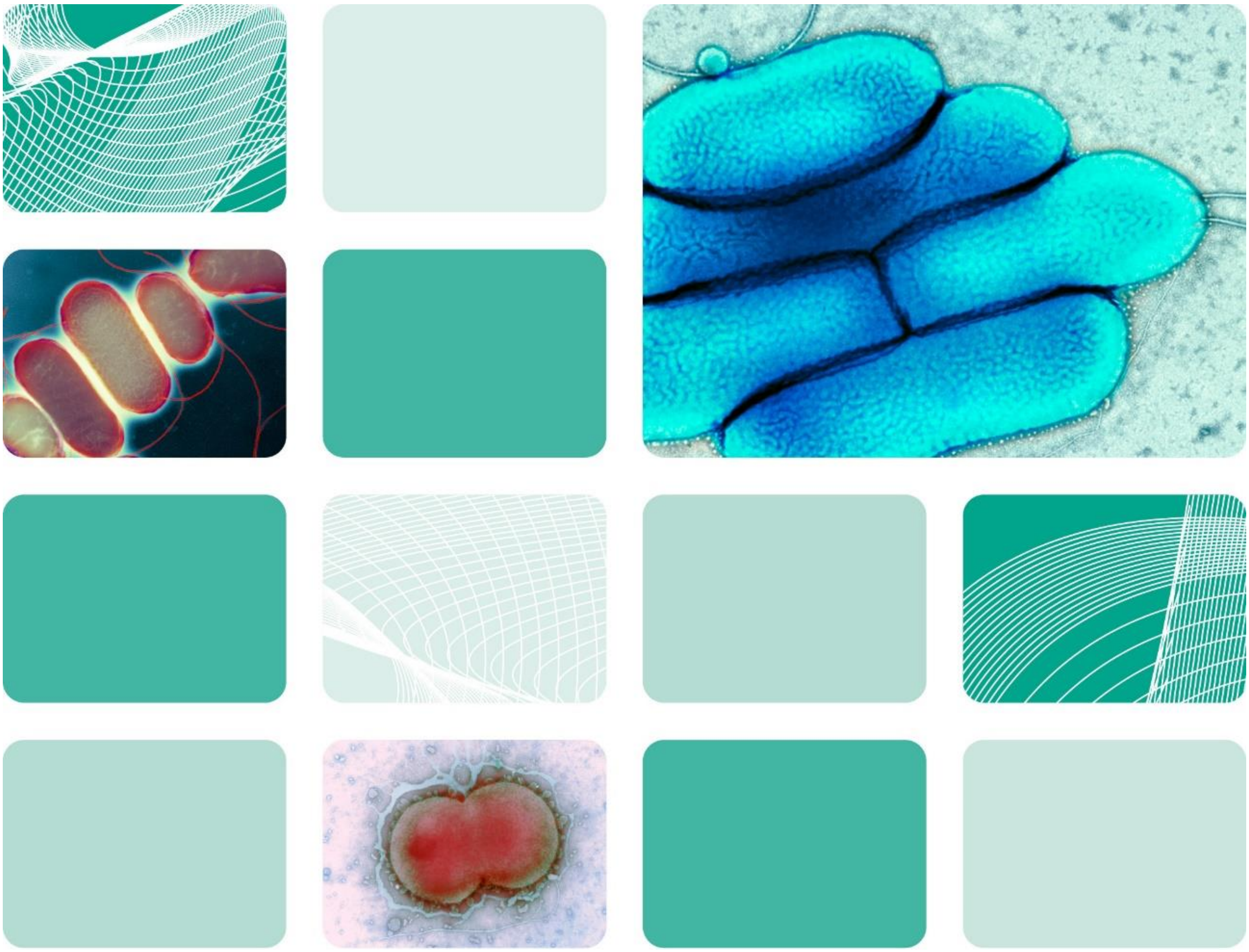
UK Health Security Agency

# UK Standards for Microbiology Investigations

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



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# Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of UKHSA working in partnership with the partner organisations whose logos are displayed below and listed on [the UK SMI website](). UK SMIs are developed, reviewed and revised by various working groups which are overseen by a [steering committee.]()

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UK SMIs are produced in association with:

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# Contents

# Amendment table

Each UK SMI document has an individual record of amendments. The amendments are listed on this page. The amendment history is available from standards@ukhsa.gov.uk.

Any alterations to this document should be controlled in accordance with the local document control process.

| | |
|---|---|
| **Amendment number/date** | 8/06.08.25 |
| **Issue number discarded** | 3 |
| **Insert issue number** | 4 |
| **Anticipated next review date*** | 06.08.28 |
| **Section(s) involved** | **Amendment** |
| **Title** | The title of this document has changed from 'Introduction to the preliminary identification of medically important bacteria and fungi from culture' to 'Introduction to the identification of medically important bacteria and fungi from culture' |
| **Whole document** | Document presented in a new format.<br>Hyperlinks updated to direct the reader to RCPath webpages.<br>Subheadings and sections have been restructured to align with laboratory practices.<br>Sections have been updated with relevant information and supporting literature. |
| **Scope of document** | The scope has been updated to clarify that the document focuses solely on culture methods, not clinical samples.<br>Links to other relevant UK SMIs that can be read in conjunction with this document have been added. |
| **Technical information** | Information has been added to the relevant subsections in Section 8: Identification or removed if no longer relevant |
| **Safety Information** | Safety references have been updated.<br>The section has been expanded for clarity. |
| **Identification** | Links to test procedure documents have been added to replace text. |

| | |
|---|---|
| | Section 8.3 MALDI-TOF MS has been moved higher in the document to reflect the order of laboratory testing. |
| **Referral to reference or specialist testing laboratories** | Hyperlinks updated as appropriate |
| **Algorithm** | An overarching algorithm covering the general identification pathway has been added |
| **References** | References have been reviewed and updated. |

*Reviews can be extended up to 5 years where appropriate

# 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 identification of the common bacteria and fungi recovered from culture of clinical specimens. It is intended to lead the user to a more detailed identification method and is designed to be used for isolated cultures of bacteria and fungi and not for direct identification of bacteria and fungi from clinical samples or smears. Direct detection, including point-of-care tests, is not included. For more information on the investigation of patient samples, please refer to the relevant bacteriology UK SMIs.

This document includes the use of culture methods, microscopy, biochemical tests and Matrix-assisted laser desorption/ionisation – time of flight mass spectrometry (MALDI-TOF MS), for the identification of microorganisms. The specific test procedure for MALDI-TOF MS is detailed in UK SMI TP 40 - Matrix-assisted laser desorption/ionisation – time of flight mass spectrometry (MALDI-TOF MS) test procedure. Some biochemical tests may not be performed routinely in the laboratory except in cases where confirmation by an alternative technique is required or automated methods are not available.

For further information on identification procedures for specific species, refer to individual identification UK SMIs. For more information on dermatophytes, refer to UK SMI B 39 – Investigation of dermatological specimens for superficial mycoses.

Please note that, following the recent update of fungal taxonomy, many species formerly part of the genus *Candida* now belong to a number of other genera. For the purposes of this document, both old and new names are mentioned as required and are collectively referred to as '*Candida* and associated ascomycetous yeasts'

UK SMIs should be used in conjunction with other relevant UK SMIs.

# 4 Introduction

The identification of microorganisms covers the initial investigations and tests that provide differentiation and an understanding of the microorganism present in culture, which can then be confirmed by further testing. Methods for identification can include macroscopic examination of colonial morphology, staining techniques, MALDI-TOF MS and biochemical tests. Further confirmation may include molecular methods.

