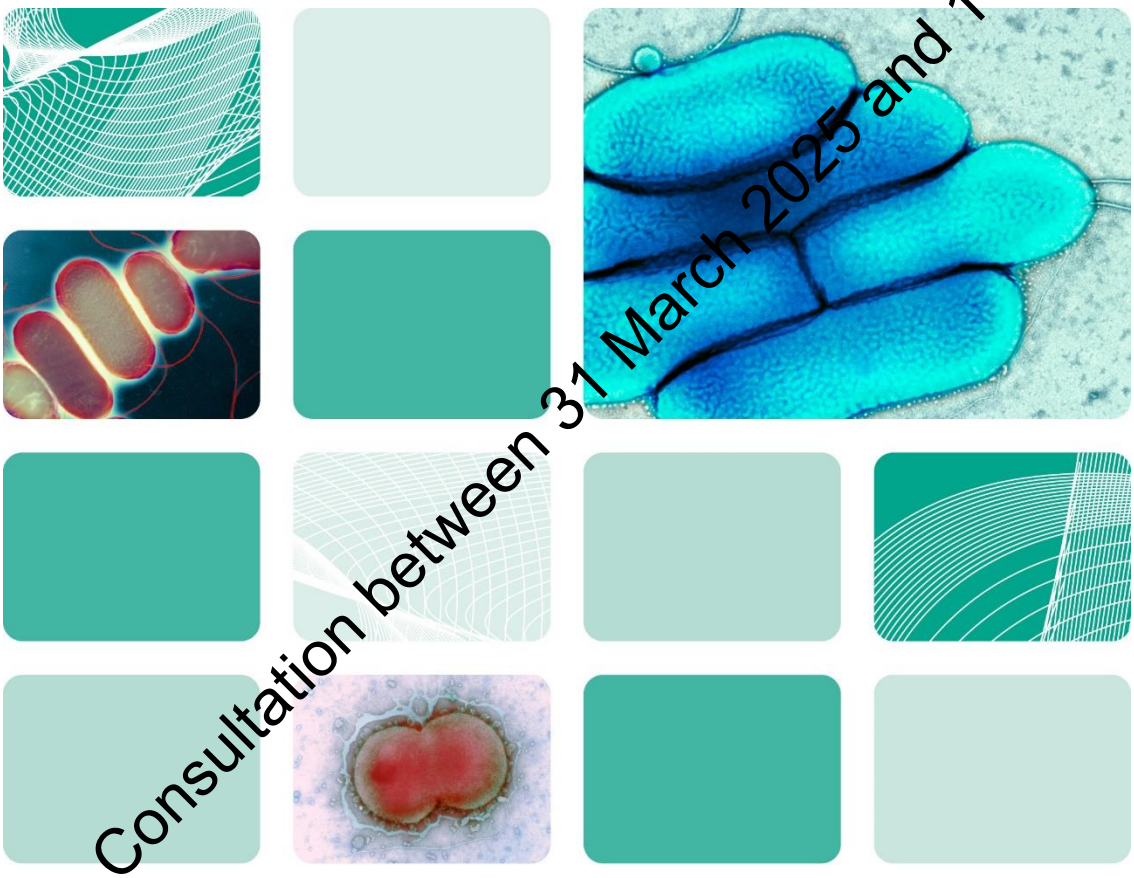
UK Health Security Agency

UK Standards for Microbiology Investigations

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



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Amendment number/date	x/dd.mm.yy
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Anticipated next review date*	dd.mm.yy
Section(s) involved	**Amendment**

*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 which may be encountered in clinical specimens following isolation on agar plates. This document covers the identification of bacterial and fungal microorganisms from culture. For guidance on identification of pathogenic parasites, refer to UK SMI B 61 - Investigation of specimens for ectoparasites. Detection and point-of-care tests are 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 30 - Investigation of dermatological specimens for superficial mycoses.

Please note, 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

Preliminary identification covers the initial investigations and tests that provide 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

Preliminary identification of microorganisms relies on one or more 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, biochemical tests and / or genotypic characteristics. Using a combination of tests, it is usually possible to place organisms, provisionally, in one of the main groups of medical importance (1).

A judgement is made on the presumptive identity of the organism based on relevant clinical presentation, travel history, colonial morphology, microscopy, and growth characteristics. MALDI-TOF MS and a limited range of tests can then be used to confirm identification; however, this relies heavily on a stable pattern of phenotypic characteristics. 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 the 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).

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 some filamentous fungi is variable (4,5). Therefore, the identification of filamentous fungi relies on colonial and microscopic morphology.

