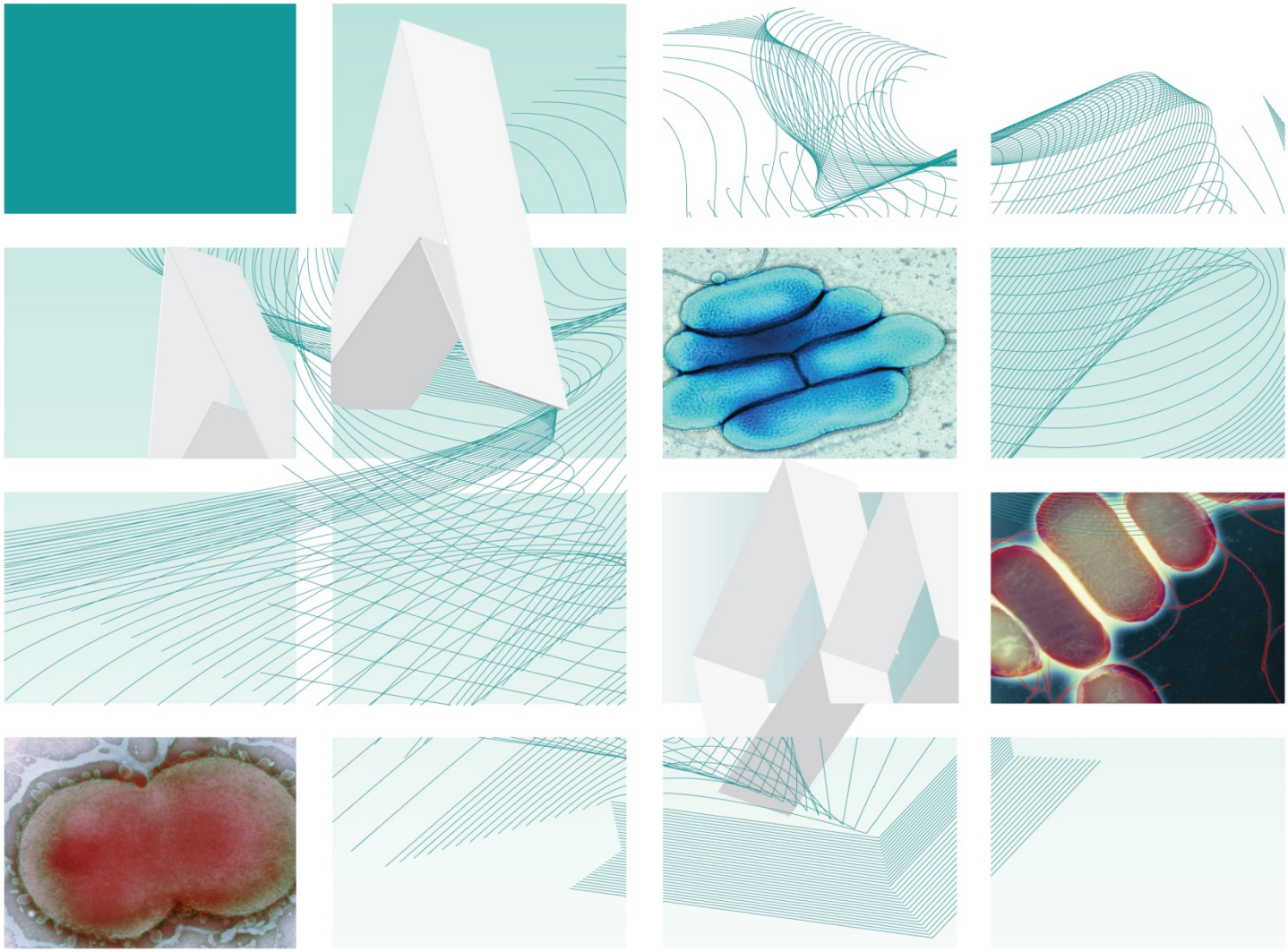
Public Health England

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	7 / 23 September 2021
Issue number discarded	2
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Section(s) involved	**Amendment**
Scope of document and appendix 6	Mention of changes in nomenclature and taxonomy for fungi added. New species have been added and names for existing species have been revised. At the time of writing it was decided to mention fungi using their former names.
Whole document	Document presented in a new format. All sections of this documents updated with current information and references.
Appendix 5	Flowchart updated
References	References updated

*Reviews can be extended up to 5 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 or 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.

Note: there have been recent changes in the nomenclature and taxonomy for fungi of medical importance. New species have been added and names for existing species have been revised(1). At the time of writing it was decided to mention fungi using their former names; however, see appendix 6 for a table of previous and revised names for the fungi mentioned in this UK SMI document.

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. These may be phenotypic characteristics such as morphology and staining pattern (for example, Gram stain reaction, lactophenol cotton blue), growth under various atmospheric conditions and temperatures, growth on various types of culture media (for example MacConkey agar, Sabouraud agar plate culture), catalase and oxidase tests or genotypic characteristics. Using these few simple tests it is usually possible to place organisms, provisionally, in one of the main groups of medical importance(2,3).