## 4.1 Principles of Identification

The identification of microorganisms relies on distinguishable characteristics to identify which microorganism is present in culture. These may be phenotypic characteristics such as growth under various atmospheric conditions and temperatures, growth on various types of culture media, colonial morphology, microscopic features using staining techniques and biochemical tests. Using a combination of diagnostic tests according to the clinical presentation and travel history of the patient, it is usually possible to provisionally classify organisms into one of the major medically significant groups (1). The presence of multiple genera/species on a single culture plate can complicate the identification process and purity plating may be required to isolate individual organisms. MALDI-TOF MS, staining methods, further biochemical tests and molecular methods can then be used to confirm identification. Please refer to the test procedure UK SMIs for further details.

When identifying microorganisms, it should be remembered that characteristics may be variable, including those of a species within a genus. For example, *Klebsiella oxytoca* is indole positive and *Klebsiella pneumoniae* is indole negative, which can be useful when differentiating these species (2). The identification of fungal species using phenotypic and microscopic methods requires extensive training and experience and for less typical species is usually performed in reference/specialist centres.

# 5 Technical information and limitations

MALDI-TOF MS has become the primary method of identification. Compared to traditional testing methods, it is rapid and accurate in the identification of bacteria and most fungi (3). However, identification of yeasts typically requires full extraction and identification of some filamentous fungi remains variable (4,5). Therefore, the identification of filamentous fungi relies primarily on colonial and microscopic morphology, supported by molecular methods for confirmation.

With the increased use of genetic sequencing methods, the taxonomy of bacteria and fungi is frequently changing, leading to the reclassification of these microorganisms. It is recommended that laboratories keep up to date with any taxonomic changes that may occur, including MALDI-TOF MS database upgrades and how these changes are reported to users by the laboratory information system. It should be noted that recent taxonomic changes may not be included in some commercial identification systems.

It is important to note that whilst MALDI-TOF MS is used as the primary method for identification of bacteria and some fungi, libraries can be limited and may not always generate an accurate result. In such cases, phenotypic or molecular methods are required for confirmation. The identification of bacteria and some fungi using MALDI-TOF MS should be interpreted in conjunction with available phenotypic information.

If identification is not made using MALDI-TOF MS, one subsequent approach is to assess microscopic appearance using staining techniques and, if required, identify the organism using molecular methods. A less common approach involves subjecting the organism to a series of biochemical tests, such as those found in commercial identification systems and rapid serological agglutinations. The data is collated and compared to standard texts or used to create a numerical profile to obtain identification. This can provide accurate identification but can be an expensive and time-consuming process (6). It should be noted that biochemical tests may not be appropriate to identify all microorganisms. For detailed identification procedures for individual organisms, please refer to the relevant identification UK SMIs.

Recent changes in nomenclature and taxonomy for medically significant fungi involve the addition of new genera and species as well as revisions to existing names (7). At the time of writing, the decision was made to refer to fungi by their former names; however, the appendix provides both the previous and updated names for all fungi mentioned in this document.

# 6 Safety considerations

The section covers specific safety considerations (8-28) related to this UK SMI, and should be read in conjunction with the general safety considerations, Control of Substances Hazardous to Health Regulations 2002 (COSHH) and ACDP guidelines approved by the HSE carrying out a suitable and sufficient risk assessment.

At containment level 2 (CL2) any primary sample that may contain Hazard Group 3 (HG3 organisms) or any manipulation of a cultured isolate suspected to be an HG3 organism that can be spread by aerosol and can cause human disease must be carried out in a Microbiological Safety Cabinet (MSC) or similar containment. Where recirculating MSCs are used, exhaust air should be passed through two HEPA filters in series.

At containment level 3 (CL3) all work with known HG3 infectious organisms that can spread by aerosol and can cause human disease must be conducted within an MSC or similar. Class I or Class II MSC will be used, but a risk assessment may indicate a

Class III cabinet is required for work with biological agents with an airborne route of transmission that can cause serious human disease, e.g., multidrug-resistant TB. Such organisms include certain *Mycobacterium* species, *Brucella* species, *Bacillus anthracis*, *Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Coccidioides immitis,* and others (8,18,29). Additionally, the type, selection and use of a microbiological safety cabinet as well as transport of biological agents should also be considered to further minimise the risk of transmission during handling of the organism.

Risk assessing the potential of a primary sample to contain a HG3 organism should be performed by a designated competent person and as a minimum should review clinical presentation, travel history and previous infections. When there is doubt the sample should be discussed with a Consultant Microbiologist before containment is derogated. If a HG3 fungus is suspected the sample should be processed at CL3.

Compliance with postal and transport regulations and waste classification and segregation is essential.

# 7 Target organisms

All medically important bacteria and fungi.

# 8 Identification

Identification of both bacteria and yeasts requires the same broad techniques carried out in a similar order. This varies according to species, laboratory equipment, specialists and local procedures. Isolates are first cultured, and the colony morphology is examined. Individual colonies can be identified using appropriate staining techniques, if required. MALDI-TOF MS or other testing methods are used where necessary. Identification can then be confirmed using molecular methods if necessary. For mould identification, examination of morphological characteristics, including colonies and microscopic structures, is critical. The use of MALDI-TOF MS for the identification of moulds is currently highly variable, dependent on the methods used and the range of fungi included in the database.

Refer to relevant identification UK SMIs for further information. It should be noted that the most encountered organisms are listed in the flowcharts (see algorithms 2 to 6) for characterisation and identification. While the list of organisms is not exhaustive, those listed are used as examples for characterisation.

## 8.1 Culture methods

Microorganisms are recovered using culture methods. Colonial morphology on non-selective, selective or chromogenic agar plates is usually the initial step when identifying most microorganisms. Following this, individual colonies can be stained and assessed using microscopy where required.

Appropriate storage and transport will enhance the recovery of fastidious organisms as excessive rough handling can reduce the yield of some fastidious bacteria and fragile moulds.

### 8.1.1 Culture media

All microorganisms have specific growth requirements, reflected in the growth media used. Agar is used as a solidifying agent and supplemented with nutrients necessary for the cultivation of microorganisms (1). It should be noted that delays in diagnosis can occur when culturing using agar media. Types of media such as selective, non-selective and differential should be carefully selected based on specimen type and suspected agent. Slight differences in media composition supplied by different manufacturers can lead to changes in the phenotypic appearance of some fungi (typically moulds), potentially complicating identification.

#### Chromogenic media

There are several commercially available chromogenic media. These are designed to target organisms with high specificity and sensitivity when present among other flora. These media contain chromogenic substrates that are broken down by specific enzymes, resulting in distinctly coloured colonies that aid in the identification of the organisms.(30).

The use of chromogenic agar has been useful in the isolation and identification of bacterial pathogens such as *Clostridioides difficile*, *Pseudomonas aeruginosa* and screening specific resistant organisms including methicillin resistant *Staphylococcus aureus,* vancomycin-resistant *Enterococcus* and Carbapenemase-producing Enterobacterales (31-34). In addition, chromogenic media are recommended for the detection of mixed yeast infections, and identification of some *Candida* and associated ascomycetous yeasts (30,35,36). Please see section 8.4 Test procedures, for detailed information about the use of chromogenic agar in the identification of *Candida* species.

Chromogenic agar can only be used to provide a presumptive identification that should be confirmed using the recommended methods.

