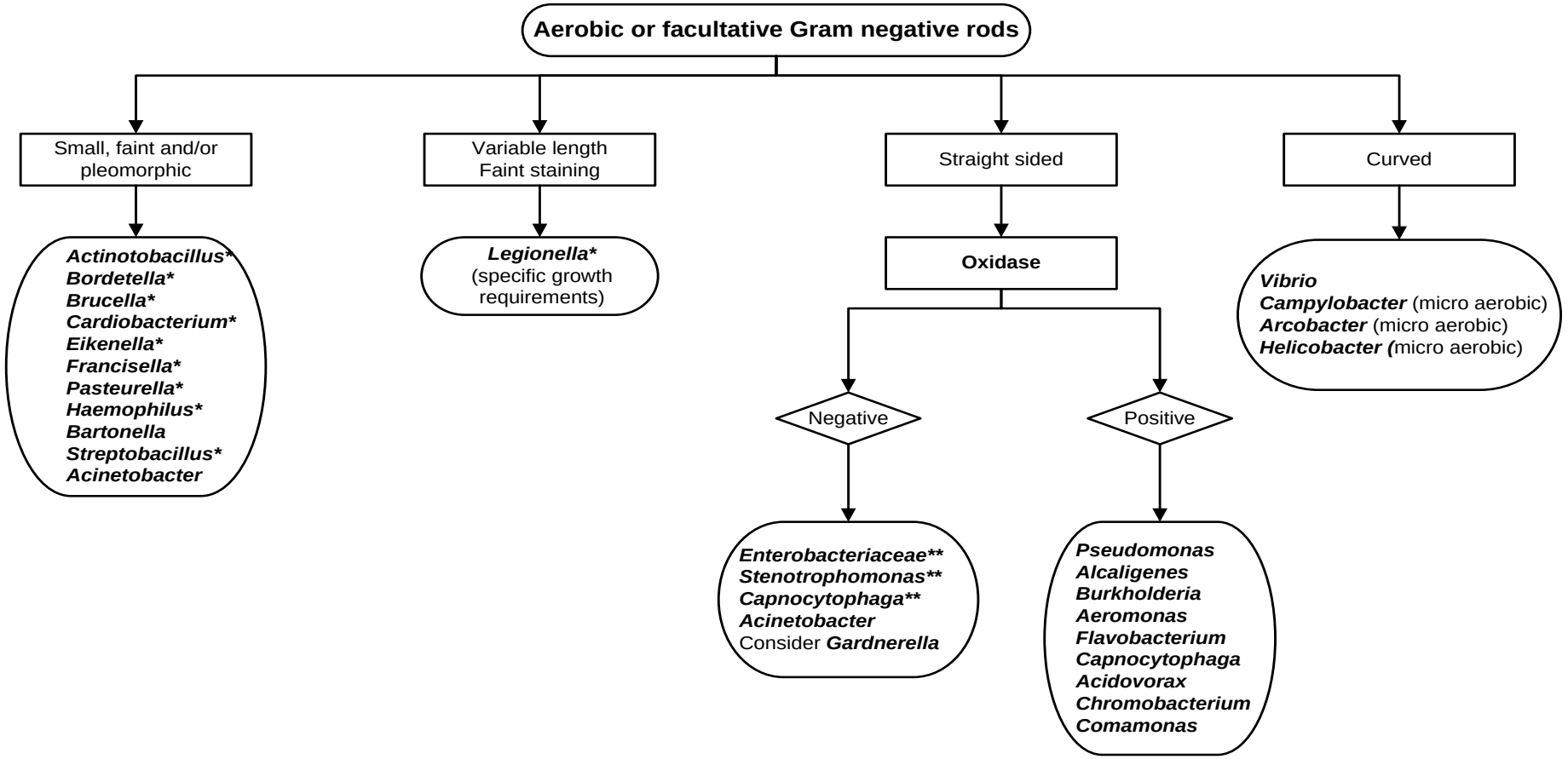
6 Characteristics of Gram Negative Bacteria[6,19,20]



The flowchart is for guidance only.

7 Characteristics of Gram Negative Bacteria[6,19,20]

(Continued from previous page)



The flowchart is for guidance only.

8 Reporting

Refer to individual UK Standard for Microbiology Investigation.

9 Referrals

Refer to individual UK Standard for Microbiology Investigation.

10 Notification to PHE[21,22] or Equivalent in the Devolved Administrations[23-26]

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 HIV & STIs, HCAIs and CJD under ‘Notification Duties of Registered Medical Practitioners’: it is not noted under ‘Notification Duties of Diagnostic Laboratories’.

Other arrangements exist in Scotland[23,24], Wales[25] and Northern Ireland[26].

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