In medical microbiology, experience of the types of specimens, the types of 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 bacteria and fungi by diagnostic laboratories is based on phenotypic characteristics, usually by direct comparison of unknown with those of type cultures. There are 3 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 on clinical data, cultural, microscopical 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 bacteria and yeasts are determined by primary identification tests such as a staining pattern and biochemical tests. Results of these tests may indicate secondary or even tertiary tests to confirm the identity of the organism, which may be a time consuming process.

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), can be applied for organism identification within diagnostic microbiology laboratories. Identification using this technology affords the opportunity to identify bacteria, most yeast species as well as some genera of filamentous fungi more rapidly and cost-effectively in comparison with more traditional techniques(4). Any identification should be considered alongside other phenotypic information that is available.

5 Technical information/limitations

Colonial morphology

For moulds identification the colonial morphology characteristics should be combined with the microscopical features. This is important due to the variation seen among isolates, colonies grown on different culture media (selective, non-selective and differential) and at different temperatures(5).

Culture technique

Culture on agar media yields results but there are inherent delays in diagnosis associated with this technique. Care must be taken when processing samples as excessive rough handling can reduce the yield of some fastidious bacteria and fragile moulds.

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. Commercial identification systems may also not be able to identify new species of organism that are not in the accompanying database(6,7).

Germ tube test

Germ tube test is a rapid screening test used to distinguish C*andida albicans* (germ tube positive) from other *Candida* species (germ tube negative). Some species, in particular *Candida tropicalis*, may form pseudohyphae; these structures may be misinterpreted as germ tubes. In contrast to germ tubes, pseudohyphae are a chain of elongated buds, constricted along their length and septate at the point of origin on the mother cell.

The incubation period must be a minimum of 2 hours to allow time for germ tubes to form. However, false positive results may be obtained if the incubation time exceeds 3 hours. In addition, over-inoculation of the serum may inhibit the formation of germ tubes, leading to false negative results. Testing of positive and negative controls should be performed.

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 be performed.

MALDI-TOF MS

MALDI-TOF MS results should be validated against the colony morphology and clinical information. MALDI-TOF MS libraries for fungi are often limited and may reduce the accuracy of identification; microscopic characteristics will provide much more 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 validated 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

Current guidance on the safe handling of all organisms documented in the safety considerations on GOV.UK.

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 relevant risk assessment, and HSE guidelines. This will normally be under full CL3 conditions. Such organisms include *Mycobacterium* species, *Brucella* species, *Bacillus anthracis*, *Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Coccidioides immitis,* and others. The above guidance should be supplemented with local COSHH and risk assessments(8-30).

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 Microscopical appearance

Bacteria

Microscopic study and staining reveal the shape (coccus or rod) and the characteristic grouping and arrangement of the cells and their shape. For example, *Streptococcus* species usually appear in pairs or short chain and *Staphylococcus* species typically form grape-like clusters(3). In addition to morphology, the Gram stained preparation also divides bacteria in 2 main categories – the Gram positive and the Gram negative bacteria(5,31). Some examples of Gram positive bacteria are *Streptococcus*, *Staphylococcus* and *Enterococcus*. Examples of Gram negative bacteria are *Haemophilus* and *Neisseria* species. However not all bacteria can be definitively classified by this technique resulting in Gram variables and indeterminate groups.

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.

Table 1: Terms used for stained preparations of bacteria(3)

Term	Description
Arrangement	Singly, in pairs, in chains, in fours (tetrads), in groups, grape-like clusters, in cuboidal packets, in bundles, palisade, filamentous.
Capsule	Presence or absence.
Endospores	Spherical, oval or ellipsoidal, equatorial, sub-terminal, terminal.
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.

Fungi

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 are 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, some fungi such as *Blastomyces dermatitidis, Sporothrix schenckii* and *Histoplasma capsulatum* are dimorphic which means they have the ability to switch between yeast and mould form growth depending on the temperature(32*)*; they grow as moulds at 30°C and as yeasts at 37 °C. These fungi are classified as hazard group 3 organisms(3,5).

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. Microscopical examination is used to observe conidia and spores using methods such as tease mounts, slide culture and adhesive tape mount (this can be one sided if a coverslip is not needed or double sided if needed). Mounts can be prepared for microscopical examination 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 – this is 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 a coverslip for examination(3). If a hazard group 3 fungus is suspected the sample must be processed in a Class III microbiological safety cabinet (MSC).

There are several types of microscopical methods such as saline mount, lactophenol cotton blue or lacto-fuchsin, calcofluor white with 10% KOH and India ink. These are used to highlight microscopical characteristics of fungi to aid identification. For more information, see TP 39 – Staining procedures. Microscopy examination includes tease mount, slide culture and adhesive tape mount. Microscopy can provide presumptive identification and should be used in conjunction with clinical history, culture, serology, biochemical or molecular testing(3,5).