### 8.1.2 Growth requirements

Microorganisms can be grouped based on their growth requirements. Some examples of growth requirements are included below; however, this is not an exhaustive list.

### Atmosphere

It is usual to divide microorganisms into 5 categories according to their atmospheric requirements (37):

- obligate aerobes grow only in the presence of oxygen
- obligate 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

### Temperature

Organisms may also be divided according to their temperature requirements (37):

- psychrophilic organisms grow at low temperatures 2 to 5°C (optimum 15°C)
- psychrotolerant species can tolerate growth at low temperatures, but grow at higher temperatures
- 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

### 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 (NAD+) for the growth of *Haemophilus* species (38).

## 8.1.3 Colonial appearance

Bacterial or fungal colonies of a single species, when grown under controlled conditions, are described by their colony morphology, characteristic size, growth rate, shape, colour, consistency, metabolic reaction, haemolysis and pigmentation. It should be noted that the growth rate and colonial morphology of certain organisms are variable, depending on the amount of inoculum (bacterial or fungal) present in a clinical specimen as well as the freshness and composition of the medium and isolation conditions (39). Strain variations should be considered when assessing colonial morphology.

## Bacteria

Colonial morphology is an important observation in the presumptive identification of bacteria. Observations include amount of growth and description, type and pattern of haemolysis on blood agar, elevation, margin, surface, consistency and size of the colony (37). Refer to Table 1 for the terms used when describing colonial morphology of bacteria.

Under favourable growth conditions, the size of bacterial colonies tends to be uniform. For example, *Streptococcus* species are small, usually 1mm in diameter, whilst *Staphylococcus* species are 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 between organisms, for example, *Campylobacter* species will yield a good growth when incubated for 48 to 72 hours uninterrupted under microaerophilic conditions at 42°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 (40,41).

**Table 1: Terms used to describe colonial morphology of bacteria (37,42)**

| 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, usually enhanced at room temperature, which 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, glabrous, 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 |

| Term | Description |
|---|---|
| **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 |
| **Haemolysis** | α - partial lysis of the red blood cells surrounding a colony causing a greenish discolouration of the medium<br>α-prime - a small zone of intact red cells with a surrounding zone of haemolysis<br>β - clear zone around the colony causing a clearing of the medium<br>non-haemolytic (previously called γ-haemolysis) - no haemolysis, no apparent change in the colour of the medium |

For individual bacterial colonial descriptions, see the relevant identification UK SMIs.

## Fungi

Fungi are broadly split into two major categories, moulds or yeasts, depending on their characteristics, as determined by colonial morphology. Yeasts exhibit a unicellular growth form and reproduce through budding to produce individual discrete colonies on culture plates (39). Pathogenic yeasts are often further categorised as either *Candida* and associated ascomycetous yeasts or *Cryptococcus* species.

Colonial morphology can be useful for categorising yeasts. Cell size, shape and colour are useful for identifying yeast. Black yeast-like fungi such as *Cyphellophora* and *Exophiala* species are easily characterised by their dark yeast-like phase that later progresses to a mycelial stage (43). Similarly, *Rhodotorula* species produce naturally pigmented colonies that are pink to red (44). The presence of a capsule around the yeast cell is useful for identifying *Cryptococcus* species. Chromogenic agar can be useful as an indicator (presumptive identification) of particular species or the presence of a mixed yeast culture. Further identification should be achieved where possible by MALDI-TOF MS. In laboratories where MALDI-TOF MS is not available, techniques such as staining for microscopic investigation, rapid screening, biochemical methods and/or specialised culture can be applied. However, the accuracy of these methods in identifying rarer yeast species is limited. If MALDI-TOF MS fails to identify a yeast, molecular methods are required for accurate identification.

Fungal colonial morphology and growth rate may vary depending on the genus, species, type and composition of culture medium used, age of culture used for subculture, amount of inoculum and the temperature of incubation (39). The required incubation time for viable growth can vary significantly. For example, *Aspergillus niger* requires as little as 72 hours for viable growth compared to some species of *Histoplasma* that require up to 6 weeks for culture (45,46). For all fungal pathogens, culture plates should be examined at regular intervals and are usually incubated for at least 48 hours (primarily for yeasts) and should be extended to 21 days for suspected respiratory fungi and dermatophyte infections.

Some fungal species are thermotolerant, such as *Aspergillus fumigatus* and can tolerate temperatures up to 45°C or higher. Therefore, culturing at higher temperatures can allow for selective isolation of fungi (46). Other fungi cannot thrive at temperatures higher than 32°C, therefore understanding the thermal tolerance of fungal species implicated in infection is important. Culture systems should routinely accommodate cultures at 30°C and 37°C. Dimorphic fungi such as *Blastomyces* species*, Sporothrix* species and *Histoplasma* species can switch between yeast and mould growth forms depending on temperature and nutrient availability (47,48). At 25 to 30°C the fungus grows as a mould but can grow as a yeast at temperatures of 37°C (47). Most dimorphic fungi, including *Blastomyces* species and *Histoplasma* species*,* are classified as hazard group 3 organisms (8). *Coccidiodes* species are also classified as hazard group 3 dimorphic fungi, however, they are not a yeast at 37°C, but instead form large, round spherules at this temperature. If there is clinical concern of dimorphic fungal infection, incubation at both temperatures is recommended to identify the different morphologies.

Moulds demonstrate a filamentous growth form with long, branching hyphae. Branching angle, the presence of cross walls, hyphal width and the presence of sporulating heads are typically used to identify moulds. A single colony may grow to fill an entire Petri dish (39). Microscopy in combination with colonial morphology is essential for the identification of moulds. Macroscopic mould morphology can vary significantly with growth conditions without major changes to microscopic appearance (39). Refer to Table 2 for the terms used to describe colonial morphology of yeast and moulds.

**Table 2: Terms used to describe colonial morphology of fungi (37,40,42,49)**

| 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 (e.g., *Talaromyces marneffei*) 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) |

| Term | Description |
| --- | --- |
| **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 (e.g., Mucorales spp.), 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 comparable to bacterial colonies

## 8.2 Microscopic appearance

### 8.2.1 Bacteria

Microscopic examination and staining reveal the shape and the characteristic grouping and arrangement of the cells. For example, *Streptococcus* species usually appear in pairs or short chains and *Staphylococcus* species typically form grape-like clusters in liquid or broth culture (37). Please see algorithms 2 to 4 for the microscopic appearance of clinically important bacteria.

When using microscopy, stains with different affinities for different organisms are used to highlight structures in clinical specimens and isolates. Gram staining improves visualisation of bacteria and allows bacteria to be categorised into two groups – Gram negative and Gram positive. However, it should be noted that not all bacteria can be characterised by Gram staining, as some bacteria do not retain the stain, resulting in Gram variable and non-stainable bacteria. (40). Other stains can be applied to microorganisms for identification such as lactophenol for fungi or Ziehl-Neelsen for Mycobacteria, please refer to UK SMI TP 39 – Staining procedures for full details on different staining methods for the identification of microorganisms

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

### 8.2.2 Fungi

#### Yeasts

Yeasts are 3-5 times the size of a bacterial cell. Making a rapid ‘wet prep’ of any colony into saline on a slide with a cover slip can very quickly confirm growth as yeast using x40 objective. Yeasts can also be visualised using Gram’s stain from cultures and specimens such as blood cultures usually as gram positive. Please refer to UK SMI TP 39 – Staining procedures for details of staining procedures used in identification of yeasts (50). In the absence of MALDI-TOF MS, laboratories can also enhance the microscopic characteristics of yeasts using growth on specialised agar.

