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NATIONAL STANDARD METHOD

INTRODUCTION TO THE PRELIMINARY IDENTIFICATION OF MEDICALLY IMPORTANT BACTERIA

BSOP ID 1

Issued by Standards Unit, Evaluations and Standards Laboratory
Centre for Infections

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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.

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INDEX

AMENDMENT PROCEDURE

Controlled document reference	**BSOP ID 1**
Controlled document title	**Introduction to the Preliminary Identification of Medically Important Bacteria**

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
3/ 25/02/08	1.3	1.4	13	**Characteristics of gram negative rods flowchart**	Reference to Table 2 removed and recommendation to refer to individual NSMs

INTRODUCTION TO THE PRELIMINARY IDENTIFICATION OF MEDICALLY IMPORTANT BACTERIA

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 SOPs and is designed to be used for cultures of bacteria isolated on agar plates and not for identification of bacteria in direct smears.

INTRODUCTION

Identification of bacteria by diagnostic laboratories is based on phenotypic characteristics, usually by direct comparison of unknown bacteria with those of type cultures[2]. 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.

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[2] 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. 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 (BSOPTP 39 - Staining Procedures) also divides bacteria in two categories - the Gram-positive and the Gram-negative bacteria[2,3]. For morphological appearance it is preferable to examine young cultures from growth on non-selective media.

Terms used for stained preparations[4]

Staining	even, irregular, unipolar, bipolar, beaded, barred
Shape	spheres, short rods (coccobacilli), long rods, filamentous, curved rods, spirals
Endospores	spherical, oval or ellipsoidal, equatorial, subterminal, terminal, cause bulging of rod
Capsule	present or absent
Size	length and breadth
Sides	parallel, bulging, concave or irregular
Ends	round, truncate, pointed
Arrangement	singly, in pairs, in chains, in fours (tetrads), in groups, grape-like clusters, in cuboidal packets, in bundles, in Chinese letters (cuneiform)
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

Cultural appearance[2,3]

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[5].

The size of bacterial colonies, assuming favourable growth conditions, is generally uniform within a species. For example streptococci are small, usually 1 mm 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[5] (eg *Pseudomonas aeruginosa*, *Serratia marcescens*), although non-pigmented strains within a species may occur.

Terms used in colonial morphology[6,7]

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

For individual colonial descriptions, see the relevant identification SOP.

Haemolysis

Some organisms produce haemolysins, which cause lysis of erythrocytes in blood-containing media[5]. 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[2,3]

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[2]

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[8]

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. (see BSOPTP 21 - Motility Test)

Nutrition[2]

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[3]

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 – (See BSOPTP 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 – (See BSOPTP 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 - 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