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

true hyphae, whereas species such as *C. parapsilsosis* and *C. krusei* produce pseudohyphae(33).

For yeasts, the colonial morphology is not always helpful, whereas their microscopic characteristics, seen directly on cornmeal agar with Tween 80 agar with a cover slip placed on a inoculum “streak”, can be useful(3). However, black yeast-like fungi such as *Cyphellophora* and *Exophiala* species (which can cause a wide range of infections, including invasive diseases in immunosuppressed patients) are easily characterised by their dark yeast-like phase that later progresses to a mycelial stage(34). Similarly, *Rhodotorula* species (which can cause infections in susceptible patients) produce naturally pigmented colonies that are pink to red(35).

It is also useful to note the colonial characteristics such as mucoid, smooth, 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 2: Terms used for stained preparations of yeasts and filamentous fungi(32,36,37)

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. Capsules are readily visible in India 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. Although sporulation is rare in some fungi there are methods applied to encourage sporulation as phenotypic identification cannot be confirmed without fruiting bodies and spore production. Note: *Trichophyton violaceum* do not produce spores
Shape of spores	Small, large, oval, spherical, cylindrical, slightly curved, ellipsoidal, crescent-shaped, septate, multi-septate. **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

Bacterial or fungal colonies of a single species, when grown on specific media under controlled conditions are described by their colony morphology, characteristic size, growth rate, shape, colour, consistency, metabolic reaction, and sometimes pigmentation. When growth conditions are carefully controlled, colonial morphology is important for preliminary identification and for differentiation of 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 as well as the freshness of the media(3,31).

Bacteria

Colonial morphology are important observations in the preliminary identification of bacteria. Colonial characteristics include amount of growth and description, type and pattern of haemolysis on blood agar, elevation, margin, surface, consistency and size of the colony(3). Table 3 details the terms used in colonial morphology of bacteria.

The size of bacterial colonies, assuming favourable growth conditions, is generally uniform within a species. For example, *Streptococcus* species are small, usually 1mm in diameter, whilst *Staphylococcus* species and species within the family, Enterobacterales are larger and usually 2 to 3mm in diameter, and those of *Bacillus* species are much larger in size and usually 2 to 7mm in diameter.

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 at 41°C, whilst *Listeria* species will grow very well when incubated in 5 to 10% CO_2 at 35°C to 37°C for 16 to 48hr(5).

Table 3: Terms used in colonial morphology of bacteria(3,31,37)

Term	Description
Colour	By reflected or transmitted light: fluorescent, iridescent, opalescent, **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, 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 documents.

Fungi

Fungal colonial morphology and growth rate may vary depending on the genus, species, type of culture medium used, age of culture used for subculture, amount of inoculum and the temperature of incubation. For example, most yeasts are detected within 5 days. Certain genera of fungi, for example *Trichophyton violaceum or T. verrucosum* can take 14 to 21 days for visible growth. The growth rate for dimorphic fungi such as *Blastomyces dermatitidis*, *Histoplasma capsulatum* and *Coccidioides immitis* is also slow and up to 8 weeks may be required for viable growth (that is, before colonies become visible).

For all other fungal pathogens, the culture plates should be examined at regular intervals, and incubated for 4 to 6 weeks before being regarded as negative(32,5). Table 4 details the terms used in colonial morphology of yeast and filamentous fungi.

Table 4: Terms used in colonial morphology of yeasts and filamentous fungi(3,5,32,37)

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)	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.
Rate 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 comparative to bacterial colonies.

Culture media

Microorganisms have specific growth factor requirements that must be added to the media such as source of nitrogen and carbon. Agar is the most important solidifying

agent used in media, meat and plant sources are also used as source of nutrients for the cultivation of microorganisms. Type of media such as selective, non-selective and differential should be carefully selected based on specimen type and suspected agent.

There are several commercially available chromogenic media; the majority of these 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 bacterial pathogens such as *Clostridioides difficile*, *Pseudomonas aeruginosa* and yeast pathogens, including some *Candida* species. In addition, chromogenic media is useful in the detection of mixed yeast infections. Sabouraud, dextrose and malt extract agar plates are the most common culture media used(38,39).

Haemolysis

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

- 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)
- β (clear zone around the colony causing a clearing of the medium),
- non-haemolytic previously called γ-haemolysis (no haemolysis, no apparent change in the colour of the medium)(3).

Note: This is more commonly used as a form of identification 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(37). This characteristic is widely used to establish preliminary identification information in bacteria but 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 as follows:

Atmosphere

It is usual to divide bacteria into 5 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 to 10% CO_2 may enhance growth)
- carboxyphilic (or capnophilic) organisms require additional CO_2 for growth(37)

Temperature

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(37).

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. Microscopical 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

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(37).