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. All identification using MALDI-TOF MS should be considered 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/species and revisions to existing names (7). At the time of writing, it was decided to mention fungi using their former names; however, see algorithm 7 for previous and revised names for fungi mentioned in this document.

6 Safety considerations

The section covers specific safety considerations (8-29) 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 the manipulation of a cultured isolate that may be a HG3 organism that can be spread by aerosol and can cause human disease must take place in a Microbiological Safety Cabinet (MSC) or similar containment. Where re-circulating 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 materials that can be spread by aerosol and can cause human disease must take place in 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., multi-drug resistant TB. Such organisms include *Mycobacterium* species, *Brucella* species, *Bacillus anthracis*,

Blastomyces dermatitidis, *Histoplasma capsulatum*, *Coccidioides immitis,* and others (8,19,30). 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 require the same broad techniques in a similar order. This varies according to species, laboratory equipment/specialists and local procedures. Isolates are first cultured and examined. Individual cultures can be identified using appropriate staining techniques, if required. MALDI-TOF MS or further testing methods are used where necessary. Identification can then be confirmed using molecular methods. 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 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.

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. 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 (31).

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 (32-35). In addition, chromogenic media is recommended for the detection of mixed yeast infections, and identification of some Candida and associated ascomycetous yeasts (31,36,37). Please see section 8.4 Test procedures, for detailed information about the use of chromogenic agar in the identification of Candida species.

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 (38):

- 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 (38):

- 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

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 (39).

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 is variable, depending on the amount of inoculum (bacterial or fungal) present in a clinical specimen as well as the freshness of the medium and isolation conditions (40). 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 (39). Table 1 details the terms used when describing colonial morphology of bacteria.

Under favourable growth conditions, the size of bacterial colonies tend 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 (41,42).

Table 1: Terms used in colonial morphology of bacteria (38,43)

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, 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, 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
Haemolysis	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

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 (40). Pathogenic yeasts are often further categorised as either Candida and associated ascomycetous yeasts, *Cryptococcus* species, or dimorphic fungi.

Colonial morphology can be useful for categorising yeasts. 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 (44). Similarly, *Rhodotorula* species produce naturally pigmented colonies that are pink to red (45). Further identification is usually achieved using chromogenic agar, mainly for the identification of common Candida (e.g., *Candida albicans*), and other yeasts are identified using MALDI-TOF MS.

In laboratories where MALDI-TOF MS is not available, techniques such as staining for microscopic investigation, biochemical methods and/or specialised culture can be applied. However, the accuracy of these methods to identify 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 of culture medium used, age of culture used for subculture, amount of inoculum and the temperature of incubation (40). 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 capsulatum* that require up to 6 weeks for culture (46,47). 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. Therefore, culturing at higher temperatures can allow for selective isolation of fungi (47). 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 dermatitidis, Sporothrix schenckii* and *Histoplasma capsulatum* can switch between yeast and mould growth forms depending on temperature and nutrient availability (48,49). At 25 to 30°C the fungus grows as a mould but can grow as a yeast at temperatures of 37°C (48). Most dimorphic fungi, including *Blastomyces dermatitidis* and *Histoplasma capsulatum,* are classified as hazard group 3 organisms (8).

Moulds demonstrate a filamentous growth form with long, branching hyphae. A single colony may grow to fill an entire Petri dish (40). Microscopy in combination with colonial morphology, is essential for the identification of moulds, especially filamentous fungi. Macroscopic mould morphology can vary significantly with growth conditions without major changes to microscopic appearance (40). Table 2 details the terms used to describe colonial morphology of yeast and moulds.

Table 2: Terms used in colonial morphology of yeasts and moulds (38,41,43,50)

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

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 chain and *Staphylococcus* species typically form grape-like clusters (38). Please see algorithms 2-4 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, which

improves visualisation of bacteria and allows bacteria to be categorised into two groups – Gram negative and Gram positive (41). 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 detail 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 as gram positive yeast-like cells. Please refer to UK SMI TP 39 – Staining procedures for details of staining procedures used in identification of yeasts (51). 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 fungi. Using these media, yeasts may be sub-cultured using the Dalmau technique or as an inoculum "streak" with a coverslip (38). 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 and chlamydospores.

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 (38,41). 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 (51). For more information, see UK SMI TP 39 – Staining procedures.

Moulds reproduce by producing spores. Microscopic examination of the sporing 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 spores and conida (38). Mounts can be prepared by either making a tease mount or a cellotape mount.

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 - placing a piece of an adhesive tape (good quality, optically clear) fungus-side down onto a drop of lactophenol cotton blue on a slide and applying an additional drop of lactophenol on top then a coverslip for examination (38).

