Public Health England

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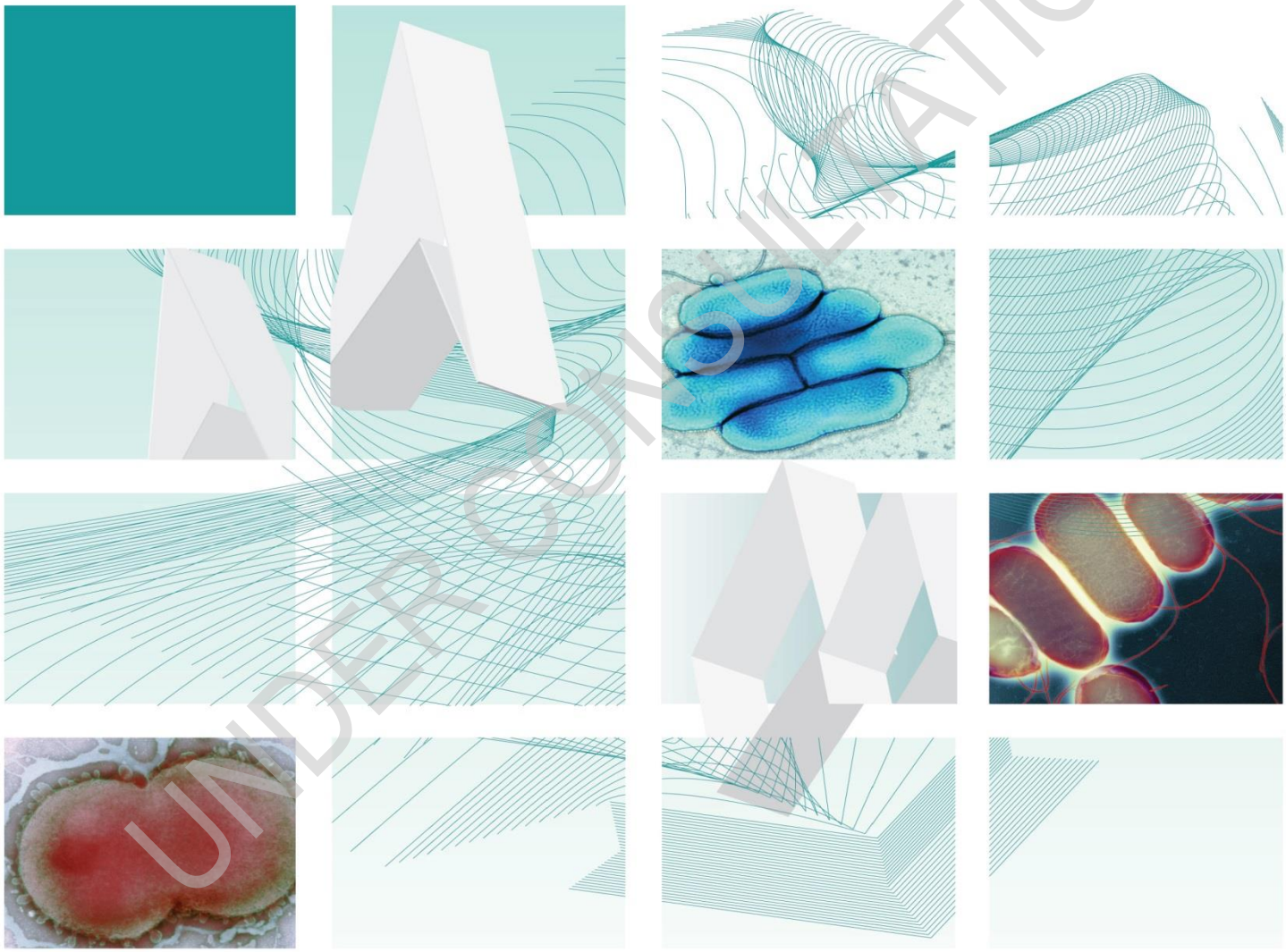
# UK Standards for Microbiology Investigations

## Introduction to the preliminary identification of medically important bacteria and fungi from culture





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# Amendment table

Each UK 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 number/date | |
| Issue number discarded | |
| Insert issue number | |
| Anticipated next review date* | |
| **Section(s) involved** | **Amendment** |
| Whole document | Document presented in a new format.<br>All sections of this documents updated with current information and references. |
| References | References updated |

*Reviews can be extended up to five years subject to resources available.

# 1 General information

View general information related to UK SMIs.

# 2 Scientific information

View scientific information related to UK SMIs.

# 3 Scope of document

This UK Standards for Microbiology Investigations (UK SMI) document describes preliminary identification of the common bacteria and fungi 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 and fungi isolated on agar plates and not for direct identification of bacteria and fungi from clinical samples/smears. It does however mention rapid methods that could be used in place of the conventional methods mentioned in this document.

For more information on dermatophytes, refer to B 39 - Investigation of dermatological specimens for superficial mycoses.

This UK SMI includes both biochemical tests and automated methods for the identification of microorganisms. Some biochemical tests may not be done routinely in laboratory except in cases where confirmation by an alternative technique is required or automated methods are not available.

# 4 Introduction

## 4.1 Taxonomy/characteristics

When classifying microorganisms, all known characteristics are taken into consideration, but certain differential and distinguishable characteristics are selected and used for the purpose of identification. Primary identification usually involves one or more characteristics. It can be phenotypic characteristics such as morphology and Gram stain reaction, or lactophenol cotton blue (fungi), growth under various atmospheric conditions and temperatures (fungi), growth on various types of culture media (for example MacConkey agar, Sabouraud agar plate culture), or ability to ferment certain sugars such as catalase and oxidase tests or genotypic characteristics including nucleotide sequence. Using these few simple tests it is usually possible to place organisms, provisionally, in one of the main groups of medical importance[1].

In medical microbiology, experience of the types of specimens, the infection and the organisms associated with those sites of infection is useful as an aid to preliminary identification. When identifying microorganisms it should be remembered that their characteristics may be variable. In addition, species within a genus may differ in some characteristics, for example *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.

## 4.2 Principles of identification

Identification of bacteraemia and fungemia is the most important and complex role of a clinical microbiology laboratory, usually by direct comparison of unknown with those of type cultures[1]. There are three basic methods of identification. The first relies heavily on the experience of the investigator: a judgement is made on the presumptive identity of the organism based and 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, the next approach will be 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, setting up tests unnecessarily, misinterpretation of results at this stage can lead the investigator down an incorrect path, which wastes both time and resources and may also lead to a misidentification and ultimately incorrect diagnosis. 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 clearly defined as reactions may vary between organisms.

Matrix-Assisted Laser Desorption/Ionisation – Time of Flight Mass Spectrometry (MALDI-TOF MS), is increasingly being applied for organism identification within diagnostic microbiology laboratories. Identification using this technology affords the opportunity to rapidly and cost-effectively identify bacteria, most yeast species as well as some genera of filamentous fungi in comparison with the more traditional techniques[2]. Any identification should be considered alongside other phenotypic information that is available.

# 5 Technical information/limitations

### Colonial morphology

Due to variation among isolates and colonies grown on different culture media, for moulds identification the colonial morphology characteristics should be combined with the microscopic morphological features for a definitive identification[3].