8.4 Tests for bacteria

Numerous biochemical tests may be used for the identification of microorganisms (refer to individual identification UK SMIs). Some tests 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(3,5,31). 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 erythrocytes 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 catalase negative. Among Gram positive bacilli, *Bacillus* are catalase positive and *Clostridioides* are catalase 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. 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 with different affinities for different organisms are used to highlight structures in clinical specimens and isolates, often viewed with the aid of different types of microscope. Bacterial morphology can be observed using stains such as Gram stain which can be used to differentiate Gram positive bacteria from Gram negative bacteria.

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 full list of all UK SMI test procedures.

8.5 Antigenic typing or serotyping tests

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(2).

8.6 Tests for fungi

Germ tube test

This is a rapid screening test commonly used to identify and differentiate C*andida albicans* (germ tube positive) from other *Candida* species (germ tube negative). It can be carried out on primary isolates or purified cultures. The formation of germ tubes is characteristic of 95 to 97% of clinical isolates of *C. albicans*. *Candida dubliniensis* and *Candida africana* also produce germ tubes, although these species are encountered less often. Further tests are required to differentiate between these 3 species.

A germ tube is a short, non-septate cylindrical filament originating from a yeast cell during germination, without any constriction at the point of origin on the mother cell and without obvious swelling along the length of the filament.

A small amount of yeast is emulsified in sterile serum (usually horse or rabbit) and incubated at 35 to 37°C aerobically for 2 to 3 hours.

Although seemingly a simple test to perform, care must be taken. The germ tube test should be performed on a single colony, using either the original isolation plate or a purified 24 hour culture. If a mixed infection is suspected, multiple colonies should be tested separately(3,5).

Rapid urease test

A presumptive identification of *Cryptococcus neoformans-Cryptococcus gattii* species complex is based on rapid urease production, whilst *Candida albicans* do not produce urease. Other species of *Cryptococcus*, *Trichosporon* and *Rhodotorula* can give a 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(3,40).

Dermatophyte test medium

This can be used for isolation and presumptive identification of dermatophytes such as *Microsporum*, *Trichophyton*, *Nannizia* 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. Dermatophytes can be isolated from hair, skin and nail specimens(41).

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

8.7 Rapid identification methods

MALDI-TOF MS

This 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. This technique has been increasingly used by the diagnostic laboratories due to its high reproducibility, speed and sensitivity of analysis and improved turnaround times

compared to phenotypic methods, it can also be directly applied to clinical samples. The range of clinical applications of MALDI-TOF MS for bacterial isolates is increasing constantly, from species identification to the promising applications of detection of antimicrobial resistance and strain typing for epidemiological studies(42).

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, some issues are faced with filamentous fungi, such as extracting protein for MALDI-TOF MS analysis, fast-changing fungal morphology and commercially available fungal reference libraries are currently not as comprehensive as the bacterial ones(43,44). The identification of moulds using this method is being developed(45).

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 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 a 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, sequencing by synthesis (SBS) technology and others.

The complete genome sequencing for the organisms such as *Salmonella*, *Streptococcus* and *Enterococcus* has provided valuable taxonomic and genomic references for infectious disease diagnostics, improved understanding of pathogenicity, supported outbreak investigations as well as references for future studies(46-48).

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 is an 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(49,50).

In addition, WGS has been used in the management of outbreaks caused by *Candida auris*, *Listeria, Cryptosporidium* and study of antibiotic resistance in bacteria(51,52).

Commercial Identification Systems (kits/rapid tests)

The use of commercially available identification kits alongside other biochemical reactions may be used to give accurate identification of bacteria and yeasts(53). 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, identification 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(3).

Note: Microscopic examination of culture is essential to differentiate between organisms with identical biochemical profiles. Results from commercial identification systems should be correlated with the results of conventional methods such as microscopic examination, colonial morphology as well as clinical presentation.

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 cost and lack of expertise amongst staff.

Examples of molecular identification methods include polymerase chain reaction (PCR) such as panfungal PCR (ITS and D1/2).

Examples of typing methods include pulsed-field gel electrophoresis, multi-locus sequence typing and multiple-locus variable-number tandem-repeat (VNTR) analysis.

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. 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(54,55).

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

Culture methods have been considered the gold standard method for bacterial identification; however, due to slow growing bacteria it can take several days or weeks to get 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(56).

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

These molecular methods work with different primer pairs in a very similar way to the 16S rRNA sequencing and they have been very useful in the identification of clinically relevant fungi (yeasts and filamentous fungi)(57). Different approaches are used for different groups of fungi with ITS1 and ITS2 proving the most useful for most fungal genera.

9 Reporting

For specific information on reporting, refer to individual UK SMI documents.

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.

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

Refer to current guidelines on SGSS reporting(58).

9.6 Infection prevention and control team

Follow locally agreed protocols for reporting to the infection prevention and control team.