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 and yeasts. It is a rapid, accurate and highly reliable identification tool for the characterisation of a diverse collection of pathogens. This technique is used for bacterial identification due to its high reproducibility, cost-effectiveness and sensitivity of analysis and improved turnaround times compared to phenotypic methods (52,53). In most instances MALDI-TOF MS is now so accurate that if an identification cannot be achieved 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 is important to be aware of the limitations of databases and to check that the MALDI-TOF MS result is in keeping with the colony morphology, microscopic characteristics and any biochemical or molecular test results. It is important this method is validated, manufacturer's instructions followed and all available updates are installed. If results are inconclusive or contradictory, further testing methods should be used to ensure accurate results. It must be noted that, MALDI-TOF MS may not discriminate between genetically similar species, such as the *Burkholderia cepacia complex* or *Shigella* species from *Escherichia coli,* causing misidentification (54,55).

The use of MALDI-TOF MS for the identification of filamentous fungi has 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 (56-58). A novel media plate to facilitate identification of moulds has recently been developed which enables direct spotting of fungal colonies onto targets plates which has demonstrated good performance (59).

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 (60). This has benefits in speeding up the time to identification of pathogens causing blood stream infection. Performance overall 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* (61,62).

8.4 Test procedures

8.4.1 Bacteria

Numerous biochemical tests may be used for the identification of microorganisms. Some tests are rapid and easy to perform and may be used for preliminary differentiation purposes. (38,41,63). Conditions under which any biochemical tests are conducted should be clearly defined as reactions may vary between organisms.

Examples of commonly used biochemical tests include:

- Catalase test
- Oxidase test
- Fermentation of glucose

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

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 (above) list the common tests used once an organism has been isolated on culture plates after colonial appearance and growth requirements have been assessed. The lists are not exhaustive and further tests may be needed in addition.

8.4.2 Fungi

Examples of some tests that aid fungal identification include:

- 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 TP 39 – Investigation of dermatological specimens for superficial mycoses

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

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

Candida chromogenic media

Chromogenic agar for the culture of *Candida* species is used widely throughout laboratories for the differentiation of *Candida albicans* from non-albicans species. *Candida* species level identification relies on morphological aspects, such as colour and texture. 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 Candida 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, increased performance in identification of *C. albicans* has been demonstrated after incubation up to 48 hours (36).

However, one limitation of most Candida chromogenic agar does not fully support the growth of multi-drug resistant species *Candida auris* (64). A novel chromogenic agar has recently been developed that offers enhanced culture of *C. auris* and colonies culture as pale cream with a distinctive blue-green halo after 48-hours incubation at 30-36°C (65). MALDI-TOF MS is required to provide final identification on all colonies with a blue-green morphology as mis-identification of *C. albicans* and *Candida parapilosis* colonies as *C. auris* have been documented (66). 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 (67,68).

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 or strain variation. Some species, in particular *Candida tropicalis*, may form pseudohyphae; these structures may be misinterpreted as germ tubes.

8.5 Further identification

8.5.1 Commercial Identification Systems (kits/rapid tests)

The use of commercially available identification kits alongside other biochemical tests may be used to give accurate identification of bacteria and yeasts (69). It should be noted that there are no commercial kits for biochemical profiling of 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 organism that are not in the accompanying database (38,70,71).

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 make 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. Commercial identification systems should be used as per manufacturer's instructions.

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 (43). For example, media supplemented with colistin or aztreonam and nalidixic acid is used to isolate Gram positive bacteria (72,73).

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 aid with 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 (74).

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, 16S rRNA sequencing 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 (75). Whilst 16S rRNA 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 (76). This process is also costly and is therefore not used for preliminary identification unless necessary (77).

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, its' performance compared to the gold standard and other currently used methods remains to be established and validated.

9 Storage

For specific storage and transport conditions, please refer to individual UK SMI identification documents or contact the appropriate reference laboratory.

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.

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 (24).

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 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 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 appropriate package and transported accordingly.