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 yeasts. Using these media, yeasts may be sub-cultured using the Dalmau technique or as an inoculum "streak" with a coverslip (37). 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 blastospore arrangement.

### Filamentous moulds

Microscopy remains the primary method for the identification of filamentous moulds in most laboratories. Microscopy should be used in conjunction with clinical history, culture, serology, biochemical or molecular testing (37,40). Staining and microscopical methods can be used to enhance the structural characteristics of fungi to aid identification. Examples include saline mount, lactophenol cotton blue or lacto-fuchsin, calcofluor white with 10% KOH (50). For more information, see UK SMI TP 39 – Staining procedures.

Moulds reproduce by producing spores. Microscopic examination of the sporulating structures and the spores themselves can aid in identification of the isolate. Methods including tease mounts, slide culture and adhesive tape are used to observe sporulating structures and conidia (37). Ensure that sporulating structures are present in the portion of the colony used for microscopic examination.

**Tease mount -** 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.

**Cellotape mount –** this involves touching a piece of an adhesive tape (good quality, optically clear) onto a fungal plate, then placing it fungus-side down onto a drop of lactophenol cotton blue on a slide and applying an additional drop of lactophenol on top followed by a coverslip for examination (37).

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

MALDI-TOF MS is used as the primary method for identification of bacteria but needs to be used in the context of colony morphology, microscopic characteristics and any biochemical or molecular test results. It is a rapid, mostly accurate and mostly reliable identification tool for the characterisation of a diverse collection of pathogens. This technique is used for bacterial identification due to its high reproducibility, costeffectiveness and sensitivity of analysis and improved turnaround times compared to phenotypic methods (51,52). If an identification cannot be achieved by MALDI-TOF MS, then the optimal pathway for unidentified isolates is referral to molecular based identification methods such as 16S pan-bacterial and ITS pan fungal PCR. It may be appropriate to use biochemical testing if MALDI-TOF MS is unavailable or inconclusive, however, this is dependent on the target organism and is not appropriate for moulds.

It is important to be aware of the limitations of databases and to check that the MALDI-TOF MS result should be evaluated with phenotypic information and clinical presentation. This method must be validated and all available updates must be installed and validated prior to use. Manufacturer's instructions must be followed. If results are inconclusive or contradictory, further testing methods should be used to ensure accurate results. It is important to note that MALDI-TOF MS may not safely discriminate between some organisms, particularly genetically similar species, such as species in the *Burkholderia cepacia complex* or *Shigella* species from *Escherichia coli,* causing misidentification (53,54). In these cases, confirmation is required using molecular methods.

The use of MALDI-TOF MS for the identification of yeasts is typically robust but generally requires full formic acid extraction. For filamentous fungi have been found to improve identification, however standardised preparation procedures and databases are still developing. Therefore, this method is being adopted mainly in specialised laboratories as the technology evolves (55-57). A novel media plate to facilitate identification of moulds has recently been developed which enables direct spotting of fungal colonies onto target plates and has demonstrated good performance (58).

It is also possible with the aid of commercial kits or in-house methods to identify microorganisms directly from blood cultures using MALDI-TOF MS (59). This has benefits in speeding up the time to identification of pathogens causing bloodstream infection. The overall performance is comparable to direct MALDI-TOF MS from agar culture for most organisms, but limited performance has been reported for yeasts and coagulase negative *Staphylococci* (60,61).

## 8.4 Test procedures

### 8.4.1 Bacteria

Numerous biochemical tests and antisera may be used for the identification of microorganisms. Some tests are rapid and easy to perform and may be used for preliminary differentiation purposes (37,40,62). Conditions under which any biochemical tests are conducted should be clearly defined as reactions may vary between organisms.

Examples of commonly used tests include:

- Catalase test
- Oxidase test
- Fermentation of glucose
- Coagulase test
- Staining procedures including modified Ziehl-Neelsen or Albert's stains

For more examples of biochemical tests, refer to full list of all UK SMI Test Procedures.

Antisera may be used for identification of some species, including *Bordetella parapertussis, Bordetella pertussis* and for serotyping *Shigella* species. A suspension of colony should be prepared according to manufacturer's instructions. Specific antiserum is added to the suspension and mixed. A positive result is indicated by agglutination in the suspected colony compared to a control. For information about the agglutination test in Salmonella species, please refer to UK SMI TP 3 - Agglutination test for Salmonella species.

Using a combination of tests, it is usually possible to place organisms, provisionally, in one of the main groups of medical importance. The biochemical tests listed above are commonly used after an organism has been isolated on culture plates and its colonial appearance and growth requirements have been assessed. The list is not exhaustive and further tests may be needed in addition.

### 8.4.2 Fungi

Examples of some tests that aid fungal identification include:

- Staining procedures including India ink and calcofluor white stains
- Rapid urease test for presumptive identification of *Cryptococcus neoformans-Cryptococcus gattii* species complex. Please refer to UK SMI TP 36 – urease test
- Dermatophyte test medium for isolation and presumptive identification of dermatophytes such as *Microsporum*, *Trichophyton*, *Nannizzia* and *Epidermophyton* genera. For further information on dermatophytes refer to UK SMI B 39 – Investigation of dermatological specimens for superficial mycoses

For other tests and procedures, please see the Test procedure UK SMIs.

For identification of yeast species, descriptions of Candida chromogenic media and germ tube test are included below.

#### Candida chromogenic media

Chromogenic agar for the culture of yeast species is used widely throughout laboratories for the differentiation of *Candida albicans* from other yeast species. Identification of yeast to species level relies on morphological aspects, such as colour and texture, but chromogenic media should not be relied on, on its own for yeast identification. Most commercially available chromogenic media for *Candida* identification are hexosaminidase-based, enabling the identification of *C. albicans* as ‘apple green’ colonies. However, discrimination between non-albicans yeast species is not recommended as these often present with similar morphological appearance on chromogenic Candida media.

Chromogenic agar should be incubated for a minimum of 24 hours at 36°C or according to manufacturer’s instructions. Improved identification of *C. albicans* has been demonstrated with incubation extended to 48 hours (35).

However, one limitation of most *Candida* chromogenic agar does not fully support the growth of multi-drug resistant species *Candidozyma auris* (63). A novel chromogenic agar has recently been developed that offers enhanced culture of *C. auris* and colonies appear as pale cream with a distinctive blue-green halo after 48 hours incubation at 30-36°C (64). MALDI-TOF MS is required to provide final identification on all colonies with a blue-green morphology as misidentification of *C. albicans* and *Candida parapsilosis* colonies as *C. auris* has been documented (65). The respective chromogenic agar should be utilised where there is a specific requirement for *C. auris* isolation i.e. screening swabs or blood cultures.

### Germ tube test

Germ tube test is a rapid screening test used primarily from blood cultures or from colonies to distinguish C*. albicans* (germ tube positive) from other *Candida* species (germ tube negative). *Candida dubliniensis* and *Candida africana* also produce germ tubes.

To perform the test, a pure single colony of yeast from either the original isolation plate or a 24 hour purified subculture is emulsified in sterile serum (rabbit or horse) and incubated at 35 to 37°C aerobically for 2-3 hours. The suspension can then be examined for germ tube formation under a microscope. Studies have shown that 1mL of blood culture can be centrifuged and the washed pellet used for germ tube testing (66,67).