### Culture technique

The culturing of bacteria on agar media yield results but there are inherent delays in diagnosis associated with this technique.

### Commercial identification systems

The use of commercially available identification kits alone may be unreliable because they may not be able to distinguish between related organisms as well as not being able to identify new species of organism that are not in the accompanying database. Therefore, commercial systems should be used alongside other tests[4,5].

**Germ tube test**

*Candida albicans* is one of the few yeast that produce germ tube and rare strains of *Candida tropicalis* may also produce a germ tube. However *C.tropicalis* may produce early pseudohyphae which may be misinterpreted as germ tube production. Experience and expertise are required for correct recognition of germ tubes.

**Conflicting findings**

When conflicting results are observed, for example; biochemical profile disagrees with serological profile, tests should be repeated from the original plate or additional tests to be performed.

**Matrix-assisted laser desorption/ionisation - time of flight mass spectrometry (MALDI-TOF MS)**

Compare MALDI-TOF MS results against the colony morphology and clinical information for accurate identification. Refer to UK SMI TP 40 - Matrix-assisted laser desorption/ionisation - time of flight mass spectrometry (MALDI-TOF MS) test procedure for more information on the technical limitations.

**Quality control**

Each new batch or shipment of commercial identification systems should be tested and verified for positive and negative reactivity using known control strains; ensuring it is fit for purpose. Laboratories must follow manufacturer's instructions when using these products.

# 6 Safety considerations

View current guidance on the safe handling of all organisms documented in the safety considerations section of the UK SMI scientific information.

As a minimum, it is recommended that the processing of any culture that may result in generation of aerosols should be processed in a microbiological safety cabinet in accordance with the relevant risk assessment, ACDP and HSE guidelines.

Processing of diagnostic sample cultures that are assessed to be at higher risk of containing hazard group 3 organisms must be undertaken under appropriate containment conditions as determined by risk assessment, and as required by Biological agents: managing the risks in laboratories and healthcare premises. This will normally be under full CL3 conditions. Such organisms include *Mycobacterium* species, *Brucella* species, *Bacillus anthracis*, *Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Coccidioides immitis,* etc. The above guidance should be supplemented with local COSHH and risk assessments.

Compliance with postal and transport regulations is essential.

# 7 Target organisms

All medically important bacteria and fungi.

# 8 Identification

Refer to relevant identification UK SMIs for further information. It should be noted that the most commonly encountered organisms are listed in the flowcharts (see appendix 1 to 5) for characterisation and identification, the list of organisms is not exhaustive but those listed are used as examples for characterisation.

## 8.1 Microscopic appearance

### Bacteria

Microscopic study and staining reveal the shape (coccus or rod) and the characteristic grouping and arrangement of the cells, their size and the presence of intracellular inclusions, for example spores. In addition to morphology, the Gram stained preparation also divides bacteria in two categories - the Gram positive and the Gram negative bacteria[3,6].

For morphological appearance, it is preferable to examine fresh cultures from growth on non-selective media. Table 1 details the terms used for stained preparations of bacteria using different types of stains.

### Fungi[3,7]

Fungal isolates from clinical samples can broadly be split into either yeast or mould depending on their predominant growth form. If they exhibit a unicellular growth form with reproduction by budding to produce individual discrete colonies on culture plates then they would be classified as yeast. Moulds demonstrate a filamentous growth form with long, branching hyphae and a single colony may grow to fill an entire Petri dish. However, when grown at different temperatures some fungi are dimorphic, i.e. grow as hyphae at ambient temperature and as yeasts at 37 °C. Dimorphic is having the ability to switch between yeast and hyphae such as *Blastomyces dermatitidis, Sporothrix schenckii* and *Histoplasma capsulatum*.

Moulds reproduce by producing spores often in a very characteristic way, the sporing structures and the spores themselves can aid in identification of the isolate. Microscopic examination is used to observe conidia and spores using methods such as tease mounts, slide culture and adhesive tape mount. Mount can be made from mould colonies by either taking a portion of the surface growth from the colony with a sharp needle and teasing it out in a drop of mounting fluid on a microscope slide and applying a coverslip, known as a tease or needle mount; or by placing a piece of an adhesive tape (good quality, optically clear) fungus-side down onto a drop of lactophenol cotton blue and apply coverslip for examination[7].

There are several types of stains that could be used to highlight microscopic characteristics of fungi to aid identification such as saline mount, lactophenol cotton blue, calcofluor white with 10% KOH and India ink. For more information, see TP 39 - Staining procedures. Microscopic examination include tease mount, slide culture and cellophane tape mount. Microscopy can provide presumptive identification therefore

microscopic methods should be used in conjunction with clinical history, culture, serology or biochemical or molecular testing.

Growth on minimal medium such as Czapek-Dox or a complex media such as cornmeal together with Tween 80 is used to examine the morphological appearance of clinically important yeast. Using these media yeast can be sub-cultured using Dalmau technique, this method of inducing production of morphological characteristics is used to look for the production of true hyphae, pseudohyphae, arthrospores, chlamydospores and capsules. Species such as *Candida albicans* and *C. tropicalis* grown on Czapek Dox or Cornmeal agar may often produce true hyphae, whereas species such as *C. parapsilsosis* and *C. krusei* produce pseudohyphae[8].

It is also useful to note the colonial characteristics such as smooth, wet, dry or wrinkled colonies and any pigment production. Table 2 details the terms used for stained preparations of fungi using different types of stains.

**Table 1: Terms used for stained preparations of bacteria[7]**

| Term | Description |
|---|---|
| **Arrangement** | singly, in pairs, in chains, in fours (tetrads), in groups, grape-like clusters, in cuboidal packets, in bundles, palisade. |
| **Capsule** | presence or absence. |
| **Endospores** | spherical, oval or ellipsoidal, equatorial, sub-terminal, 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, for example filamentous forms interspersed with coccobacillary forms. |
| **Shape** | spheres (cocci), short rods (coccobacilli), long rods (bacilli), filamentous, curved rods, spirals. |
| **Sides** | parallel, bulging, concave or irregular. |
| **Size** | length and breadth. The diameter is measured in micrometres (µm). |
| **Staining** | even, irregular, unipolar, bipolar, beaded, barred, acid-fast. |

**Table 2: Terms used for stained preparations of yeasts and filamentous fungi[9-11]**

| Term | Description |
|---|---|
| **Arrangement** | it should be noted that presence of budding is a feature that is useful in identification of yeasts which may show budding on a narrow or broad base. The arrangement and mechanism of production of spores is key to the identification of mould species. |
| **Capsule** | presence or absence.<br>Capsules are readily visible in Indian ink preparations or in some histological stains. |

| | |
|---|---|
| **Hyphae** | presence or absence and with or without branching, whether septate or pauci-septate, true or pseudohyphae in the case of yeast isolates. |
| **Sporangia /sporangiospores, Conidia /conidiospores, Aleuriospores, Arthrospores, Multiseptate conidia, Chlamydospores** | presence or absence.<br>Although sporulation is rare in some fungi there are ways to encourage sporulation and phenotypic identification will not be possible without spore production. |
| **Shape of spores** | small, large, oval, spherical, cylindrical, slightly curved, ellipsoidal, crescent-shaped, septate, multi-septate.<br>**Note:** some moulds will have more than one type of spore. |
| **Size** | There is great variation in size of cells. The diameter is measured in micrometres (µm). |

## 8.2 Cultural appearance[6,7]

Bacterial or fungal colonies of a single species, when grown on specific media under controlled conditions are described by their colony morphology, characteristic size, shape, colour, consistency, odour, metabolic reaction, and sometimes pigmentation. When growth conditions are carefully controlled, colonial morphology is important for preliminary identification and for differentiating organisms. However, it should also be noted that the growth rate of certain organisms is variable, depending on the amount of inoculum (bacterial or fungal) present in a clinical specimen.