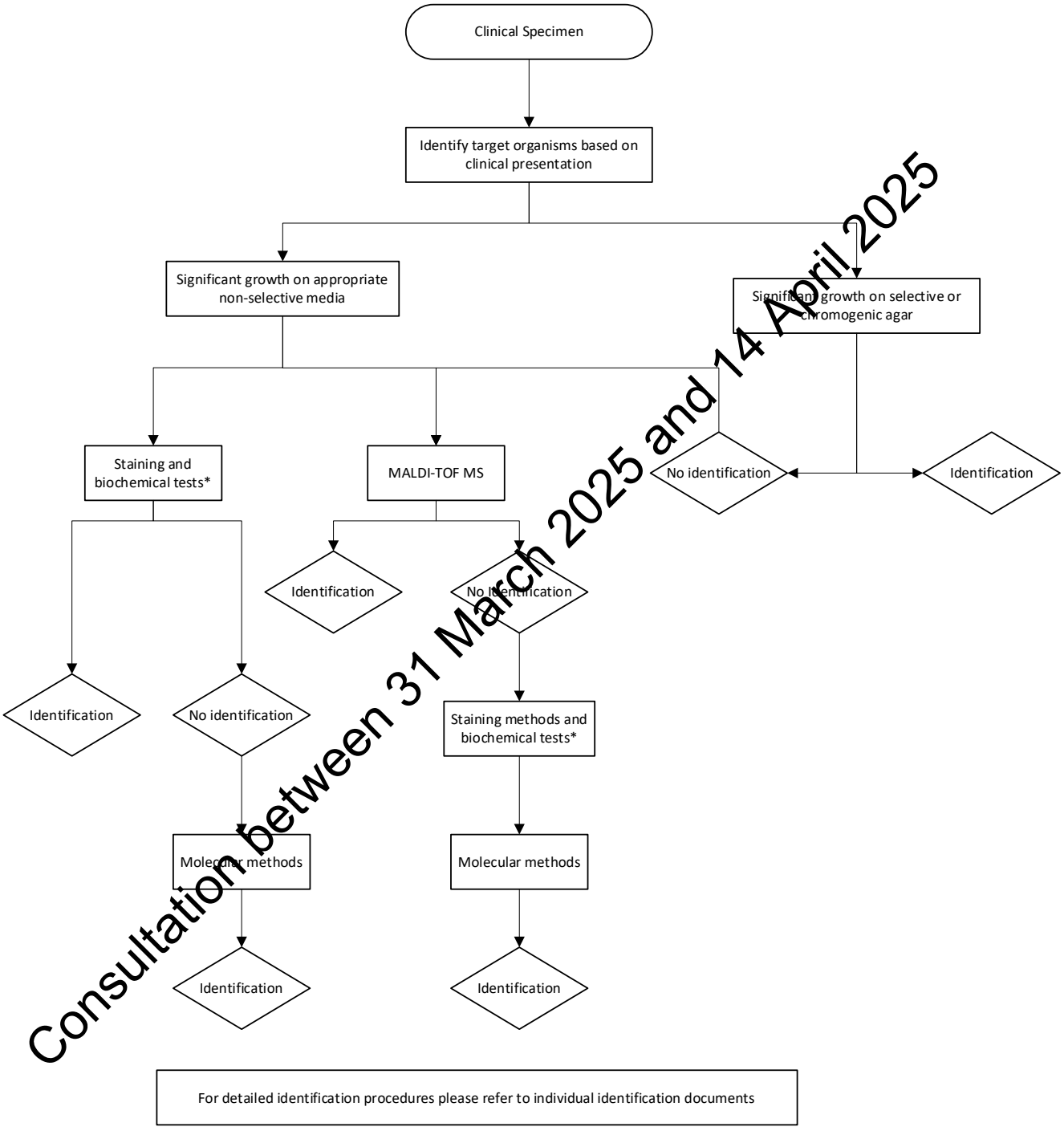
12 Public health responsibilities of diagnostic laboratories

Diagnostic laboratories have 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 has 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.

