Public Health England

UK Standards for Microbiology Investigations

Review of users' comments received by Working group for microbiology standards in clinical bacteriology

ID 01 Introduction to the preliminary identification of medically important bacteria and fungi from culture

"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011**."

This publication was created by Public Health England (PHE) in partnership with the NHS.
Recommendations are listed as ACCEPT/ PARTIAL ACCEPT/DEFER/ NONE or PENDING

© Crown copyright 2021

Consultation: 23/04/2021 – 07/05/2021

Version of document consulted on dk+

Proposal for changes

1. Section for comments: 4.1 Taxonomy and characteristics

Comment number: 1

Date received: 06/05/2021
Laboratory/organisation name: UKCMN

a) ability to ferment certain sugars such as catalase and oxidase tests.
Comment: This sentence is incorrect: catalase and oxidase are not examples for sugar fermentation tests

Recommended action

a) ACCEPTED: this has been updated in the document

Comment number: 2

Date received: 13/05/2021
Laboratory/organisation name: IBMS

a) The document highlights fungi in brackets but this may also apply to bacteria. It is not clear why Fungi is in brackets
b) Catalase and oxidase are not sugar reactions. This should be amended to biochemical tests.
c) Line 5 there is no mention of Lactofuchsin (? Check on the use of lactoPHENOL cotton blue).

Recommended action

a) ACCEPTED: brackets around word 'fungi' removed
b) ACCEPTED: this has been updated in the document
c) ACCEPTED: sentence amended and mention of lacto-fuchsin added to section 8

2. Section for comments: 4.2 Principles of identification

Comment number: 3

Date received: 06/05/2021
Laboratory/organisation name: UKCMN

a) Identification of bacteria and fungemia: these conditions are 'detected' and the causative agents are 'identified'
b) fungaemia spelling

Recommended action

a) ACCEPTED: this has been updated in the document

b) ACCEPTED: this has been updated in the document

Comment number: 4

Date received: 13/05/2021
Laboratory/organisation name: IBMS

a) Line 2 does not make sense; words appear to be missing from sentence.
b) The IBMS is unsure of the point of the first sentence why is fungaemia the most important and complex role
c) Line 5 extra and in the sentence.
d) Paragraph 2. Not true for moulds, although yeasts can be identified using commercial kits.
e) Paragraph 3. A step by step approach should be taken for the identification of any organism. The final method follows a step-by-step approach to identification. Fundamental characteristics of the organism are determined by primary identification tests such as a Gram stain, oxidase or catalase. Results of these tests indicate secondary or even tertiary tests to confirm the identity of the subject. This is a systematic approach and does not rely on the expertise of the investigator Comment: The IBMS would disagree with the highlighted sentence. Expertise is required to understand the test outcome This is a systematic approach and does not rely on the expertise of the investigator. It is the view of the Institute of Biomedical Science that the difficulties with Section 4 revolve around a general approach to identification, which is not necessarily right for fungi.

Recommended action

a) ACCEPETD: this has been updated in the document
b) ACCEPTED: this has been updated in the document
c) ACCEPTED: extra ‘and’ removed
d) NONE: sentence says ‘commercial identification systems’, MALDI-TOF MS can identify moulds
e) ACCEPTED: text in section 4 amended where necessary

3. Section for comments: 5 Technical information and limitations

Comment number: 5

Date received: 06/05/2021
Laboratory/organisation name: UKCMN

a) Colonial morphology: and different temperatures (for fungi)
b) Cultural technique: So, this is not a problem for fungi?

Recommended action
a) ACCEPTED: words ‘different temperatures’ added
b) ACCEPTED: this has been updated in the document

Comment number: 6

Date received: 13/05/2021

Laboratory/organisation name: IBMS

a) Colonial Morphology - Identification of moulds- Isolates should be sub-cultured on to the routine media (usually Sabourauds dextrose agar with chloramphenicol), utilised in an individual laboratory. This is so that the staff get used to the morphology of the moulds on their own agar. Sub-culturing often induces the mould to produce sporing structures with which they can be identified. Identification should be made on microscopic examination for typical sporing structures.
b) Microscopic and morphological identification is not necessarily definitive, because of the now recognised species complexes.
c) Culture Technique - The construction of this sentence should be reviewed. The current structure is ambiguous in its meaning.
d) Germ Tube Test - There is no mention of the fact that a distinction is required between C.albicans, C.africana and C.dubliniensis , which are all clinically significant yeasts. (C.tropicalis produces true hyphae).
e) MALDI-ToF For yeasts, the colonial morphology is not always helpful. Whereas their microscopic characteristics, seen directly on Cornmeal tween agar with a cover slip placed on an inoculum streak, is extremely useful. The use of chromogenic media to identify mixtures is also useful as the MALDI-ToF will give unreliable results in this situation.