The germ tube test performance is limited as false positive results may occur if the incubation time exceeds 3 hours. False negative results occur due to over-inoculation of the serum, strain variation or bacterial contamination. Some species, in particular *Candida tropicalis*, although produce true hyphae, may also form pseudohyphae, which are chains of yeast cells where separation after budding is incomplete; these structures may be misinterpreted as germ tubes (68,69).

## 8.5 Further identification

### 8.5.1 Commercial Identification Systems (kits/rapid tests)

Commercially available identification kits alongside other biochemical tests may be used for identification of bacteria and yeasts (70). It should be noted that there are no commercial kits for biochemical profiling of most filamentous fungi. 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 and may not be able to identify new or uncommon species of organisms that are not in the accompanying database (37,71,72).

Laboratories must be aware of limitations of the specific commercial identification system that is used. 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.

The inability of commercially available identification kits to distinguish between related organisms makes them unreliable for stand-alone testing. 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.

### 8.5.2 Resistance properties

Microorganisms can exhibit a characteristic inherent resistance to specific antibiotics, heavy metals, or toxins, which can be used to aid preliminary identification in bacteria (42). For example, media supplemented with colistin or aztreonam and nalidixic acid is used to isolate Gram positive bacteria (73,74).

Testing the susceptibility of an isolate to a particular antibiotic is also useful in identification. For example, resistance to vancomycin or susceptibility to colistin or polymyxin can assist in the presumptive identification of most clinically significant Gram negative bacteria. Susceptibility to metronidazole is commonly used to detect the presence of anaerobic bacteria and to facilitate the recovery of Actinomyces in mixed anaerobic cultures (75).

For *Aspergillus fumigatus*, VIP multi-well plates are available that contain itraconazole, voriconazole and posaconazole are breakpoint concentrations and can be used for a determination of azole resistance.

### 8.5.3 Molecular Methods

Whilst molecular methods are usually associated with detection of a species from patient samples, some methods can be used for preliminary identification of organisms in culture. These methods have made initial identification of species more rapid and precise than possible with phenotypic methods. 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.

For example, sequencing of the 16S rRNA gene can be used to identify rarely encountered bacteria when MALDI-TOF MS is unavailable and phenotypic identification is not sufficient. Nuclear ribosomal internal transcribed spacer (ITS) region pan-fungal sequencing can also be utilised for rarely encountered yeasts when other identification methods fail (76). Whilst 16S and ITS sequencing can be beneficial in these scenarios, not all organisms have been sequenced and some homologous species may still be difficult to distinguish (77). This process is also costly and is therefore not used for preliminary identification unless necessary (78).

For identification of filamentous fungi, multiple ribosomal and chromosomal targets such as 18S, ITS, D1/2, calmodulin, and tubulin need to be used in combination.

### Next generation sequencing (NGS)

Whilst currently limited to reference laboratories, NGS could become more common in clinical laboratories. NGS provides a quick and accurate means of identifying pathogens that could be potentially beneficial for routine diagnostics in the future. However, it is currently costly, and its performance compared to the gold standard and other currently used methods remains to be established and validated.

## 9 Storage

For storage and transport conditions of specific microorganisms, please refer to the relevant individual UK SMI identification documents or contact the appropriate reference laboratory. For information on the storage and retention of specimens, please see the Royal College of Pathologists retention and storage of pathological records and specimens guidance. Careful consideration should be given to the storage of containment level 3 pathogens and any Schedule 5 pathogens should be notified in compliance with the Anti-terrorism, Crime and Security Act (21).

## 10 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. This ensures 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.

## 10.1 Infection Specialist

Certain clinical conditions must be notified to the laboratory associated infection specialist. Follow local protocols for reporting to the patient's clinician.

## 10.2 Presumptive identification

If appropriate growth characteristics, colonial appearance, Gram stain of the cultured isolate, biochemical and serological results are demonstrated, presumptive identification can be reported according to local protocols until identification is confirmed.

## 10.3 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.

## 10.4 Health Protection Team (HPT)

Refer to local agreements in devolved administrations.

## 10.5 UK Health Security Agency

Refer to current guidelines on Second Generation Surveillance System (SGSS) reporting (23).

## 10.6 Infection prevention and control team

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

# 11 Referral to reference laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory see user manuals and request forms

Organisms that are difficult to identify, have 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 reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

England

Wales

Scotland

Northern Ireland

Note: It is good practice to notify the reference/specialist laboratory before sending an isolate. Please ensure the referring paperwork contains all relevant clinical information, and the hazard group of the presumptive identification.

In case of sending away to laboratories for processing, ensure that specimen is placed in an appropriate package and transported accordingly.

# 12 Public health responsibilities of diagnostic laboratories

Diagnostic laboratories have a public health responsibility as part of their duties. Amongst these are additional local testing, or referral to further characterise the organism as required, primarily for public health purposes e.g. routine *Cryptosporidium* detection; serotyping or microbial subtyping; and a duty to refer appropriate specimens and isolates of public health importance to a reference laboratory.

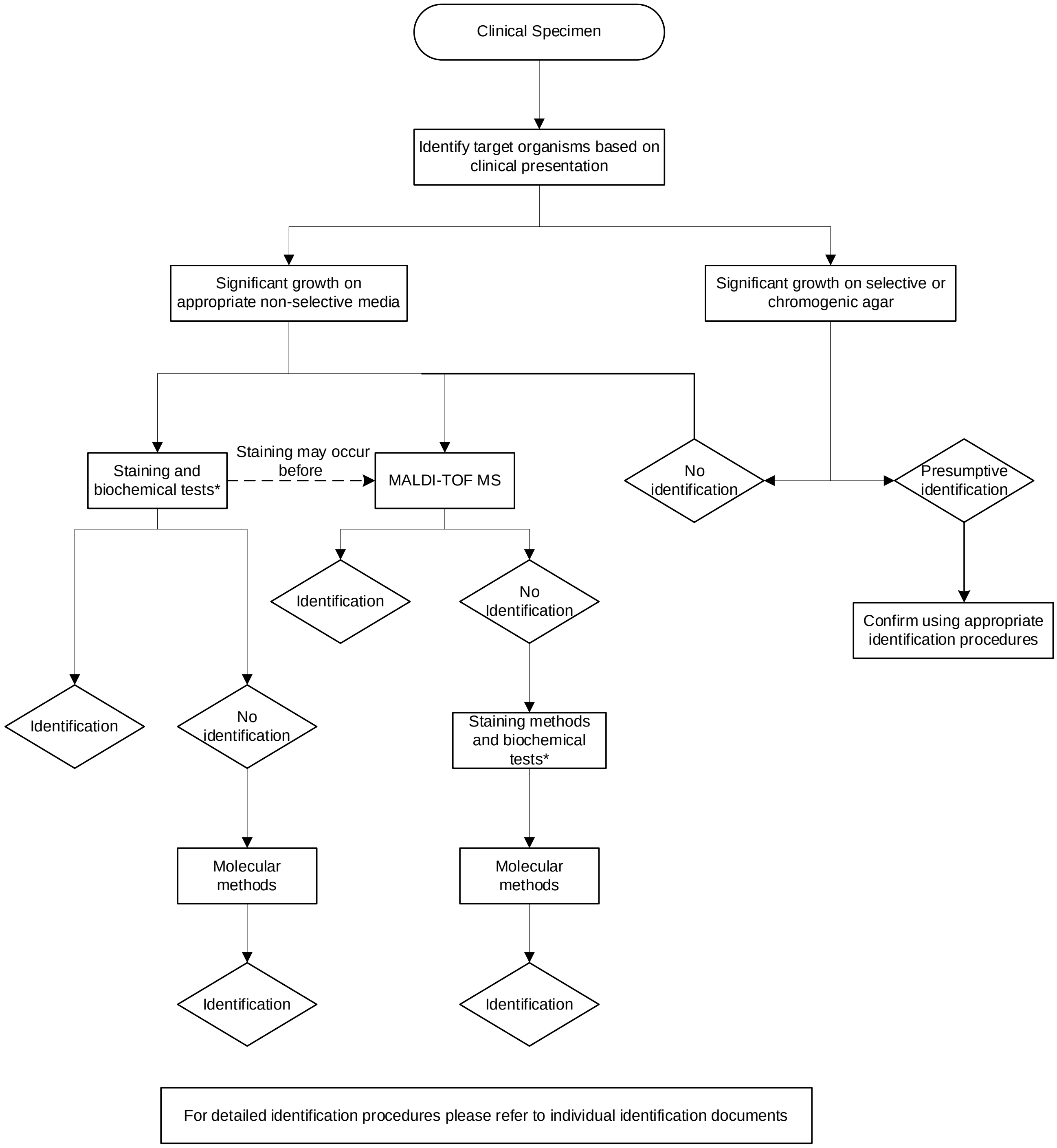
Diagnostic laboratory outputs inform public health intervention, and surveillance data is required to develop policy and guidance forming an essential component of healthcare. It is recognised that additional testing and referral of samples may entail some costs that have to be borne by the laboratory but in certain jurisdictions these costs are covered centrally.