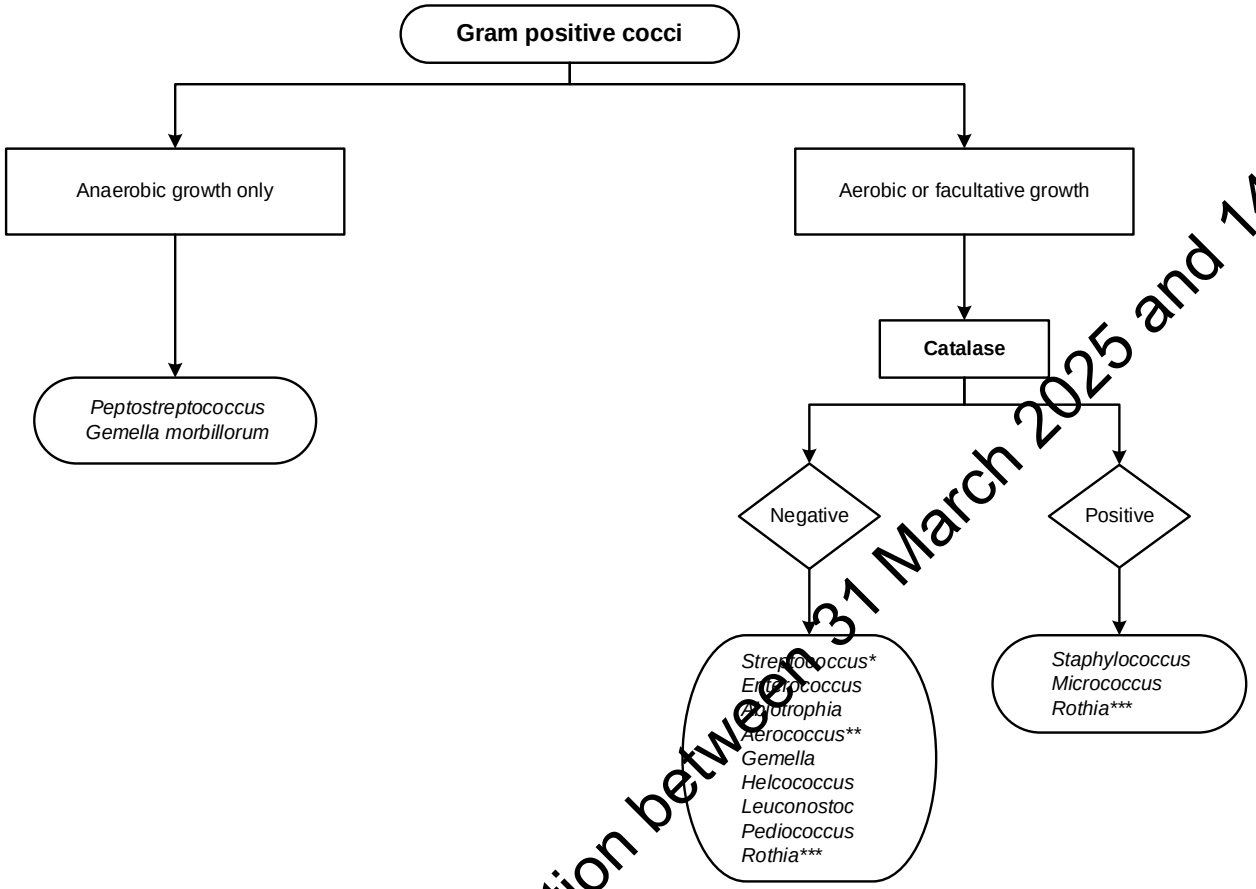
Algorithm 1: Preliminary identification of bacteria and yeasts



*Please note: Biochemical tests may not be appropriate for all microorganisms.

The flowchart is for guidance only.