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

Scotland *(for bacteria only)*

Northern Ireland

UK Clinical Mycology Network

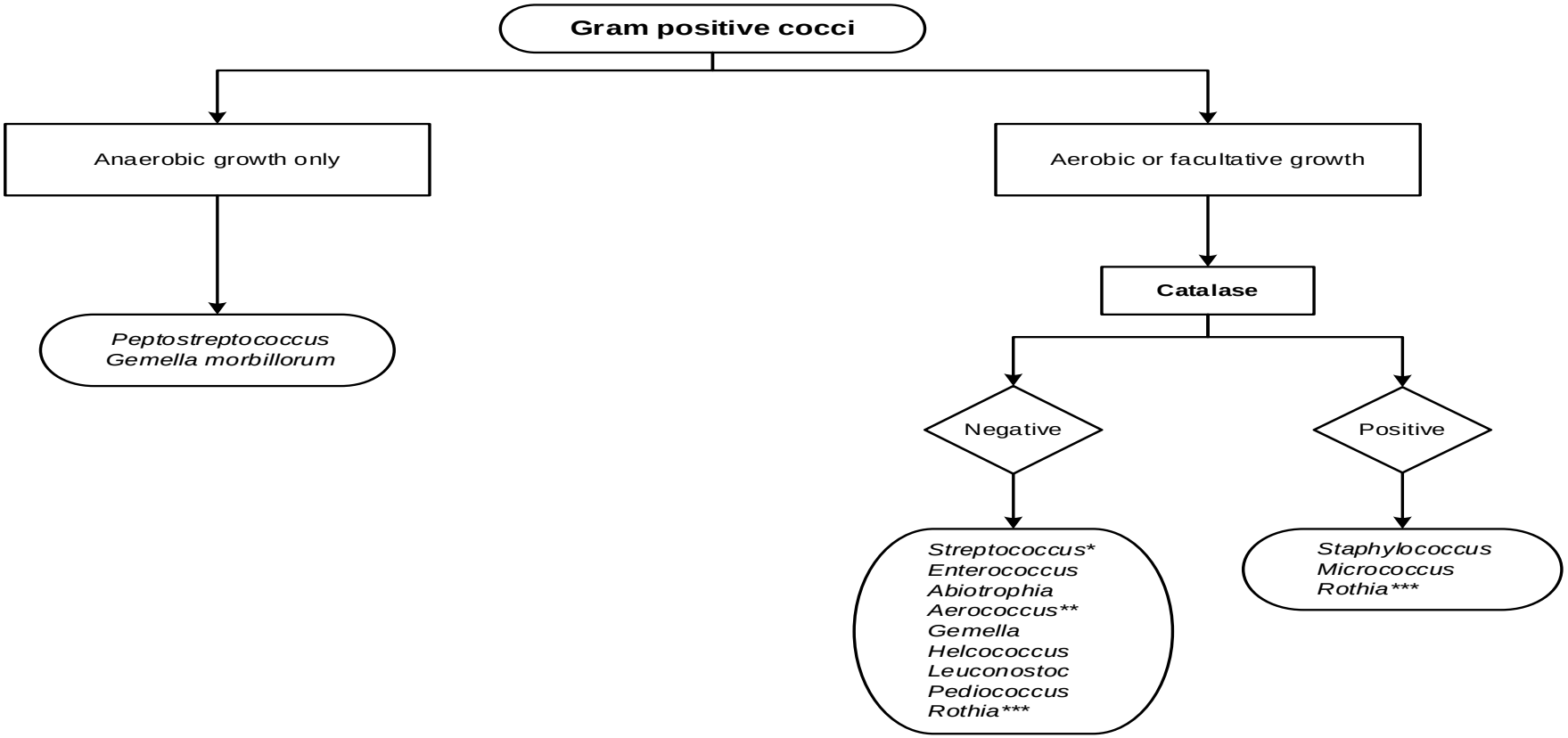
UKCMN Secretariat
c/o PHE Mycology Reference Laboratory
Infection Sciences
Pathology Sciences Building
Southmead Hospital
Bristol
BS10 5NB

PHE Mycology Reference Laboratory

National Infection Services, PHE South West Laboratory
Science Quarter
Southmead Hospital
Bristol
BS10 5NB