Diagnostic laboratories should be mindful of the impact of laboratory investigations on public health and consider requests from the reference laboratories for specimen referral or enhanced information.



UK Standards for Microbiology Investigations | Issued by the Standards Unit, UK Health Security Agency

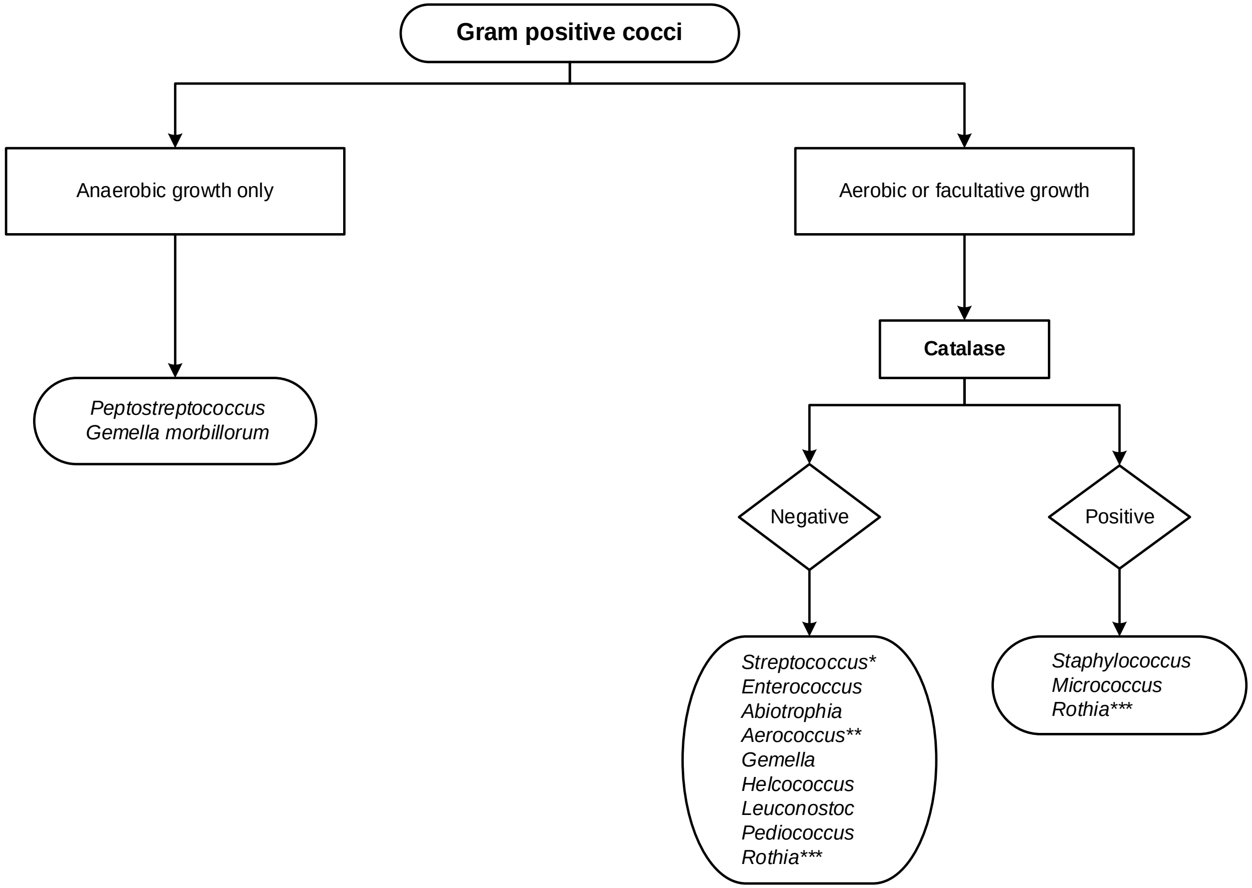
# Algorithm 1: Identification of bacteria and yeasts




*Please note: Biochemical tests may not be appropriate for all microorganisms. For yeasts, biochemical identification is secondary to the use of MALDI-TOF MS.

The flowchart is for guidance only.