Algorithm 2: Characteristics of Gram positive cocci



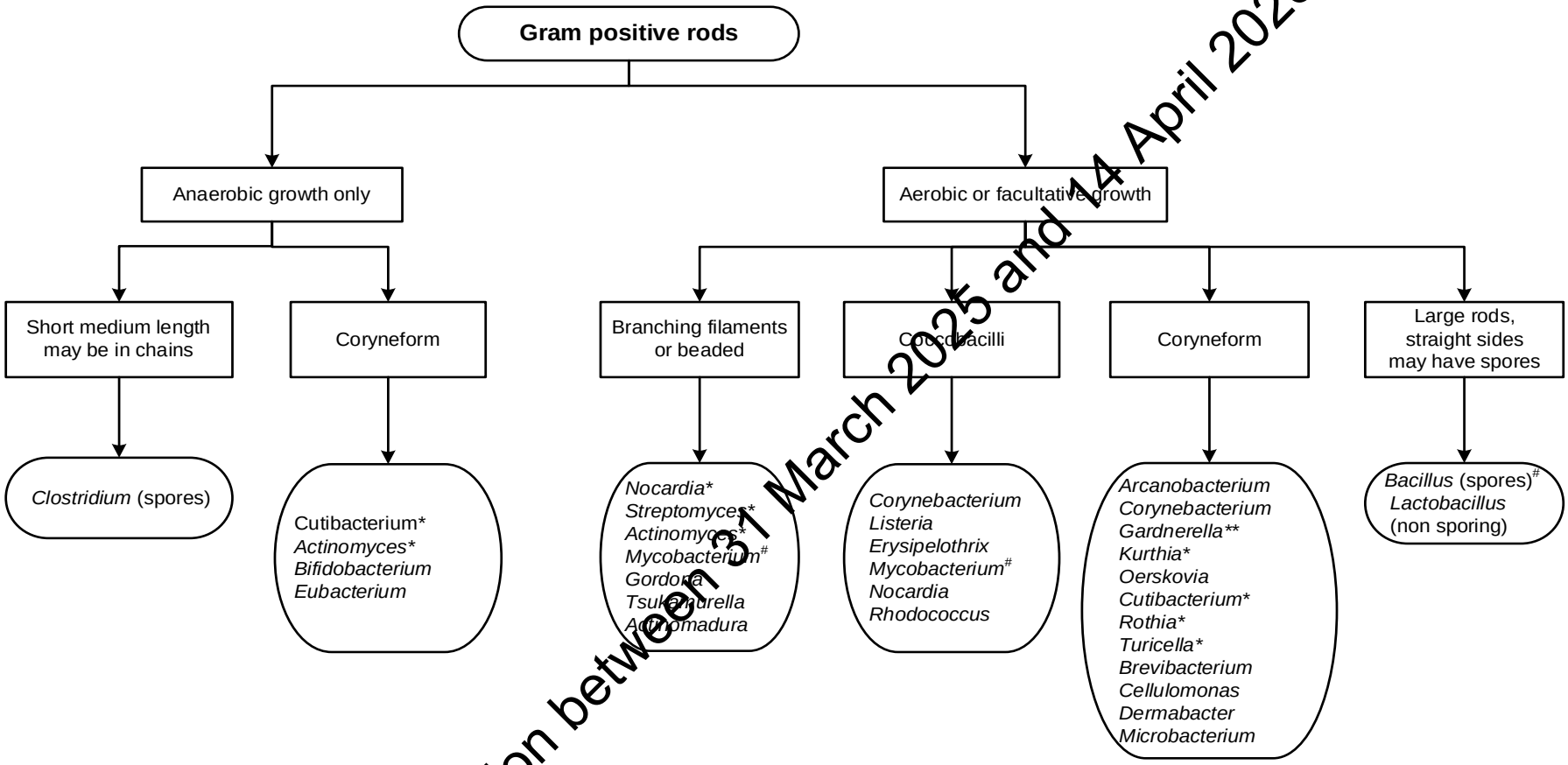
***** Some species may be anaerobic

****** 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 (63,78,79).

Algorithm 3: Characteristics of Gram positive rods