### Bacteria

Bacteria can be characterised into various shapes and sizes. For example, *Streptococcus* species are small, usually 1mm in diameter and usually appear in pairs or short chain. *Staphylococcus* species and species within the family, Enterobacteriaceae are larger and usually 2 to 3mm in diameter. *Staphylococci* exhibit grape like clusters and Enterobacteriaceae are long with rounded ends. *Bacillus* species are much larger in size and usually 2 to 7mm in diameter and are rod shaped[7]. The growth rate for bacteria vary from organism to organism, for example, *Campylobacter* species will yield a good growth when incubated for 48 to 72 hours uninterrupted under microaerophilic conditions while *Listeria* species will grow very well when incubated in 5 to10% $CO_2$ at 35°C to 37°C for 16 to 48hr[3]. Table 3 details the terms used in colonial morphology of bacteria

### Fungi

Fungal colonial morphology and growth rate may vary depending on the type of culture medium used, age of culture, amount of inoculum and the temperature of incubation, for example *Histoplasma capsulatum* colonies exhibit thermal dimorphism and appear as white to tan mould colonies when grown on Sabouraud agar at 25 to 30°C.For example, the growth rate for dimorphic fungi such as *Blastomyces dermatitidis*, *Histoplasma capsulatum* and *Coccidioides immitis* is slow, it is common that culture remain negative for weeks before colonies appear; about 1 week to 4 weeks are usually required for viable growth (that is, before colonies become visible)[11]. Certain genera of fungi, for example *Trichophyton violaceum or T. verrucosum* take up to 14 to 21 days for visible growth and re-incubation for longer growth. Fungal culture should be examined at regular intervals, plates should be

incubated for 4 to 6 weeks before being regarded as negative[3]. Table 3 details the terms used in colonial morphology of yeast and filamentous fungi.

**Table 3: Terms used in colonial morphology of bacteria[6,7,10]**

| Term | Description |
|---|---|
| **Colour** | by reflected or transmitted light: fluorescent, iridescent, opalescent,<br>**Note:** There are many colours ranging from white to yellow, pink, orange, red or purple. |
| **Pigmentation** | Some organisms produce a pigmented colony which is usually enhanced at room temperature, this can be seen on the topside and reverse side of the colony. For example Pseudomonas aeruginosa green pigment and , Serratia marcescens red pigment,, Rhodoturula mucilaginosa, Aspergillus nidulans), although non-pigmented strains within a species may occur |
| **Consistency (texture)** | butyrous (buttery), fluffy, mucoid (thick, stringy, and wet), friable, membranous, rugose (wrinkled), dry, moist, brittle, viscous, powdery, velvety, glabrose, granular, floccose. |
| **Edge/margin** | entire, undulate, lobate, crenated, erose, fimbriate, effuse, filiform, curled, wavy. |
| **Elevation (topography)** | flat, raised, low convex, convex or dome-shaped, umbonate, with or without bevelled margin, pulvinate, crateriform. |
| **Emulsifiability** | easy or difficult, forms homogeneous or granular suspension or remains membranous when mixed in a drop of water. |
| **Shape/form** | Colonial shape is determined by the edge and thickness of the colony: smooth, filiform, spreading, rhizoid, circular, irregular, filamentous, spindle, punctiform, radiate. |
| **Opacity** | transparent, translucent, opaque. |
| **Size** | The diameter is usually measured in millimetres. Colony size varies and it is also described in terms such as pinpoint, small, medium and large. |
| **Structure** | amorphous, granular, filamentous, curled. |
| **Surface** | smooth, glistening, rough (fine, medium or coarsely granular), concentric (ringed), papillate, dull or wrinkled, heaped up, contoured, veined. |
| **Degree of growth** | scanty, moderate or profuse. |

For individual bacterial colonial descriptions, see the relevant identification UK SMI using link: https://www.gov.uk/government/collections/standards-for-microbiology-investigations-smi#identification.

**Table 4: Terms used in colonial morphology of yeasts and filamentous fungi[3,7,10,11]**

| Term | Description |
|---|---|
| **Colour** | yeast colonies are usually white, cream, yellow, red, pink or brown. Mould colonies vary greatly, often in shades of green, red, brown or black and the surface colour usually reflects the colour of the spores. For some groups such as the dermatophytes looking for reverse pigmentation on the underside of colonies can be helpful. |

| | |
|---|---|
| **Pigmentation** | pigment production may colour the entire colony as with yeast or in some moulds it may only be the spores that are pigmented. Colonies of some moulds may produce diffusing pigments. |
| **Consistency (texture)** | fungal colony characteristics are dependent upon whether it is yeast or a filamentous fungus. They range from cottony or woolly (floccose), granular, chalky, velvety, powdery, silky, glabrous (smooth), or waxy. |
| **Edge/margin** | entire, undulate, filamentous, lobate, erose (serrated). |
| **Elevation (topography)** | It is observed on the reversed side of the agar and described as: flat, raised, convex, crateriform, heaped, grooved, folded or wrinkled. |
| **Size** | the diameter is usually measured in millimetres. Colony size varies and it is also described in terms such as slow-growing, small, medium and large. |
| **Degree of growth** | some fungal colonies are fast growing, covering the entire surface of the agar and taking up all the air-space in a petri-dish whilst other fungi may grow in a restricted manner. |

**Note:** Yeast colony descriptions can be very similar to bacterial colonies.

## Culture media

Microorganisms have specific growth factor requirements that must be added to the media for growth such as source of nitrogen and carbon. Agar is the most important solidifying agent used in media, meat and plant are also used as source of nutrients for the cultivation of microorganisms. Type of media should be carefully selected based on specimen type and suspected agent.

There are several commercially available chromogenic media, majority of them are selective and differential. These are designed to target organisms with high specificity and sensitivity when present among other flora. Chromogenic substrates are incorporated into these media that are broken down by enzymes imparting a distinct visible colour to the growing colonies to help in their identification. The use of chromogenic agar has been very useful in the isolation and presumptive detection of bacteria and yeasts pathogens such as *Clostridium difficile*, *Pseudomonas aeruginosa* and direct identification of some Candida species. One of the primary test for the diagnosis of fungal infections is fungal culture; Sabouraud dextrose and malt extract agar plates are the most common culture media used[12,13].

## Haemolysis[7]

Some organisms produce haemolysins, which cause lysis of erythrocytes in blood-containing media. This haemolysis may be:

- β (clear zone around the colony causing a clearing of the medium),
- α (partial lysis of the red blood cells surrounding a colony causing a greenish discolouration of the medium),
- α-prime (a small zone of intact red cells with a surrounding zone of haemolysis) or
- non-haemolytic previously called γ-haemolysis (no haemolysis, no apparent change in the colour of the medium).

