INTRODUCTION TO THE PRELIMINARY IDENTIFICATION OF MEDICALLY IMPORTANT BACTERIA

BSOP ID 1

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

Representatives of several professional organisations, including those whose logos appear on the front cover, are members of the working groups which develop National Standard Methods. Inclusion of an organisation’s logo on the front cover implies support for the objectives and process of preparing standard methods. The representatives participate in the development of the National Standard Methods but their views are not necessarily those of the entire organisation of which they are a member. The current list of participating organisations can be obtained by emailing standards@hpa.org.uk.

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.

Whereas every care has been taken in the preparation of this publication, the Health Protection Agency or any supporting organisation cannot be responsible for the accuracy of any statement or representation made or the consequences arising from the use of or alteration to any information contained in it. These procedures are intended solely as a general resource for practising professionals in the field, operating in the UK, and specialist advice should be obtained where necessary. If you make any changes to this publication, it must be made clear where changes have been made to the original document. The Health Protection Agency (HPA) should at all times be acknowledged.

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 conditions.

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.

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.

Suggested citation for this document:
# INDEX

<table>
<thead>
<tr>
<th>Status of National Standard Methods</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Index</td>
<td>3</td>
</tr>
<tr>
<td>Amendment Procedure</td>
<td>4</td>
</tr>
<tr>
<td>Scope of Document</td>
<td>5</td>
</tr>
<tr>
<td>Introduction</td>
<td>5</td>
</tr>
<tr>
<td>Technical Information</td>
<td>8</td>
</tr>
<tr>
<td>1 Safety Considerations</td>
<td>9</td>
</tr>
<tr>
<td>2 Target Organisms</td>
<td>9</td>
</tr>
<tr>
<td>3 Identification</td>
<td>9</td>
</tr>
<tr>
<td>4 Identification Flow Chart</td>
<td>10</td>
</tr>
<tr>
<td>5 Reporting</td>
<td>14</td>
</tr>
<tr>
<td>6 Referrals</td>
<td>14</td>
</tr>
<tr>
<td>7 Acknowledgements and Contacts</td>
<td>15</td>
</tr>
<tr>
<td>References</td>
<td>16</td>
</tr>
</tbody>
</table>
## AMENDMENT PROCEDURE

<table>
<thead>
<tr>
<th>Amendment Number/Date</th>
<th>Issue no. Discarded</th>
<th>Insert Issue no.</th>
<th>Page</th>
<th>Section(s) involved</th>
<th>Amendment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3/25/02/08</td>
<td>1.3</td>
<td>1.4</td>
<td>13</td>
<td>Characteristics of gram negative rods flowchart</td>
<td>Reference to Table 2 removed and recommendation to refer to individual NSMs</td>
</tr>
</tbody>
</table>

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.
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. 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 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. For morphological appearance it is preferable to examine young cultures from growth on non-selective media.

Terms used for stained preparations

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

Cultural appearance

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.

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 (eg Pseudomonas aeruginosa, Serratia marcescens), although non-pigmented strains within a species may occur.
**Terms used in colonial morphology**

- **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. 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**

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% CO₂ may enhance growth)
- Carboxyphilic (or capnophilic) organisms require additional CO₂ for growth

**Temperature**

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

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

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

Numerous biochemical tests may be used for the identification of microorganisms (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

Gram-positive cocci

- Anaerobic growth only
  - *Peptostreptococcus*
  - *Gemella morbillorum*

- Aerobic or facultative growth
  - Catalase
    - Negative
      - *Streptococcus*  
      - *Enterococcus*
      - *Abiotrophia*
      - *Aerococcus***
      - *Gemella*
      - *Helcococcus*
      - *Leuconostoc*
      - *Pediococcus*
      - *Rothia***
    - Positive
      - *Staphylococcus*
      - *Micrococcus*
      - *Rothia***

* 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[^1][^2][^3]

**Gram-positive rods**

- **Anaerobic growth only**
  - Short—medium length
    - May be in chains
    - **Clostridium** (spores)
      - *Propionibacterium*
      - *Actinomyces*
      - *Bifidobacterium*
      - *Eubacterium*
      - *Mobiluncus*
  - **Coryneform**
    - *Nocardia*
      - *Streptomyces*
      - *Actinomycetes*
      - *Mycobacterium*
      - *Gordona*
      - *Tsukamurella*
      - *Actinomadura*

- **Aerobic or facultative growth**
  - Branching filaments or beaded
    - **Cocccobacilli**
      - *Corynebacterium*
      - *Listeria*
      - *Erysipelothrix*
      - *Mycobacterium*
      - *Nocardia*
      - *Rhodococcus*
  - **Coryneform**
    - *Arcanobacterium*
    - *Corynebacterium*
    - *Gardnerella*[^2]
    - *Kurthia*[^2]
    - *Oerskovia*
    - *Propionibacterium*[^2]
    - *Rothia*[^2]
    - *Turicella*[^2]
    - *Brevibacterium*
    - *Cellulomonas*
    - *Dermabacter*
    - *Microbacterium*
  - **Large rods**
    - Straight sides
    - May have spores
    - **Bacillus** (spores)
      - *Lactobacillus* (non-sporing)

---

[^1]: This organism is pleomorphic
[^2]: G. vaginalis is a Gram-variable rod and may usually be differentiated by its microscopic appearance
[^3]: *Mycobacterium* species should be referred to the Reference Laboratory for full identification
Flowchart 3 - Characteristics of Gram-negative bacteria\textsuperscript{6,9,10}

```
Gram-negative bacteria

Cocci / coccobacilli
- Aerobic or facultative
  - Acinetobacter
  - Kingella
  - Moraxella
  - Neisseria

- Anaerobic growth only
  - Veillonella

Rods
- Aerobic or facultative
- Anaerobic growth only
  - Refer to flowchart 4
  - Bacteroides
  - Fusobacterium
  - Porphyromonas
  - Privotella
```
Flowchart 4 - Characteristics of Gram-negative rods\(^6,9,10\) (continued from previous page)

**Aerobic or facultative Gram-negative rods**

- Small, faint and/or pleomorphic
- Variable length; faint staining
- Straight sided
- Curved

**Legionella**
(specific growth requirements)

**Enterobacteriaceae**
- *Stenotrophomonas*
- *Capnocytophaga*
- *Acinetobacter*
Consider *Gardnerella*

**Pseudomonas**
- *Aloigenes*
- *Burkholderia*
- *Aeromonas*
- *Flavobacterium*
- *Capnocytophaga*
- *Acidovorax*
- *Chromobacterium*
- *Comamonas*

**Vibrio**
- *Campylobacter* (micro aerobic)
- *Arcobacter* (micro aerobic)
- *Helicobacter* (micro aerobic)

---

* 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.

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

For further information please contact us at:
Standards Unit
Evaluations and Standards Laboratory
Centre for Infections
Health Protection Agency
Colindale
London
NW9 5EQ
E-mail: standards@hpa.org.uk
REFERENCES