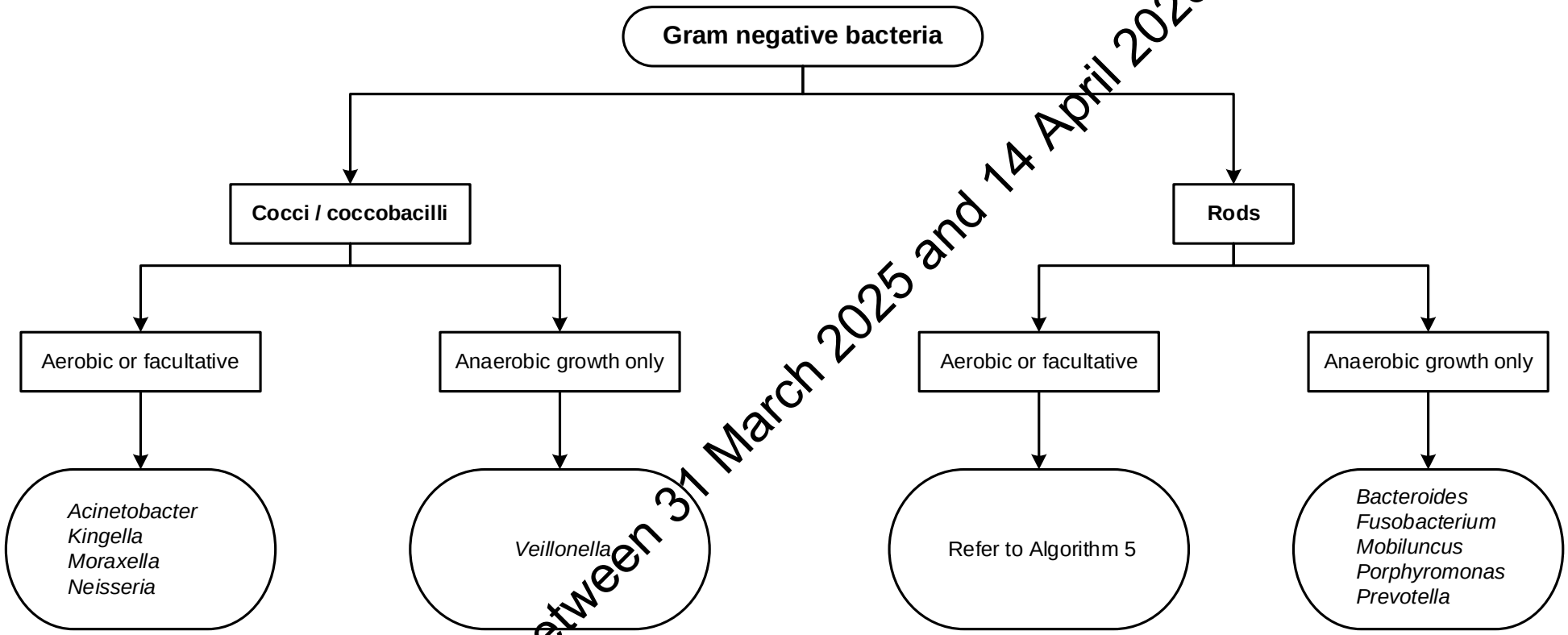
*This organism is pleomorphic

***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

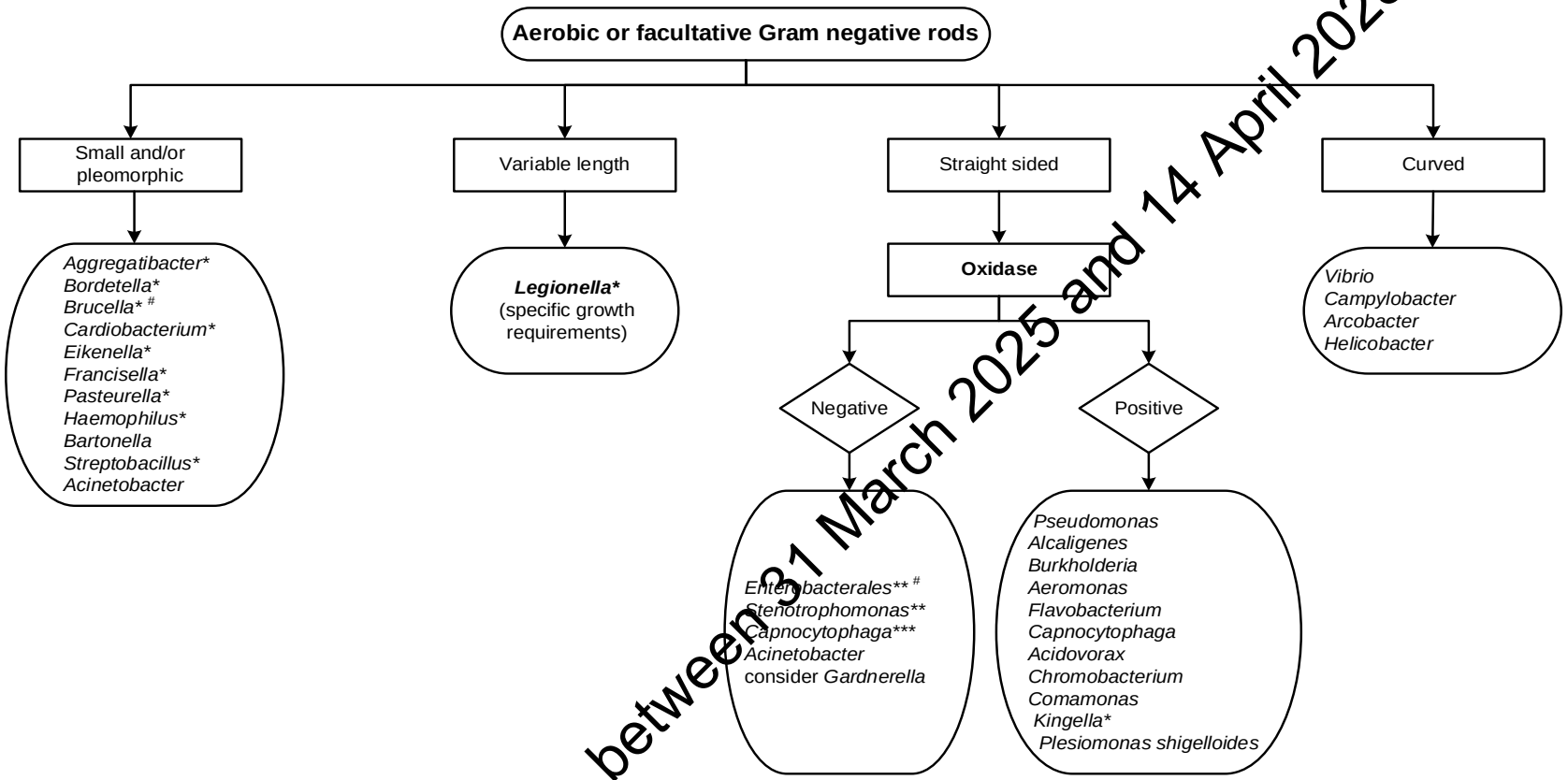
The flowchart is for guidance only (63,78,79).