N/A

1 SAFETY CONSIDERATIONS

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

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

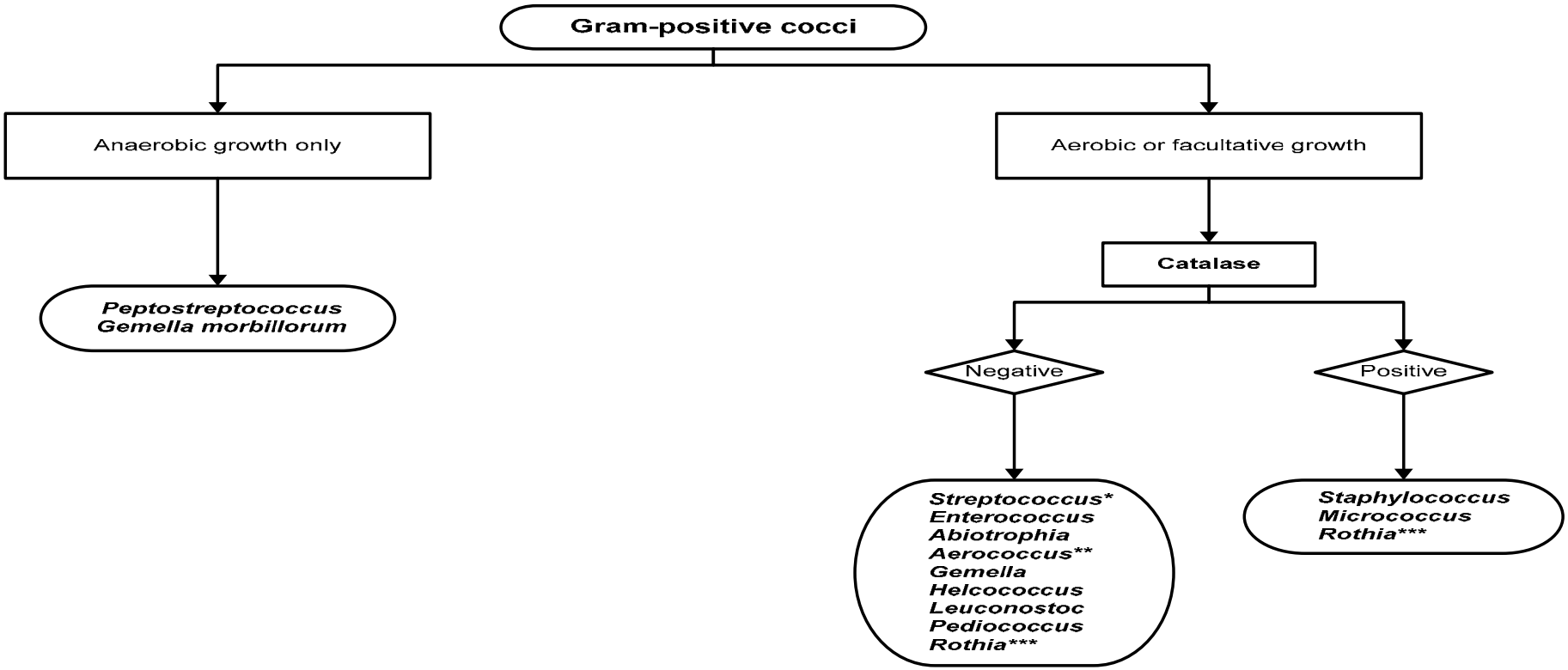
N/A

3 IDENTIFICATION

N/A

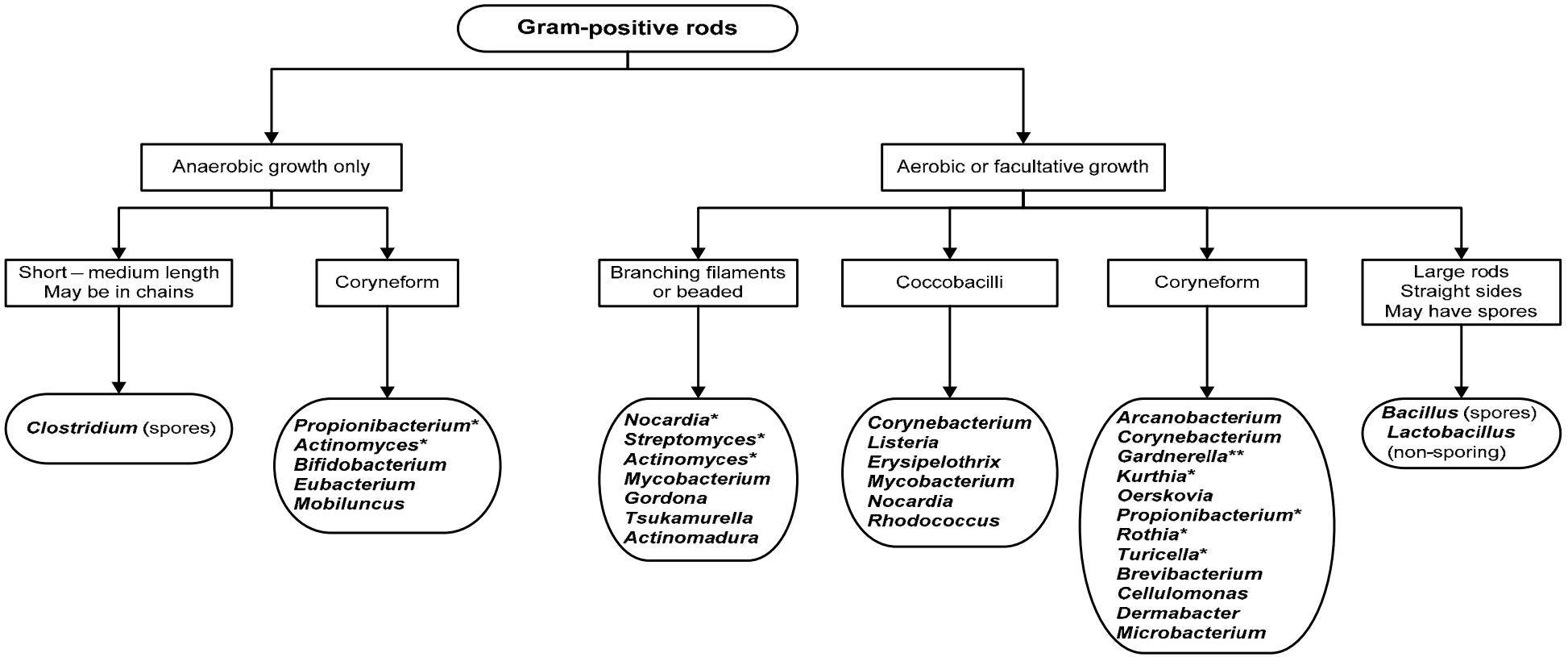
4 IDENTIFICATION FLOW CHART