**Note:** This is used as a form of identification more in bacteria (particularly streptococci and related Gram-positive cocci) although yeasts can occasionally cause haemolysis.

### Resistance properties

Certain organisms exhibit a characteristic inherent resistance to specific antibiotics, heavy metals, or toxins[10]. This characteristic is widely used to establish preliminary identification information in bacteria and no longer used for yeasts. For example, Gram positive organisms grow on Columbia blood agar supplemented with colistin and nalidixic acid inhibiting the growth of Gram-negative bacilli.

Testing the susceptibility of an isolate to a particular antibiotic is also useful in identification, for example most clinically significant Gram-negative bacteria are resistant to vancomycin and susceptible to the antibiotics, colistin or polymyxin.

## 8.3 Growth requirements

Microorganisms can be grouped on the basis of their growth requirements. They are as follows:

### Atmosphere[10]

It is usual to divide organisms into 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
- microaerophilic organisms grow best in an atmosphere with reduced oxygen concentration (addition of 5 to10% $CO_2$ may enhance growth)
- carboxyphilic (or capnophilic) organisms require additional $CO_2$ for growth

### Temperature[10]

Organisms may also be divided according to their temperature requirements:

- psychrophilic organisms grow at low temperatures 2 to 5°C (optimum 10 to 30°C).
- mesophilic organisms grow at temperatures between 10 to 45°C (optimum 30 to 40°C).
- thermophilic organisms grow very little at 37°C (optimum 50 to 60°C).
- hyperthermophilic organisms grow at temperatures of 80°C or higher

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 flagella shown by a darting “zigzag” movement or peritrichate flagella, which cause a less vigorous and more vibratory movement. Some bacteria may be motile at different temperatures, for

example motile at ambient temperature but not at 37°C, or vice versa (TP 21 - motility test).

### Nutrition[10]

Study of the nutritional requirements of an organism is useful in identification, for example 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 (Nicotinamide Adenine Dinucleotide (NAD)) for the growth of *Haemophilus* species.

## 8.4 Tests for bacteria[3,6,7]

Numerous biochemical tests may be used for the identification of microorganisms (refer to individual identification UK SMIs). 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. Examples of biochemical tests are:

- **Catalase** (TP 8 - Catalase test)

  The catalase test is used to detect the presence of catalase enzymes by the decomposition of hydrogen peroxide to release oxygen and water as shown by the following reaction:

  $2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

  Hydrogen peroxide is formed by some bacteria as an oxidative end product of the aerobic breakdown of sugars. If allowed to accumulate it is highly toxic to bacteria and can result in cell death. Catalase either decomposes hydrogen peroxide or oxidises secondary substrates, but it has no effect on other peroxides. Blood must be avoided, as erythrocyte produce catalase and can give a false positive reaction.

  This test is essential for differentiating between Gram positive cocci: *Staphylococcus* are catalase positive and *Streptococcus* are negative. As well as Gram-positive bacilli: *Bacillus* is catalase positive and *Clostridium* is negative

- **Oxidase** (TP 26 - Oxidase test)

  The oxidase test is used to determine if an organism possesses the cytochrome oxidase enzyme. 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 and fermentation of glucose test)

  The oxidative-fermentative test is used to determine if bacteria metabolise carbohydrates oxidatively, by fermentation, or are non-saccharolytic and therefore have no ability to use the carbohydrate in the media.

  Oxidative organisms can only metabolise glucose or other carbohydrates under aerobic conditions, that is, oxygen is the ultimate hydrogen acceptor. Other organisms ferment glucose and the hydrogen acceptor is then another substance, for example sulphur. This fermentative process is independent of

oxygen and cultures of organisms may be aerobic or anaerobic. The end product of metabolising a carbohydrate by fermentation is an acid.

- **Different staining methods** (TP 39 - Staining procedures)
  There are many staining procedures commonly used for the identification of microorganisms. These stains are used to highlight structures in clinical specimens/isolates, often when viewed with the aid of different types of microscopes. Stains have different affinities for different organisms and are used to differentiate types of organisms or to view specific parts of organisms. Bacterial morphology can be observed using stains such as Gram stain which can be used to differentiate Gram positive bacteria from negative bacteria. For more information on staining procedures, refer to the relevant UK SMI.

Using these few simple tests it is usually possible to place organisms, provisionally, in one of the main groups of medical importance. The biochemical tests (above) list the common tests used once an organism has been isolated on culture plates and colonial appearance and growth requirements have been assessed. The lists are not exhaustive and further tests may be needed in addition to the common ones described. Refer to https://www.gov.uk/government/collections/standards-for-microbiology-investigations-smi#test-procedures for the full list of all UK SMI test procedures.

## 8.5 Antigenic typing or Serotyping tests[1]

Serotyping is a subtyping method based on the immuno-reactivity of various antigens, different strains of a species may have different antigens. These antigens can be determined by specific antisera. Selected antisera can be used to classify different bacterial species. This may be based on either carbohydrate or protein antigens from the bacterial cell wall or the capsular polysaccharide. There are commercially available serotyping identification kits for the rapid detection of organisms.

## 8.6 Tests for fungi

### Diagnostic tests[13,14]

Diagnostic tests such as targeted pan-fungal PCR and latex agglutination are rapid and valuable diagnostic tests for the identification of fungal diseases. These tests are often more sensitive and specific but the results may vary depending on the species, sample and host. Therefore these tests should be used in conjunction with culture, microscopic observations made by clinical mycologist, assessment of the host and radiographic features.

### Germ tube test[3]

This is a rapid presumptive identification test used to differentiate *Candida albicans, Candida africana* and *Candida dubliniensis* from other *Candida* species and other yeasts. Strains of all three of these species produce the germ tube when incubated with serum at 35ºC to 37 ºC for 1 to 3 hours. This test is presumptive because not all isolates of *Candida albicans* will be germ tube positive. Results should be read between 2 to 3 hours to reduce false positives. Heavy inoculum of yeast and presence of bacteria may lead to false negatives. It should be noted that further tests to be performed to confirm any initial results and to differentiate Candida strains[7].

### Rapid urease test[7,15]

This can be used for differentiation between the yeasts, *Candida albicans* and *Cryptococcus neoformans*. A presumptive identification of *C. neoformans* is based on rapid urease production, whilst *Candida albicans* do not produce urease. Other species of *Cryptococcus*, *Trichosporon* and *Rhodotorula* can give positive result for urease test. Occasionally, *Candida krusei* can give a positive result. This test should be used in conjunction with other tests. For more information on urease test, refer to TP 36 - urease test.

### Dermatophyte test medium[16]

This can be used for isolation and presumptive identification of dermatophytes filamentous fungi such as *Microsporum*, *Trichophyton*, and *Epidermophyton* genera because of a distinct colour change in the medium. Rapidly growing species may produce a complete colour change in the medium within days while the slower growing species will take longer periods to change the indicator. Care should be taken during interpretation, as some non-dermatophytes can also cause a colour change. Dermatophyte can be isolated from hair, skin and nail specimens.