# Algorithm 2: Characteristics of Gram positive cocci



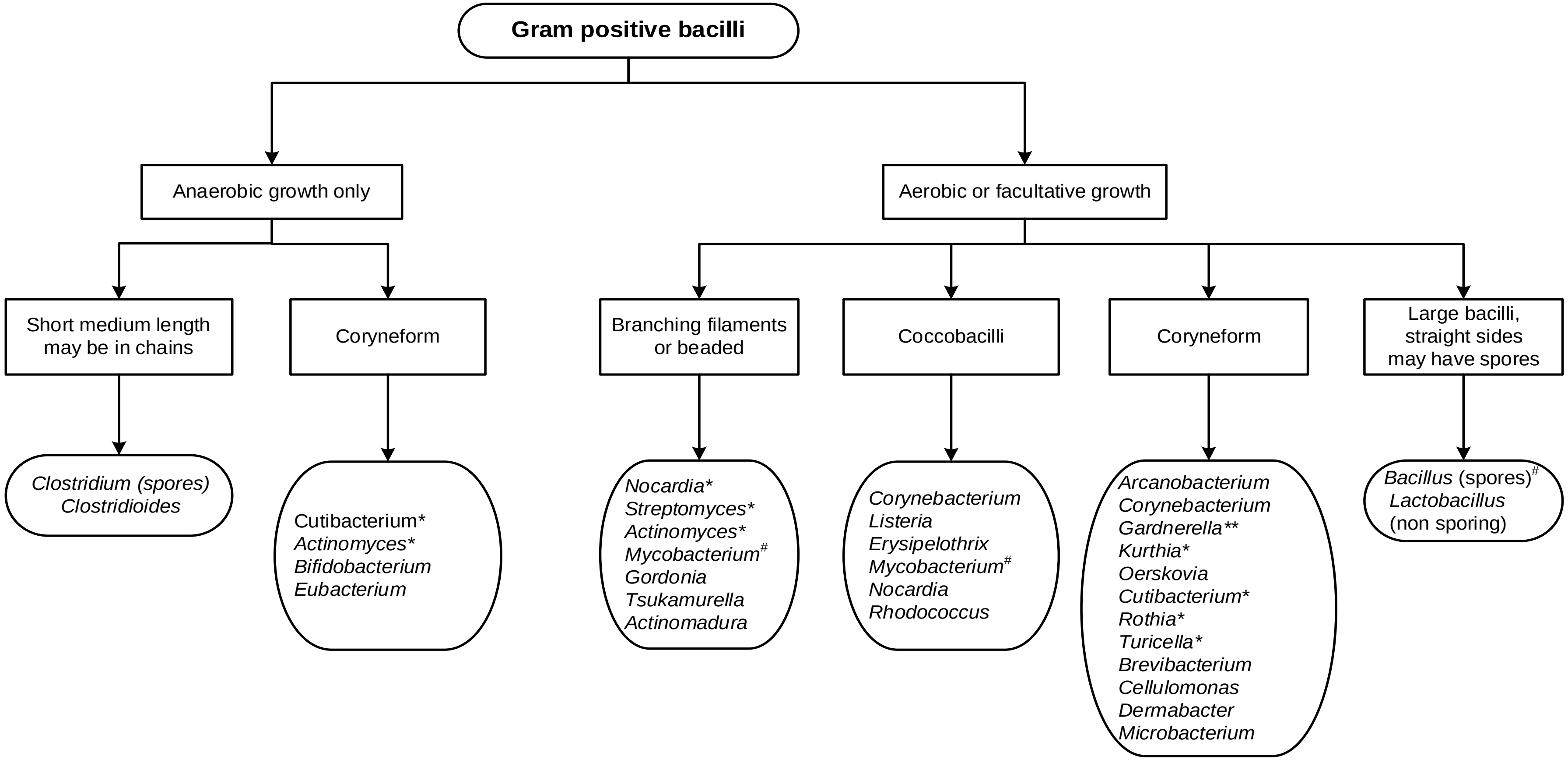

***** Some species may be anaerobic

****** 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 (62,79,80).

# Algorithm 3: Characteristics of Gram positive bacilli



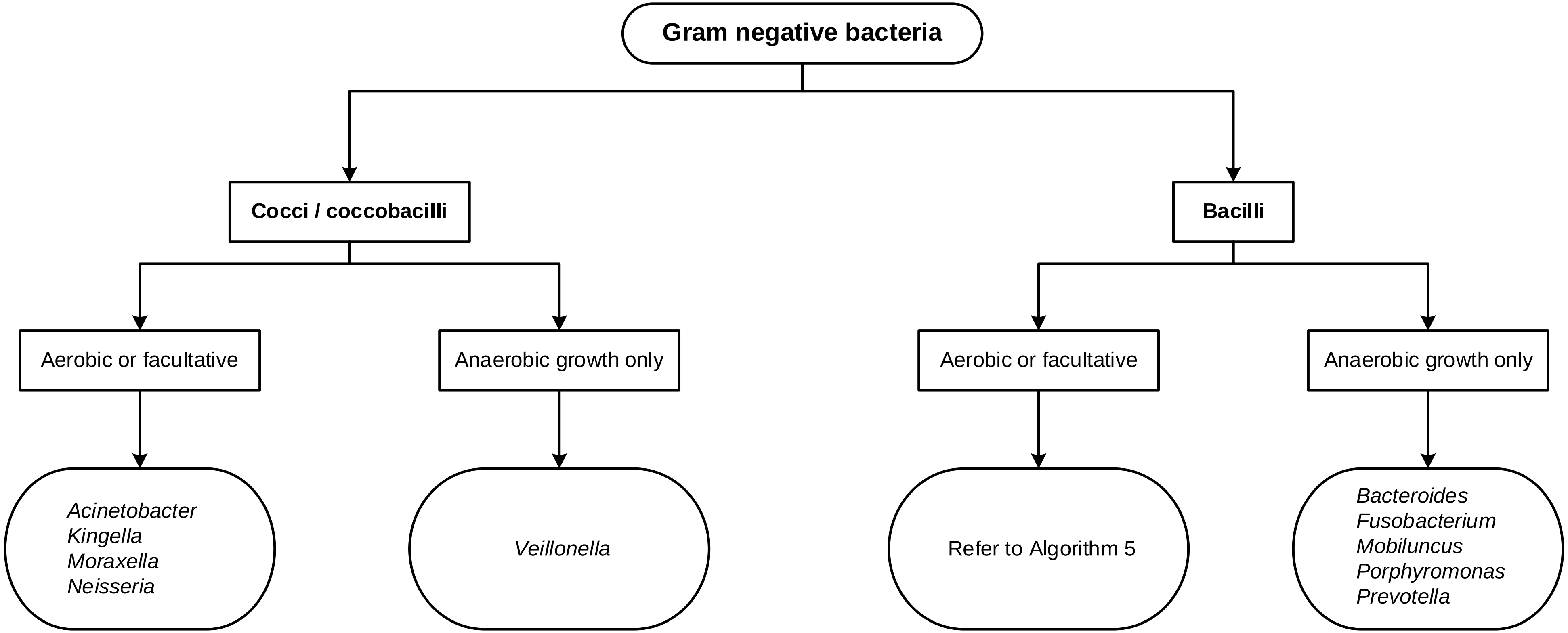

*This organism is pleomorphic

***Gardnerella vaginalis* is a Gram variable bacilli 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

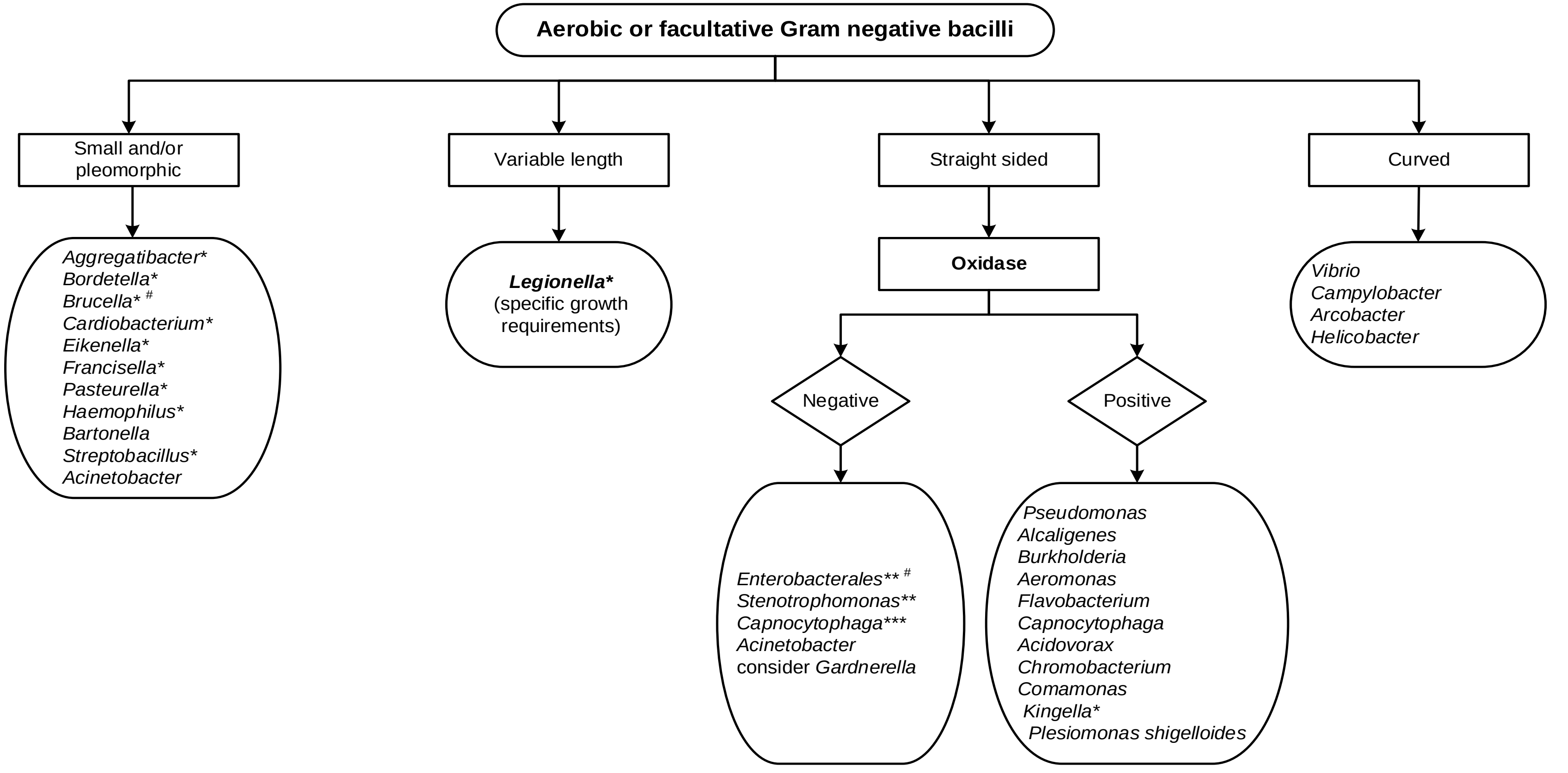
The flowchart is for guidance only (62,79,80).

# Algorithm 4: Characteristics of Gram negative bacteria




The flowchart is for guidance only (79,81).

# Algorithm 5: Characteristics of Gram negative bacteria




***** Some species may be anaerobic

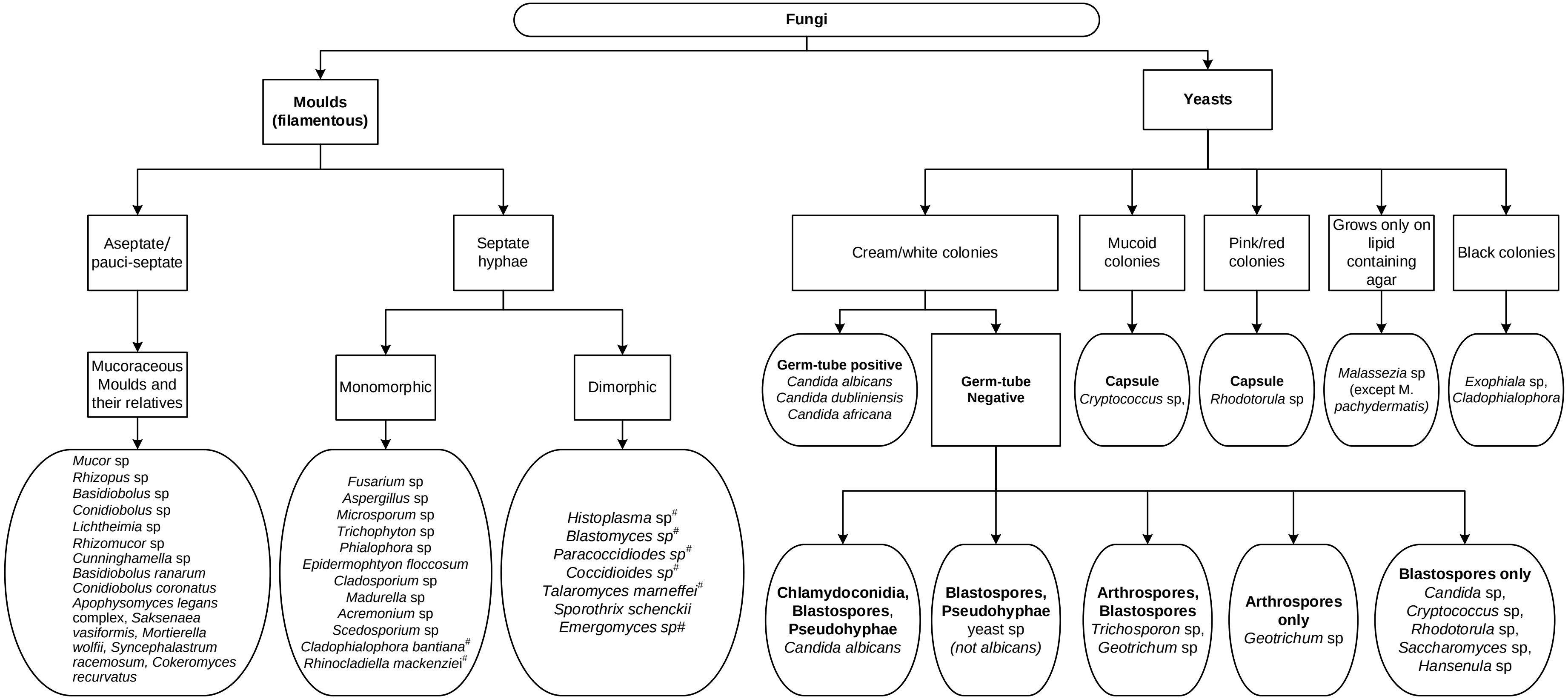
****** May be weak catalase positive

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

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

The flowchart is for guidance only (79,81).

# Algorithm 6: Morphological characteristics of fungi




#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 obtained from MALDI-TOF MS and other commercial identification systems. The nomenclature for some species has changed, see appendix 6 (6,7,39,44,50).