Appendix 1: Characteristics of Gram positive cocci

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



***** Some species may be anaerobic

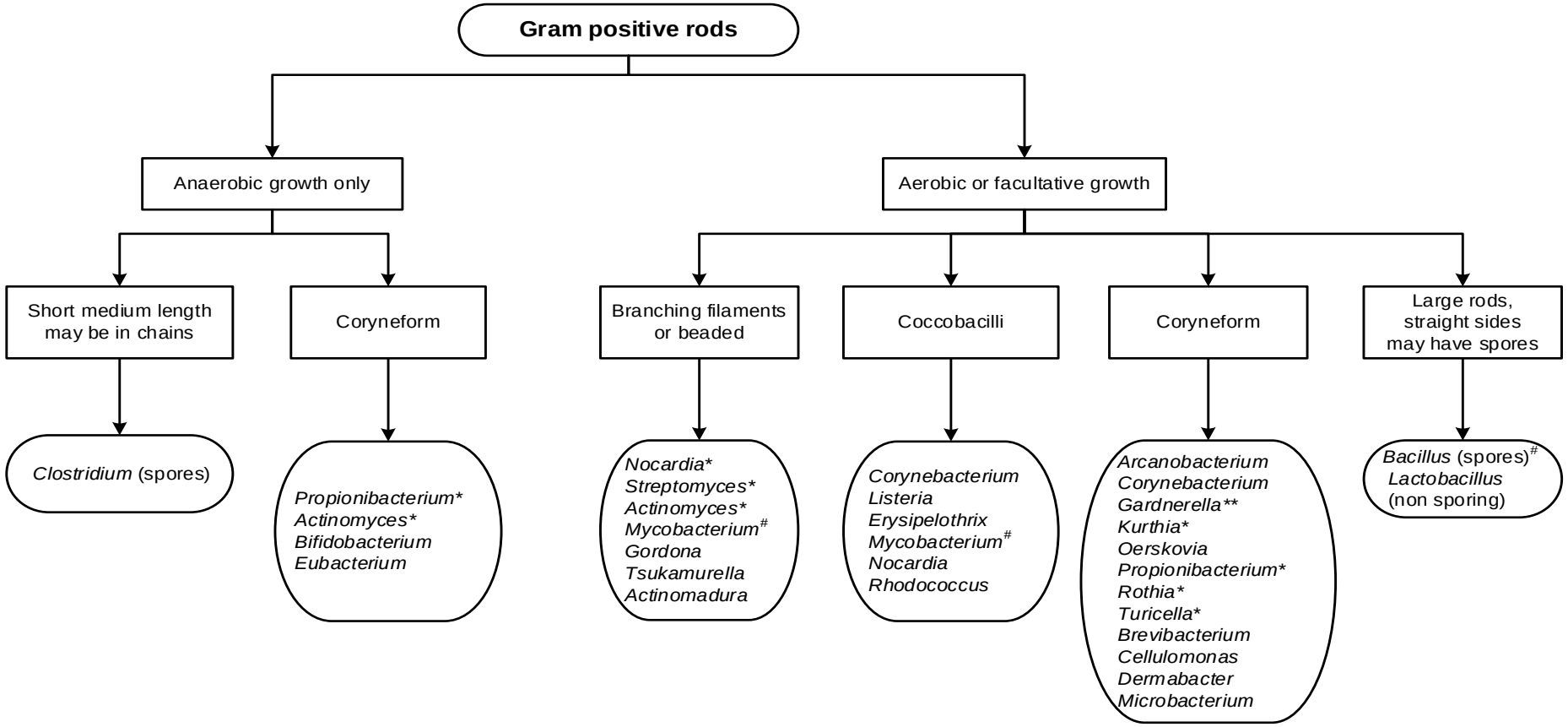
****** May be weak catalase positive

******* 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(8,31,59).

Appendix 2: Characteristics of Gram positive rods

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



*This organism is pleomorphic

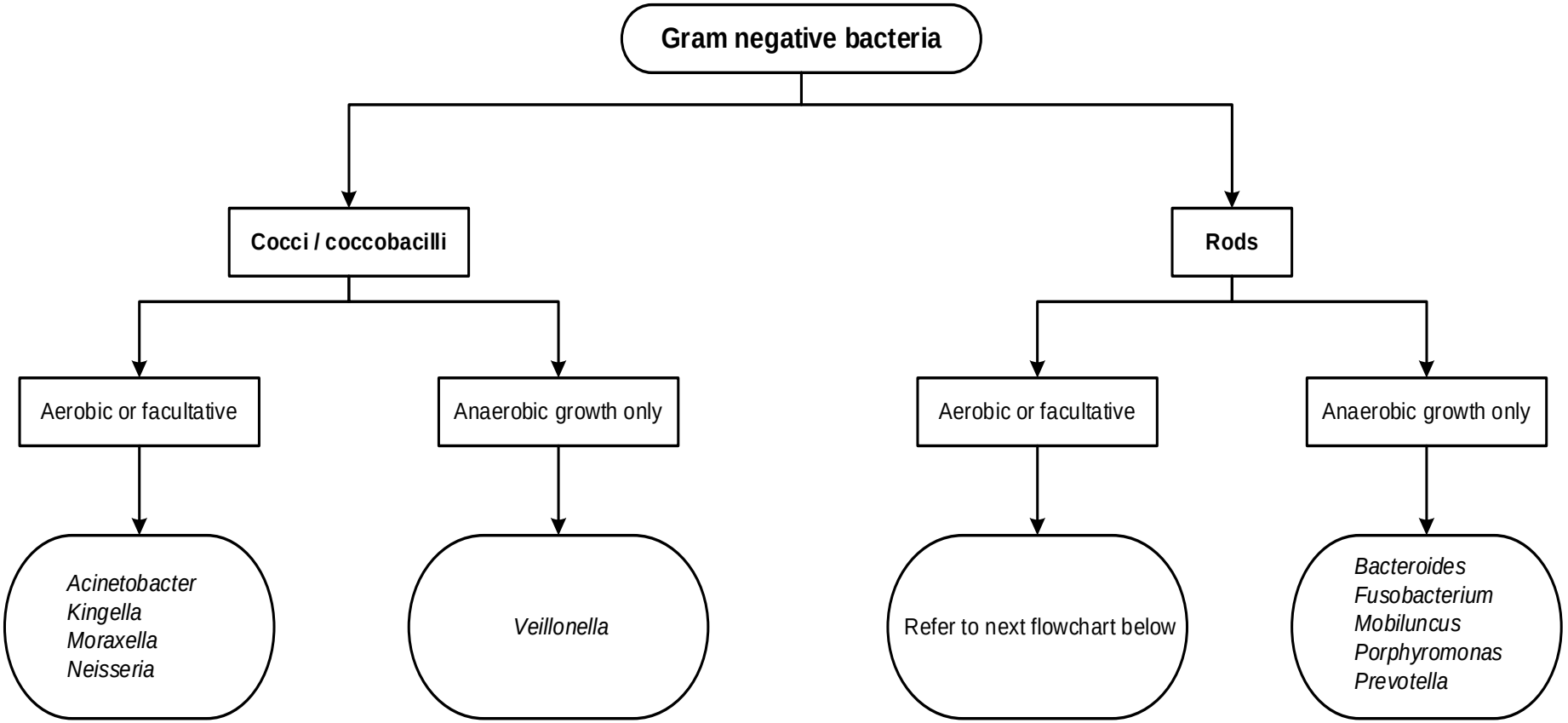
***Gardnerella vaginalis* is a Gram variable rod and may usually be differentiated by its microscopic appearance