**Note:** The complete classification of dermatophytes is dependent upon microscopic observations of direct and slide culture preparations, along with clinical symptoms presentation and other biochemical tests.

## 8.7 Rapid identification methods

### Matrix-assisted laser desorption/ionisation - time of flight mass spectrometry (MALDI-TOF MS)

MALDI-TOF MS is a simple, rapid, accurate and highly reliable identification tool for the characterisation of a diverse collection of pathogens, it combines the advantages of phenotypic assays with the rapidity and accuracy of molecular assays. Over the past years this technique has been increasingly used by the diagnostic laboratories due to its high reproducibility, speed and sensitivity of analysis and increased turnaround times compare to phenotypic methods, it can also be directly applied to clinical samples. MALDI-TOF MS also has the ability to perform more tests, requiring less staff with minimal expertise and guarantee traceability throughout the process. Some drawback are high initial cost of the equipment but low cost per test, lack of universal primers and high bacterial load. The range of clinical applications of MALDI-TOF MS for bacterial isolates is increasing constantly, from species identification to the two most promising applications in the near future: detection of antimicrobial resistance and strain typing for epidemiological studies[17].

MALDI-TOF MS is rapidly becoming a standard method for yeast identification such as differentiating *Candida albicans* from *Candida dubliniensis* and it is increasingly implemented for moulds. Although fungi can be identified by MALDI-TOF MS but some issues are faced with filamentous fungi, such as extracting protein for MALDI-TOF MS analysis, fast-changing fungi morphology and commercially available fungal reference libraries are currently not as comprehensive as the bacterial ones[18,19]. The identification of moulds using this method is being developed. It has equally been very useful for direct testing of clinical specimens such as urine, cerebrospinal fluid and blood which has resulted in significant improvements to patient care and reduced turnaround time to result[2].

MALDI-TOF MS (with an extensive database) has also been used for the identification to the species level of rare yeast such as Cryptococcus, Saccharomyces and Trichosporon[20].

For more information, refer to UK SMI TP 40 - Matrix-assisted laser desorption/ionisation - time of flight mass spectrometry (MALDI-TOF MS) test procedure.

## Whole genome sequencing (WGS)

This is also known as full genome sequencing, complete genome sequencing, or entire genome sequencing. This technique determines the complete DNA sequence of an organism's genome at a single time. WGS is a rapid, affordable and accurate genotyping tool that provides information on pathogen detection, identification, epidemiological typing and drug susceptibility. WGS is becoming widely used technique in research, clinical diagnostics and public health laboratories. There are several high-throughput techniques that are available and used to sequence an entire genome such as pyrosequencing, nanopore technology and sequencing by synthesis (SBS) technology etc.

WGS has been used successfully to explore the genome of organisms as well as to identify candidate genes responsible for pathogenesis, and to develop better methods of strain detection and to advance the understanding of the evolution of organisms. For example WGS has the ability to provide appropriate method for sequencing of *Shigella sonnei, Escherichia coli* O157 in terms of outbreaks detection, epidemiological surveillance, study of antimicrobial resistance and the detection of emerging phenotypes[21,22]. WGS has been used by the Centers for Disease Control and Prevention in outbreaks caused by *Candida auris*, *Listeria, Cryptospordium* and study antibiotics resistance in bacteria[23,24].

## Commercial identification Systems (kits/rapid tests)[7]

The use of commercially available identification kits alongside other biochemical reactions may be used to give accurate identification of bacteria and yeasts[25]. It should be noted that there are no commercial kits for biochemical profiling of filamentous fungi. Laboratories should follow manufacturer's instructions. Ideally, where possible, scores should be available and easily accessible during the authorisation process and for audit purposes. In many cases, the commercial identification system may not reflect recent changes in taxonomy.

**Note:** If a commercial yeast identification kit provides a biochemical profile but also specifies examination of key morphological features to obtain a numerical profile it is not sufficient to identify the organism based only on the biochemical profile.

## Other identification methods

Molecular methods have had an enormous impact on bacterial and fungal taxonomy and analysis of gene sequences has increased understanding of the phylogenetic relationships of bacteria, fungi and other related organisms. These methods have made identification of many species more rapid and precise than is possible with phenotypic techniques and have also aided the recognition of numerous new species. Some of these methods remain accessible to reference laboratories only and are difficult to implement for routine microbial identification in a clinical laboratory due to costs and lack of expertise amongst staff.

Molecular methods include both detection and typing methods and examples include real-time polymerase chain reaction, pulsed-field gel electrophoresis, Multi-locus sequence typing, multiple-locus variable-number tandem-repeat analysis also known as VNTR, 16S rDNA sequencing and 18S rRNA sequencing.

## Real-time polymerase chain reaction (RT- PCR)

Real-time polymerase chain reaction (RT-PCR) is the method of choice for sensitive detection and precise quantification of minute amounts of targeted DNA sequence. When combined with reverse transcription (RT) real-time PCR is the preferred method also for the detection and quantification of RNA. Benefits of this procedure over conventional methods include sensitivity, enhanced specificity, and the potential for high throughout as well as accurate quantification. An additional advantage of real-time PCR is the relative ease and convenience of use compared to some older methods. This method has been used successfully in the identification of bacteria and fungi[26,27].

For more information on the key laboratory elements needed when performing molecular PCR assays can be accessed from Q 4 - Good laboratory practice when performing molecular amplification assays.

## 16S rRNA gene sequencing[28]

Culture method has been considered the gold standard method for bacterial identification however due to slow growing bacteria it can take up to days/weeks to get the results therefore to complement culture 16S rRNA based characterisation of bacterial species has been universally accepted as an accurate and faster method of bacterial identification and phylogenetic classification. The 16S rRNA gene is a universal gene found in all bacterial chromosomes. It is used to identify the presence of conserved and variable regions in the 16S rRNA gene for phylogenetic identification.

Some drawbacks are that this method fails to distinguish between some closely related species with similar sequence identity.

## 18S ribosomal RNA (18S rRNA), intergenic spacer region (ITS), large ribosomal subunit (D1-D2) sequencing

These molecular method work with different primer pairs in a very similar way to the 16S rDNA sequencing and they have been very useful in the identification of clinically relevant fungi (yeasts and filamentous fungi)[29]. Different approaches are used for different groups of fungi with ITS1 and ITS2 proving the most useful for most fungal genera.

# 9 Reporting

Refer to individual UK Standard for Microbiology Investigation.

**Note:**

The results of any identification tests should be entered in the pathology IT system and should be available to staff validating those results. For automated identification systems, identification scores (that identify the probability of a correct identification) and organisms in the differential list should be entered so that the likelihood of the preferred and alternative identifications can be considered in the context of the clinical circumstances and consideration can be given as to when alternative identification tests are required. However it should be noted that it is not always feasible to store all the alternative identifications from the various identification systems onto the IT system as these systems should have already been validated before put in routine use.

## 9.1 Infection Specialist

Certain clinical conditions must be notified to the laboratory associated infection specialist.

Follow local protocols for reporting to the patient's clinician.

## 9.2 Preliminary identification

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

## 9.3 Confirmation of identification

For confirmation and identification please see Specialist and reference microbiology: laboratory tests and services page on GOV.UK for reference laboratory user manuals and request forms.