Algorithm 4: Characteristics of Gram negative bacteria



The flowchart is for guidance only (78,80).

Algorithm 5: Characteristics of Gram negative bacteria



***** Some species may be anaerobic

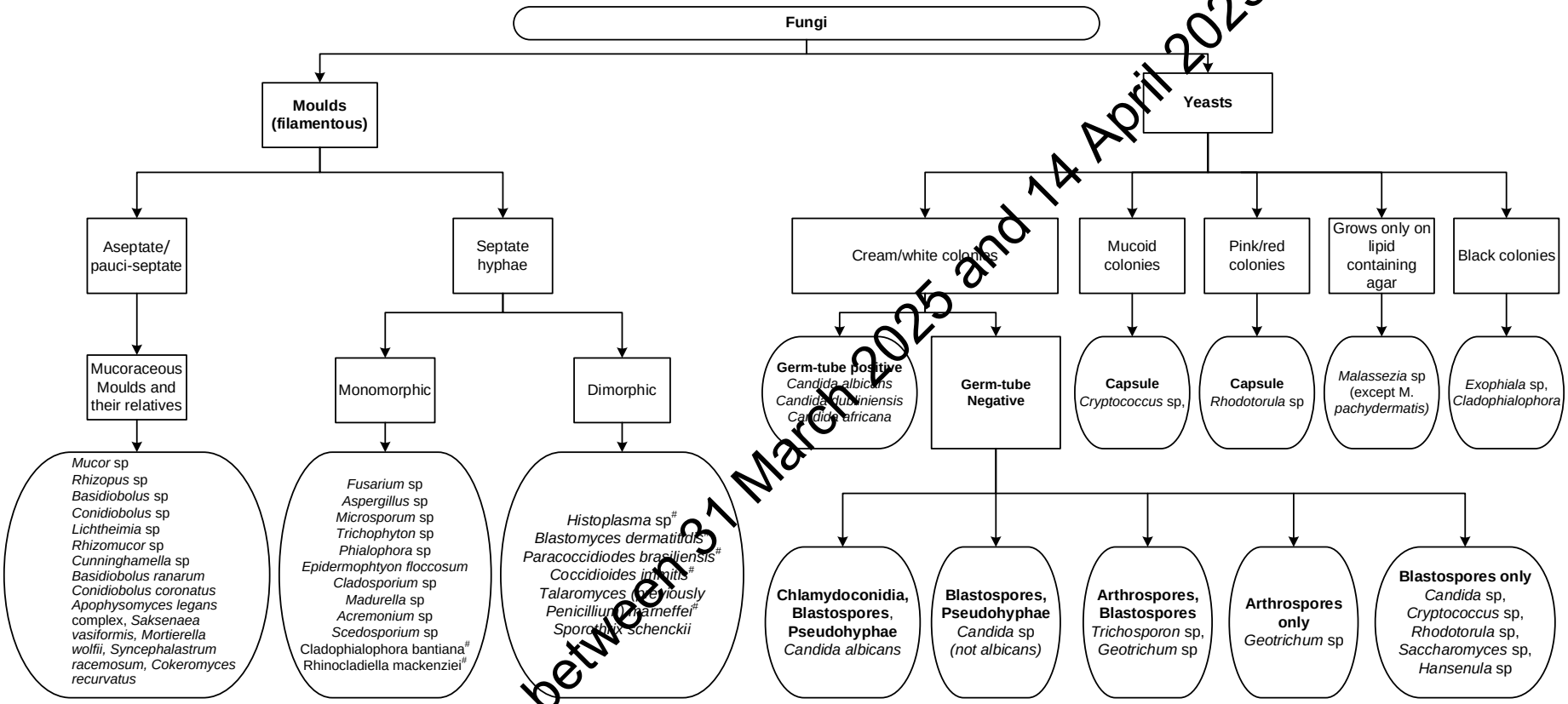
****** May be weak catalase positive

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

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 (6,7,40,45,51).

Appendix: List of revised fungal taxa mentioned in this document

Previous species name	Revised species name (7)
Candida krusei	*Pichia kudriavzevii*
*Candida glabrata (*for full list of *Candida* species see reference(7))	*Nakaseomyces glabratus*
Candida guilliermondii	*Meyerozyma guilliermondii*
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*

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

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