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(8,31,59).

Appendix 3: Characteristics of Gram negative bacteria

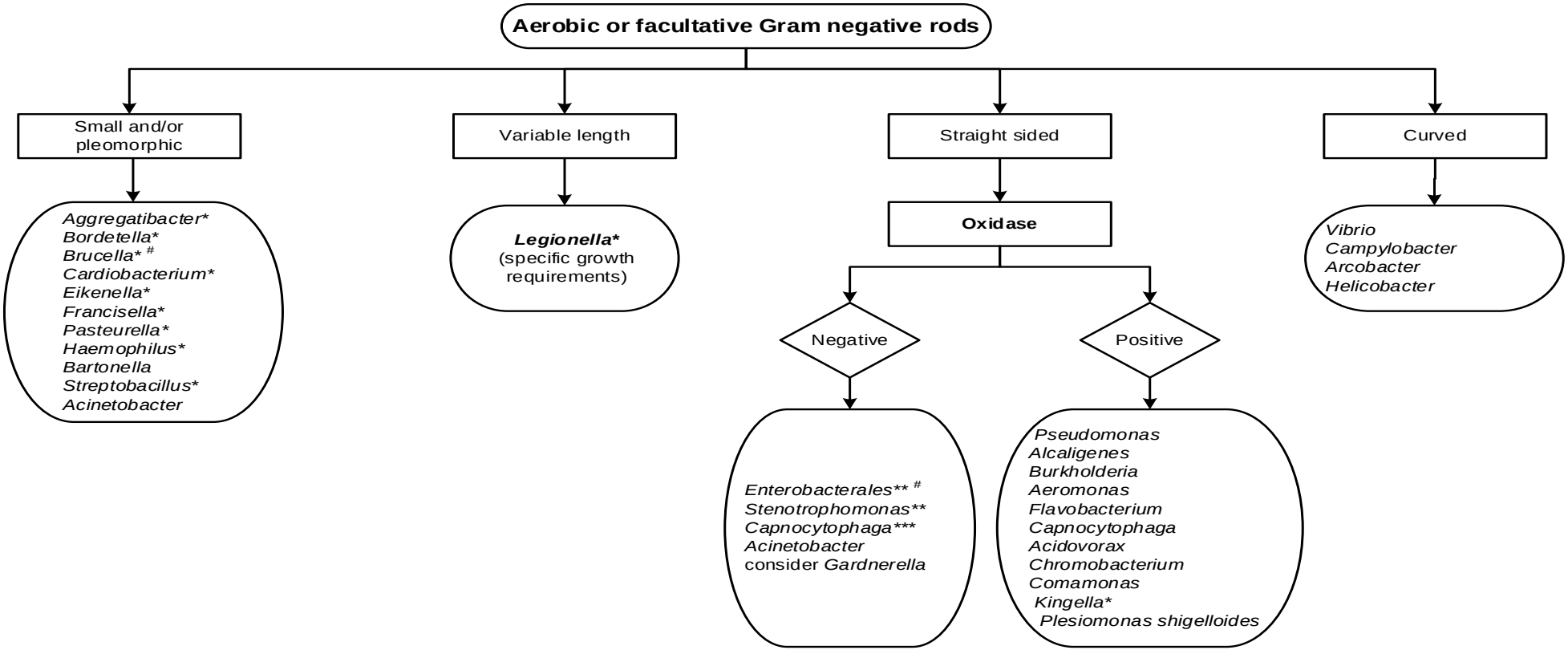
An accessible text description of this flowchart is provided with this document.