## 9.4 Health Protection Team (HPT)

Refer to local agreements in devolved administrations.

## 9.5 Public Health England[30]

Refer to current guidelines on SGSS reporting.

## 9.6 Infection prevention and control team

N/A

# 10 Referral to reference laboratories

Refer to individual UK Standard for Microbiology Investigation for further information.

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory see the Specialist and reference microbiology: laboratory tests and services page on GOV.UK for user manuals and request forms

Organisms with unusual or unexpected resistance, or associated with a laboratory or clinical problem, or an anomaly that requires investigation should be sent to the appropriate 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:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Scotland

https://www.hps.scot.nhs.uk/a-to-z-of-topics/reference-laboratories/

Northern Ireland

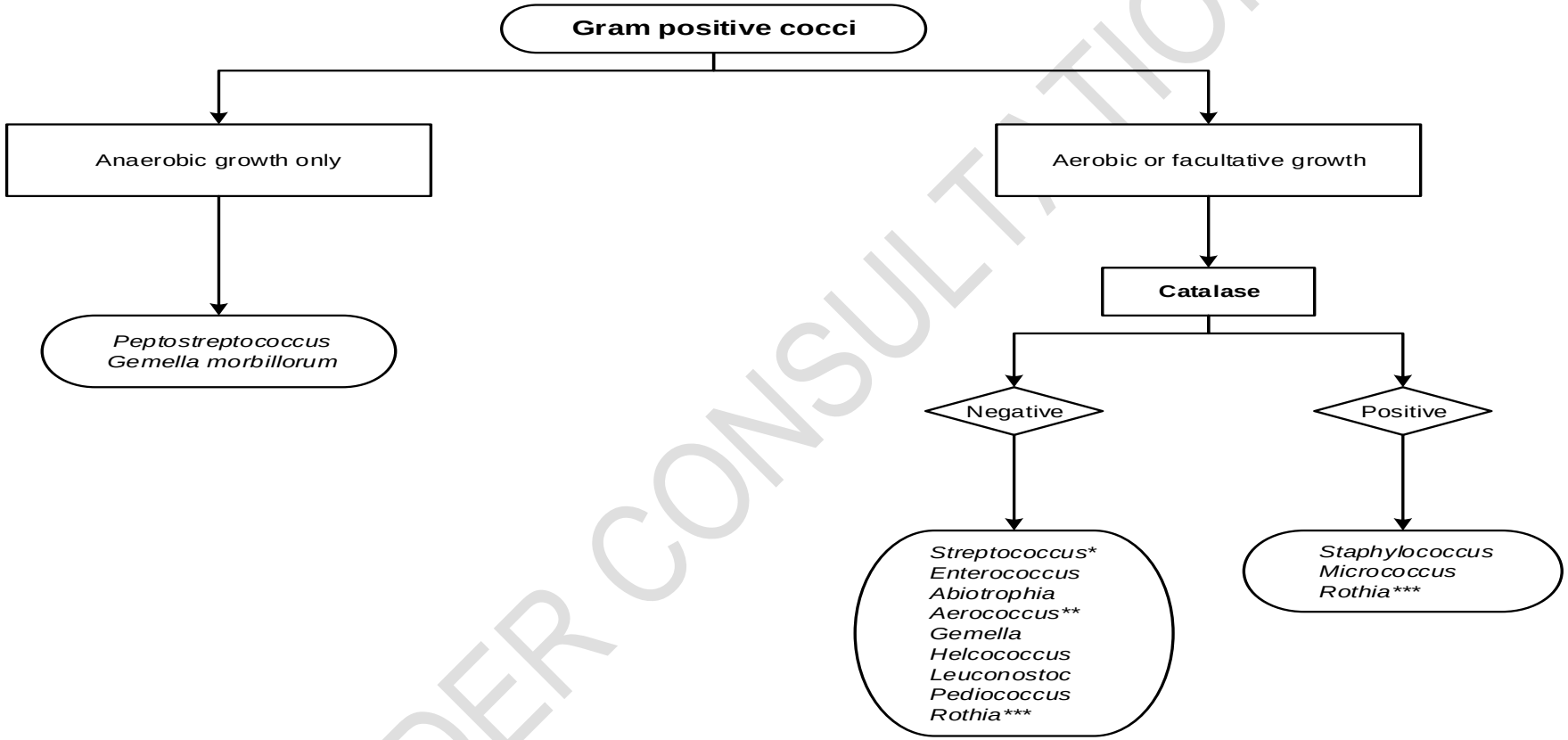
https://www.publichealth.hscni.net/directorates/public-health/health-protection

**UK Clinical Mycology Network**
UKCMN Secretariat
c/o PHE Mycology Reference Laboratory
Infection Sciences
Pathology Sciences Building
Southmead Hospital
Bristol
BS10 5NB
https://www.gov.uk/government/groups/uk-clinical-mycology-network

**PHE Mycology Reference Laboratory**
National Infection Services, PHE South West Laboratory
Science Quarter
Southmead Hospital
Bristol
BS10 5NB
https://www.gov.uk/government/publications/mycology-reference-laboratory-mrl-service-user-handbook/mycology-reference-laboratory-service-user-handbook

# Appendix 1: Characteristics of Gram positive cocci[6,31,32]

An accessible text description of this flowchart is provided with this document.