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

| Former species name | Revised species name (7) |
|---|---|
| *Candida auris* (82) | *Candidozyma auris* |
| *Candida glabrata* (for full list of *Candida* species see reference(7)) | *Nakaseomyces glabratus* |
| *Candida guilliermondii* | *Meyerozyma guilliermondii* |
| *Candida krusei* | *Pichia kudriavzevii* |
| *Candida lusitaniae* | *Clavispora lusitaniae* |
| *Candida rugosa* | *Diutina rugosa* |
| *Cryptococcus albidus* | *Naganishia albida* |
| *Cryptococcus curvatus* | *Cutaneotrichosporon curvatum* |
| *Cryptococcus cyanovorans* | *Cutaneotrichosporon cyanovorans* |
| *Cryptococcus laurentii* | *Papiliotrema laurentii* |
| *Geotrichum capitatum* | *Magnusiomyces capitatus* |
| *Geotrichum clavatum* | *Magnusiomyces clavatus* |
| *Trichosporon cutaneum* | *Cutaneotrichosporon cutaneum* |
| *Trichosporon dermatis* | *Cutaneotrichosporon dermatis* |
| *Trichosporon loubieri* | *Apiotrichum loubieri* |
| *Trichosporon mucoides* | *Cutaneotrichosporon mucoides* |
| *Trichosporon montevideense* | *Apiotrichum montevideense* |
| *Trichosporon mycotoxinivorans* | *Apiotrichum mycotoxinivorans* |

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