The flowchart is for guidance only(8,60).

Appendix 4: Characteristics of Gram negative bacteria *(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

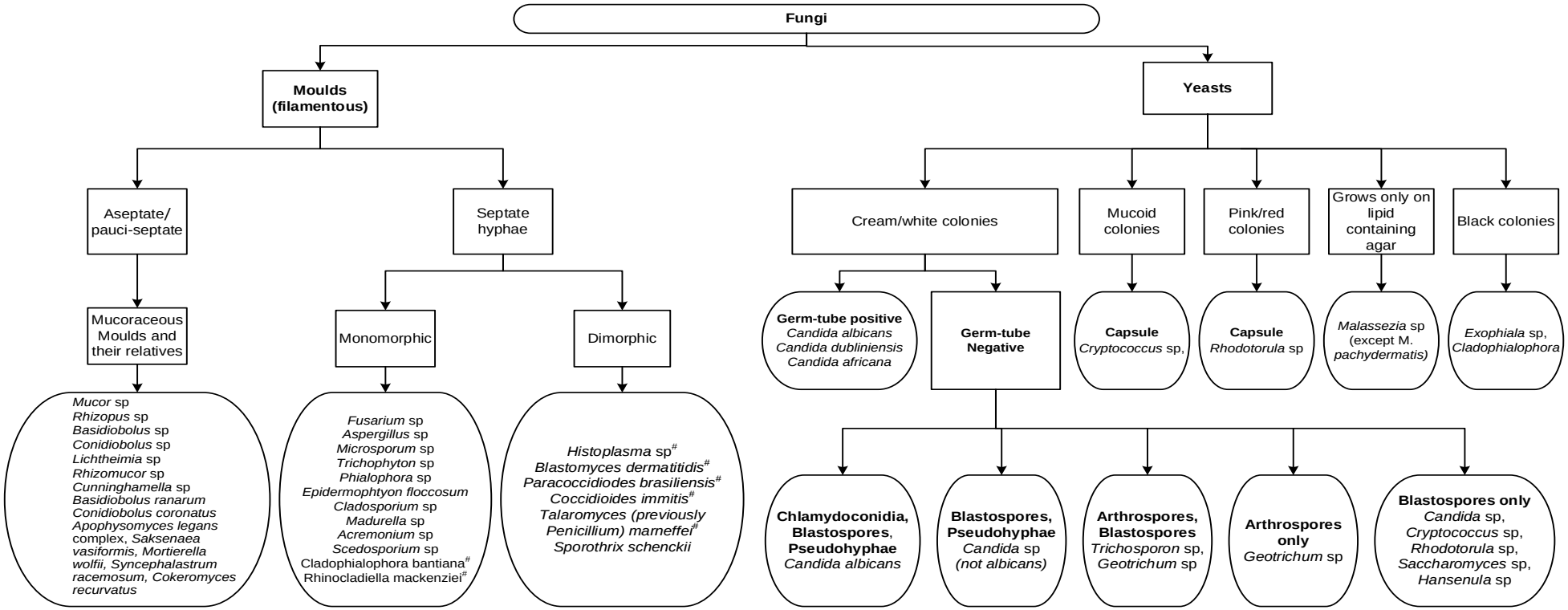
******* This organism is pleomorphic, catalase variable and a facultative anaerobe

[#] *Brucella* species and species within the family Enterobacterales are hazard group 3 organisms and should be processed in Containment level 3 laboratories.

The flowchart is for guidance only(8,60).

Appendix 5: Morphological characteristics of fungi

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



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

The flowchart is for guidance only and to assist in validation of results obtain from MALDI-TOF MS and other commercial identification systems. The nomenclature for some species has changed, see appendix 6(1,5,34,35).

Appendix 6: List of revised fungal taxa mentioned in this document

Previous species name	Revised species name(1)
*Candida krusei (*for full list of *Candida* species see reference[1])	*Pichia kudriavzevii*
Rhodotorula minuta	*Cystobasidium minutum*
Rhodotorula slooffiae	*Cystobasidium slooffiae*
Cryptococcus albidus	*Naganishia albida*
Cryptococcus curvatus	*Cutaneotrichosporon curvatum*
Cryptococcus diffluens	*Naganishia diffluens*
Cryptococcus laurentii	*Papiliotrema laurentii*
Trichosporon cutaneum	*Cutaneotrichosporon cutaneum*
Trichosporon loubieri	*Apiotrichum loubieri*
Trichosporon mucoides	*Cutaneotrichosporon mucoides*
Trichosporon mycotoxinivorans	*Apiotrichum mycotoxinivorans*
Rhodotorula minuta	*Cystobasidium minutum*
Rhodotorula slooffiae	*Cystobasidium slooffiae*

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For the information for the evidence grade ratings given, scientific information section on the UK SMI website.

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