***** Some species may be anaerobic

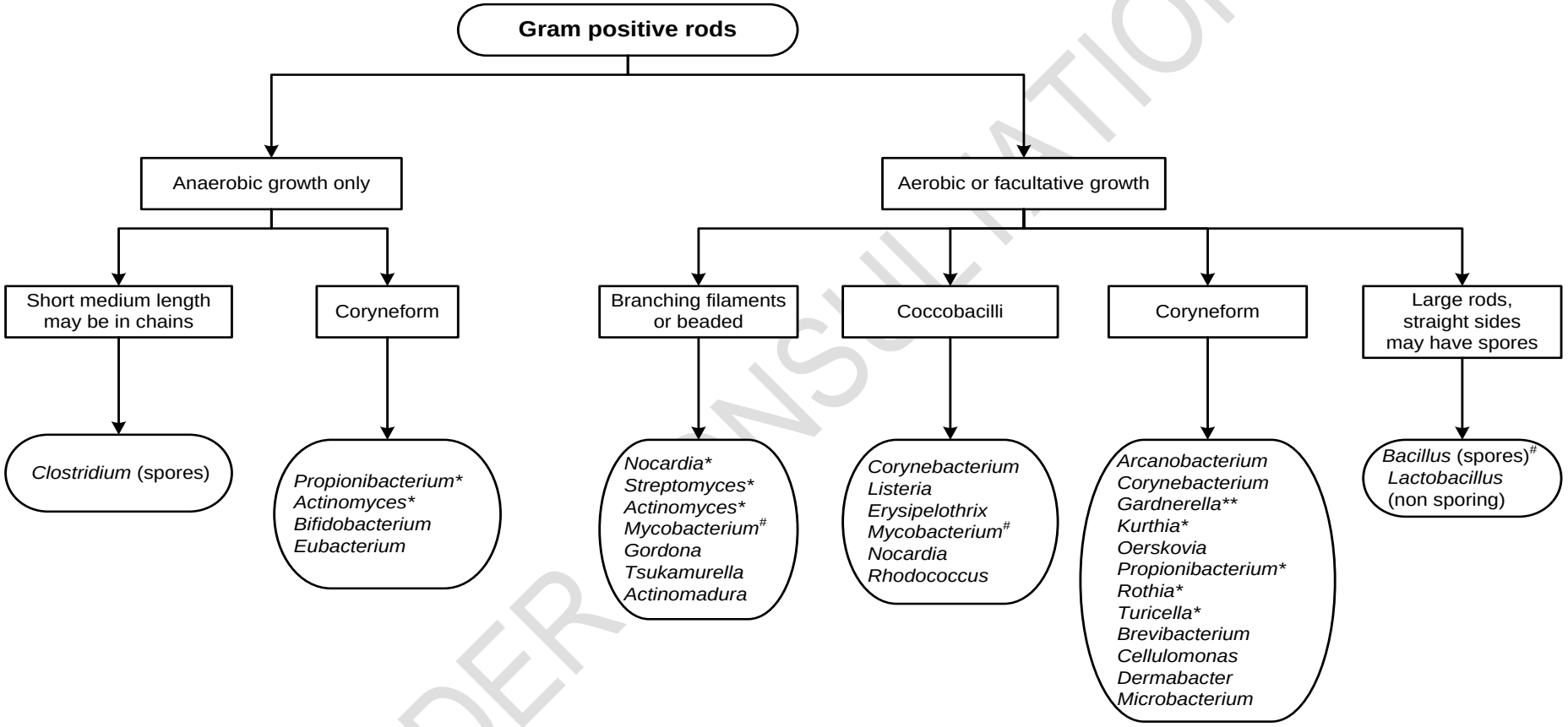
****** May be weak catalase positive

******* This organism is pleomorphic (with a variation in the size and shape of cells) catalase variable, catalase test may not be helpful for differentiation

The flowchart is for guidance only.

# Appendix 2: Characteristics of Gram positive rods[6,31,32]

An accessible text description of this flowchart is provided with this document.




*This organism is pleomorphic

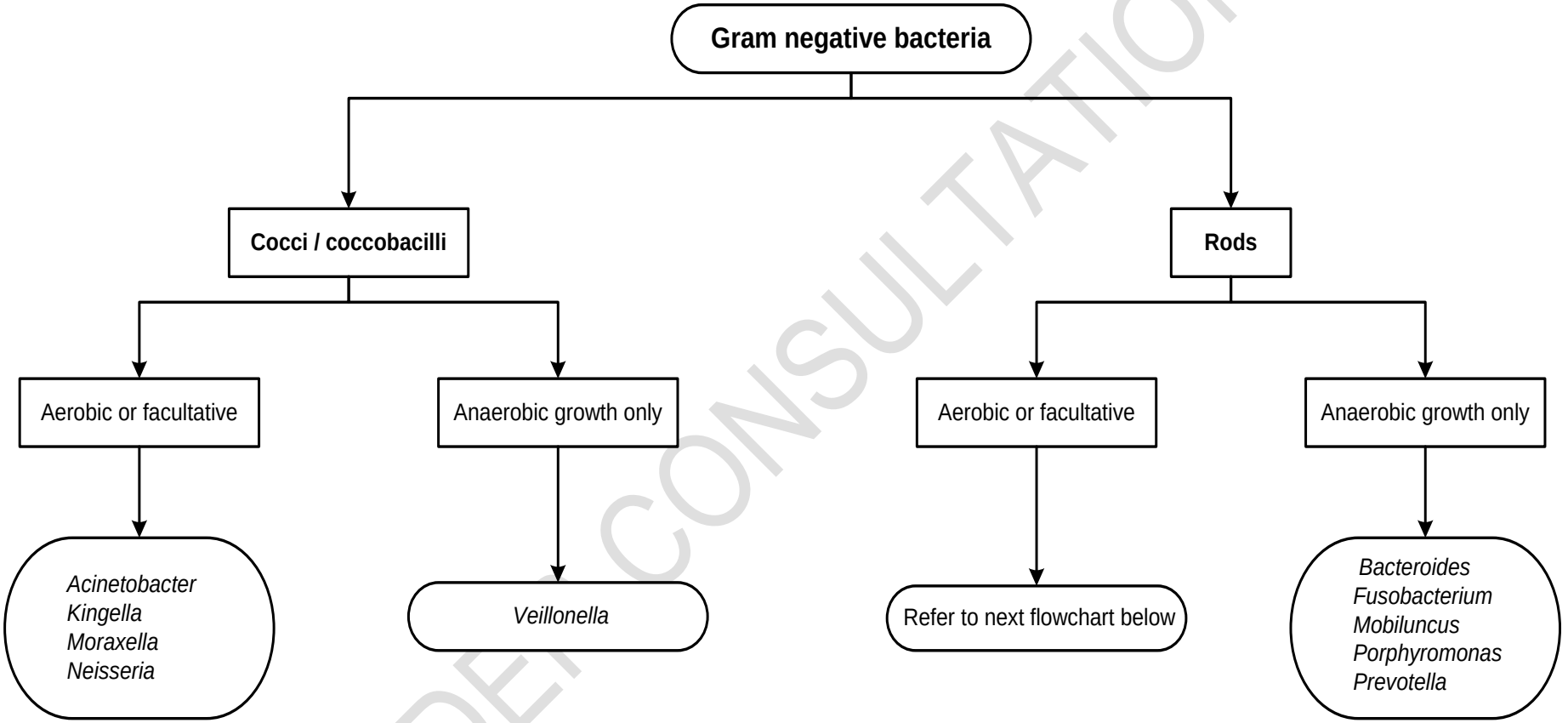
***Gardnerella vaginalis* is a Gram variable rod and may usually be differentiated by its microscopic appearance

# These organisms (that is, *Mycobacterium tuberculosis* and *Bacillus anthracis*) are hazard group 3 organisms and should be processed in a Containment level 3 laboratory. *Mycobacterium* species should be referred to the Reference Laboratory for full identification

The flowchart is for guidance only.