Flowchart 1 - Characteristics of Gram-positive cocci[6,9,10]



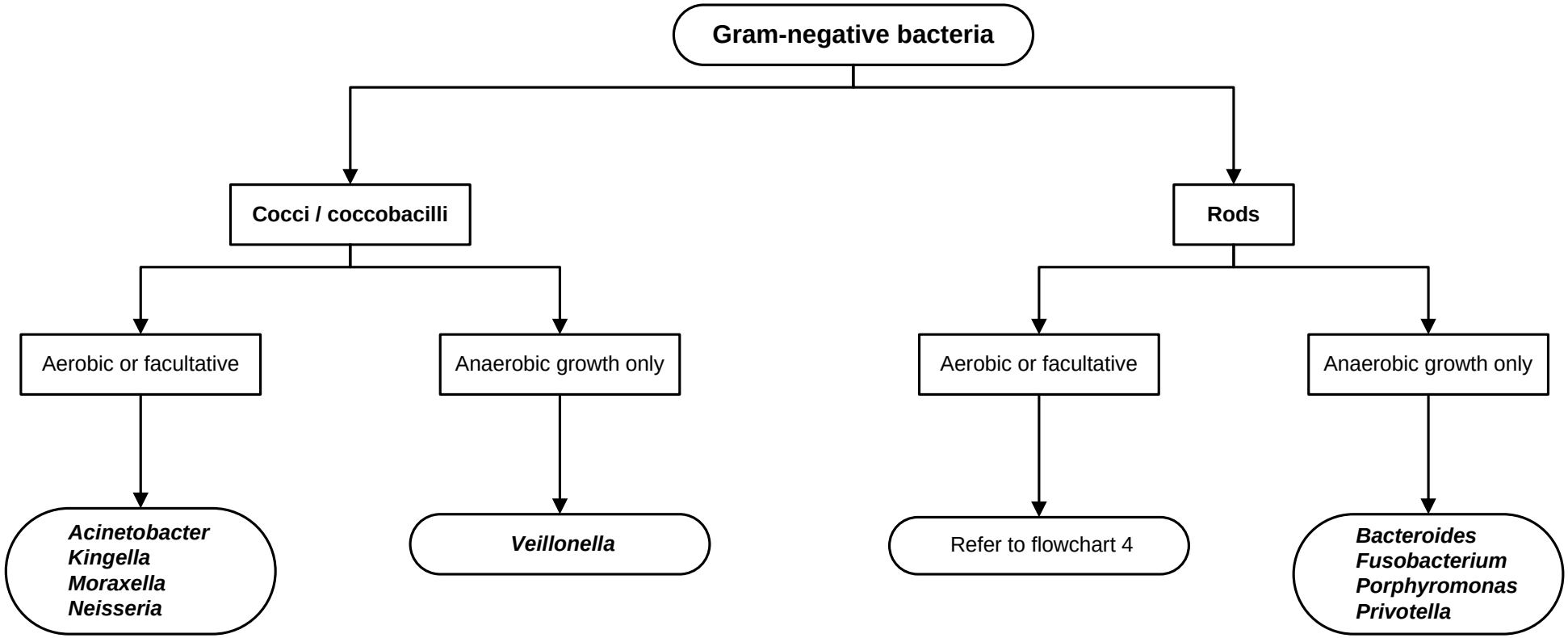
***** Some species may be anaerobic
****** May be weak catalase positive
******* This organism is pleomorphic and catalase variable, catalase test may not be helpful for differentiation

Flowchart 2 - Characteristics of Gram-positive rods[6,9,10]

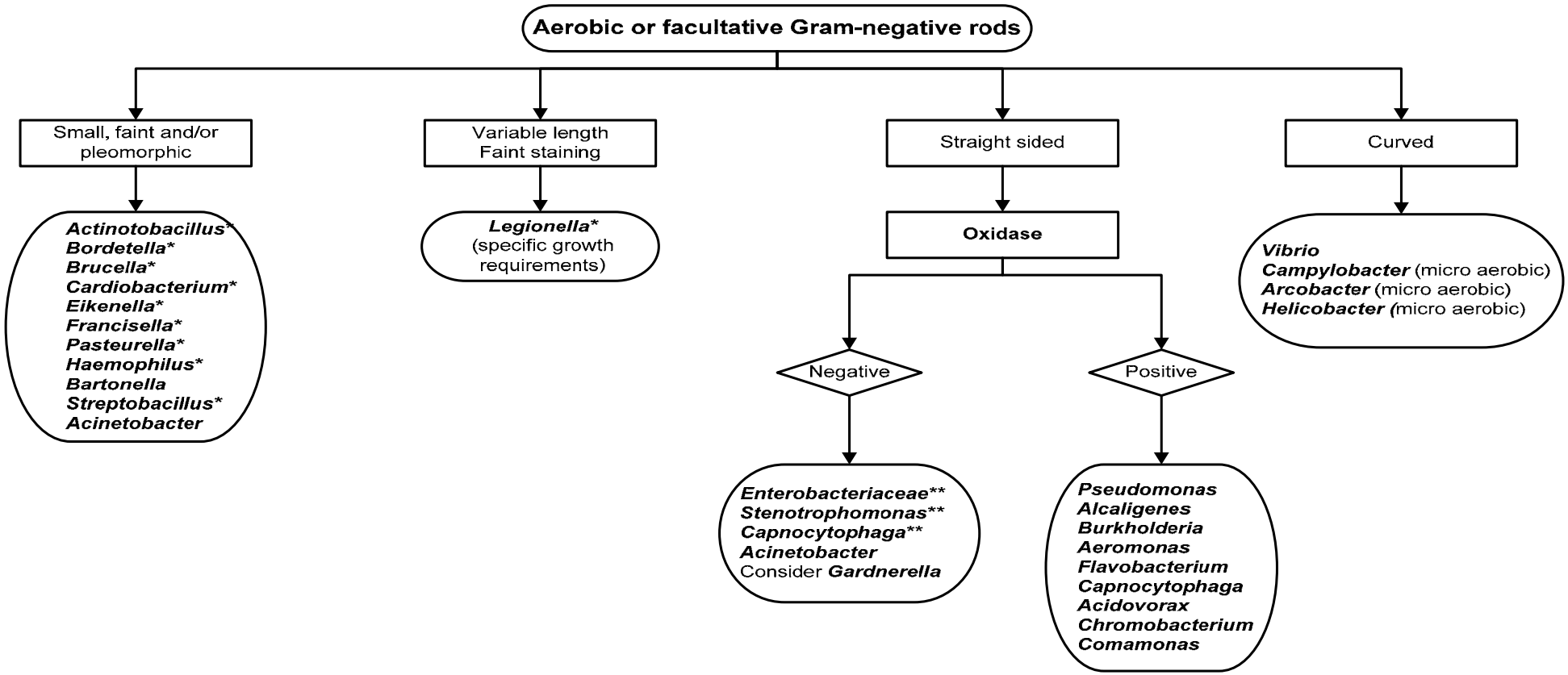


* This organism is pleomorphic
** *G. vaginalis* is a Gram-variable rod and may usually be differentiated by its microscopic appearance
Mycobacterium species should be referred to the Reference Laboratory for full identification

Flowchart 3 - Characteristics of Gram-negative bacteria[6,9,10]



Flowchart 4 - Characteristics of Gram-negative rods[6,9,10] (continued from previous page)



* This is a diverse group of bacteria, which are often difficult to identify.
** For differential characteristics - see individual BSOP IDs

5 REPORTING

Refer to individual National Standard Methods

6 REFERRALS

Refer to individual National Standard Methods

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.

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