# Appendix 3: Characteristics of Gram negative bacteria[31,33]

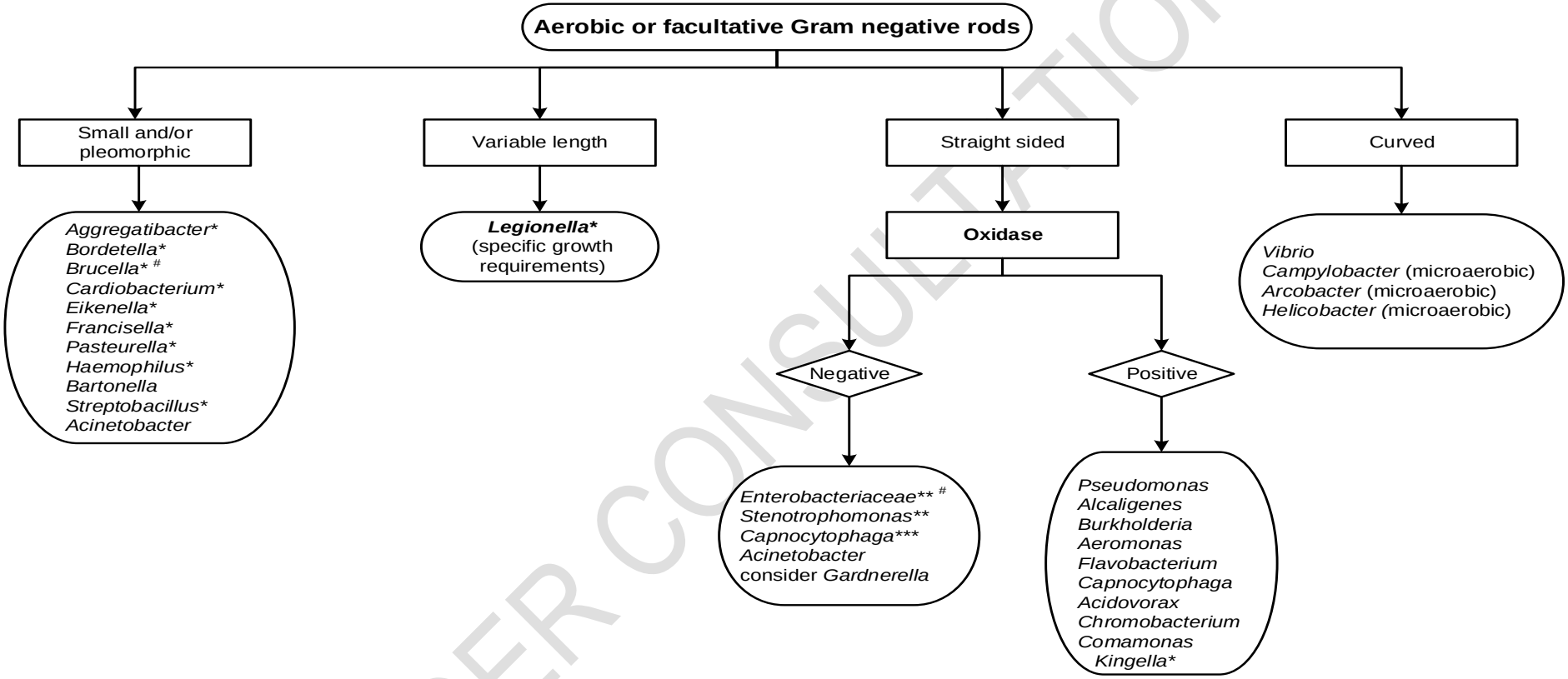
An accessible text description of this flowchart is provided with this document.




The flowchart is for guidance only.

# Appendix 4: Characteristics of Gram negative bacteria[31,33] *(Continued from previous page)*

An accessible text description of this flowchart is provided with this document.




***** Some species may be anaerobic

****** May be weak catalase positive

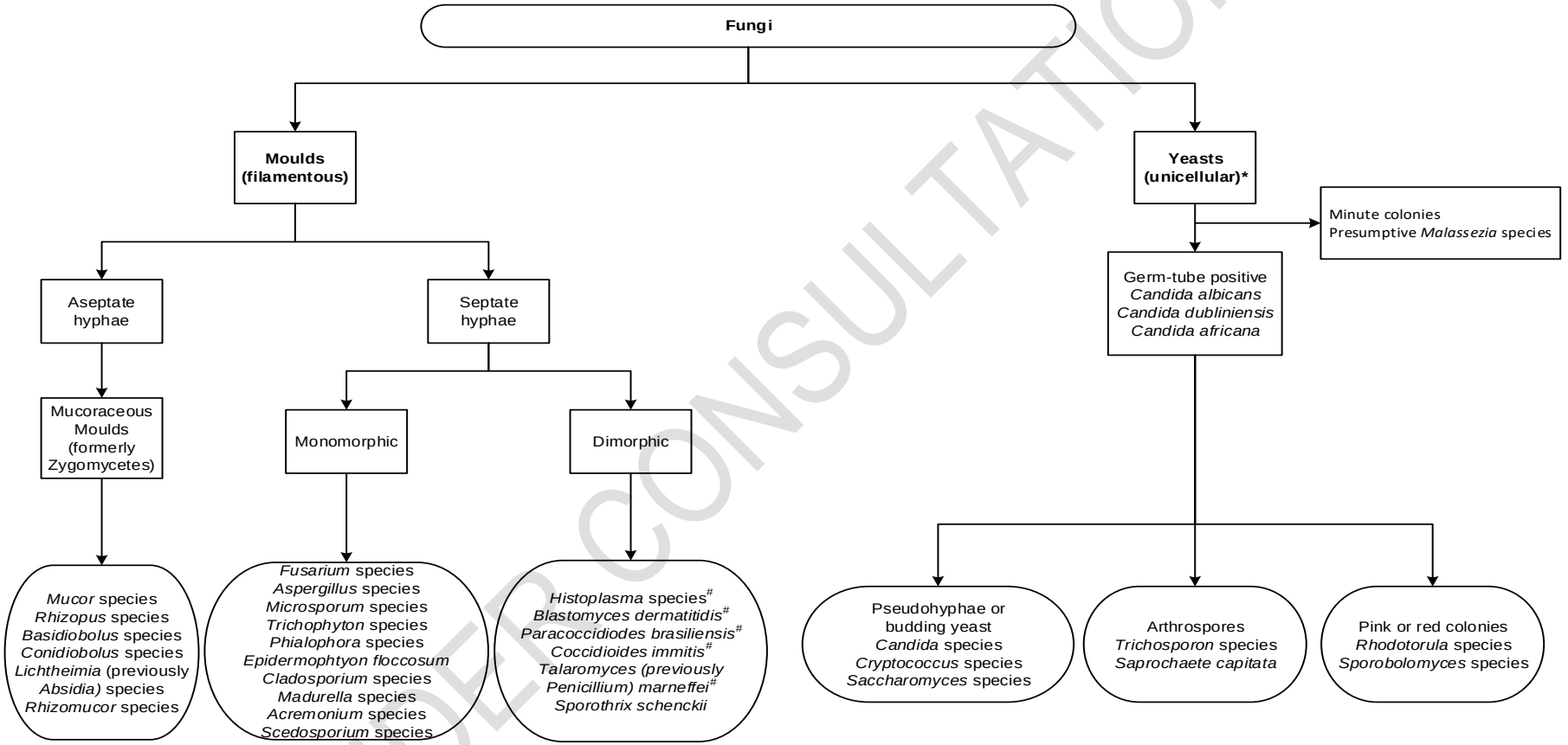
******* This organism is pleomorphic, catalase variable and a facultative anaerobe

[#] These organisms (that is, *Brucella* species and species within the family Enterobacteriaceae) are hazard group 3 organisms and should be processed in Containment level 3 laboratories.

The flowchart is for guidance only.

# Appendix 5: Characteristics of fungi[3,34]

An accessible text description of this flowchart is provided with this document




#These fungi are hazard group 3 organisms and should be processed in a Containment level 3 laboratory.

*_C. glabrata_ does not form pseudohyphae or a germ tube[7].

The flowchart is for guidance only.

# References

For the information for the evidence grade ratings given, refer to the scientific information link above in section 2.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