Recommended action

a) NONE: paragraph amended where necessary
b) ACCEPTED: this has been updated in the document
c) ACCEPTED: this has been updated in the document
d) NONE: further information on 'Germ tube test' can be found in section 8.6
e) ACCEPTED: this has been updated in the document

4. Section for comments: 8.1 Microscopic appearance

Comment number: 7

Date received: 06/05/2021
Laboratory/organisation name: UKCMN

a) presence of intracellular inclusions, for example spores: endospore is not an intracellular inclusion. Also visualising these requires special staining
b) Gram positive and the Gram negative bacteria: What about Gram-variables?
c) saline mount: saline is not a stain!
d) calcofluor white with 10% KOH and India ink: CFW with KOH and India ink are only used for direct examination of clinical samples and not identification of fungal isolates in culture.
e) Czapek-Dox: for yeasts?
f) Dalmau technique: Do we expect NHS laboratories to set up Dalmau agar? Is this realistic?
g) Table 2 Indian: India ink NOT Indian ink

Recommended action

a) ACCEPTED: this has been updated in the document
b) ACCEPTED: this has been updated in the document
c) ACCEPTED: this has been updated in the document
d) ACCEPTED: this has been updated in the document

e) ACCEPTED: this has been updated in the document
f) ACCEPTED: sentence amended to say Dalmau technique 'may be' rather than 'is used'.
g) ACCEPTED: this has been updated in the document

Comment number: 8

Date received: 13/05/2021
Laboratory/organisation name: IBMS

a) Fungi - The use of double-sided sticky tape is better. A drop of lactophenol cotton blue or Lactofuchsin is put on to a microscope slide; the tape is placed on the drop of stain (fungus side UP); a further drop of stain is put on the fungus; a coverslip is placed on top. Then examined microscopically.
b) Microscope preparations from suspected HG3 fungi MUST be made using needle mounts in a Class 1 microbiological safety cabinet (MSC) and sealed (e.g. with nail varnish) and wiped with disinfectant, before removal from the MSC for microscope examination.
c) Paragraph 3. There is no mention of Lactofuschsin as a stain.

Recommended action
a) ACCEPTED: this has been updated in the document
b) ACCEPTED: this has been updated in the document
c) ACCEPTED: mention of Lacto-fuchsin added

5. Section for comments: 8.2 Cultural appearance

Comment number: 9

Date received: 06/05/2021
Laboratory/organisation name: UKCMN

a) odour: odour of colony for fungal culture?
b) 1 week to 4: This is an average, cultures may be kept for up to 8 weeks if there is clinical indication (Ref: McGinnis, Laboratory Handbook of Medical Mycology)
c) longer growth: This sentence does not make sense: what is longer growth?
d) 4 to 6 weeks before being regarded as negative: This general statement is problematic: 4-6 weeks incubation for what sample types? Superficial samples for dermatophytes? sterile tissue/fluid samples for dimorphics? respiratory samples in CF? All samples??
e) Pseudomonas: Is this correct? Chromogen agar for Pseudomonas?
f) identification: not just identification, chromogenic media also allows to detect mixed yeast cultures

Recommended action
a) ACCEPTED: word 'odour' removed
b) ACCEPTED: this has been updated in the document
c) ACCPTED: this has been updated in the document
d) ACCEPTED: this has been updated in the document
e) NONE: *Pseudomonas* can be isolated using chromogenic agar
f) ACCEPTED: this has been updated in the document

Comment number: 10

Date received: 13/05/2021
Laboratory/organisation name: IBMS

a) Fungi - Although the information is acceptable and growth rate can be used as an aid to mould identification, this paragraph is more about the culturing of the organisms from clinical samples rather than identification per se.
b) Line 6. Grammar needs checking.
c) An incorrect unit of measurement is used. It should be µm not mm
d) Culture media Page 12, paragraph 2, line 8 Although the use of chromogenic media is good for screening yeast species and can give a presumptive identification, the IBMS would not promote its use for direct identification of yeast species. Its use is in the detection of mixed cultures.
e) Line 8. test should be pleural tests
f) The IBMS would state a positive fungal microscopy is more important in the diagnosis of fungal infection than fungal culture. It is not unusual to grow environmental contaminants from clinical samples also, in the instance where fungi are not grown, as is the case in some dermatology samples, a positive microscopy is diagnostic for a fungal infection.
g) This paragraph is not directly related to identification.

Recommended action

a) NONE: paragraph amended where appropriate
b) ACCEPTED: sentence amended
c) ACCEPTED: µm replaced with mm for *Streptococcus* species
d) ACCEPTED: word 'direct' removed
e) ACCEPTED: this has been updated in the document
f) ACCEPTED: this has been updated in the document
g) ACCEPTED: this has been updated in the document

6. Section for comments: 8.3 Growth requirements

Comment number: 11

Date received: 06/05/2021
Laboratory/organisation name: UKCMN

a) Microorganisms can be grouped on the basis of their growth requirements. They are as follows: Generally speaking, these are relevant for bacteria and not fungi.

Recommended action

a) ACCEPTED: this has been updated in the document

7. Section for comments: 8.6 Tests for fungi

Comment number: 12

Date received: 06/05/2021
Laboratory/organisation name: UKCMN

a) What is a 'targeted' panfungal PCR? This means there is also 'non-targeted' panfungal PCR! It may refer to species-specific PCR?
b) identification of fungal diseases: Does this refer to cryptococcal antigen test? If so, why just latext and not LFA?
c) Germ tube test: In addition, Germ tube test should be carried out on a single isolated colony.
d) Candida in italic
e) C. neoformans: C. neoformans / C. gattii complex
f) slide culture preparations: We are giving the message that for identification of dermatophytes we need slide cultures! This is not realistic for routine use...

Recommended action

a) ACCEPTED: this has been updated in the document
b) ACCEPTED: mention of 'lateral flow assay' added
c) ACCEPTED: this has been updated in the document
d) ACCEPTED: this has been updated in the document
e) ACCEPTED: this has been updated in the document
f) ACCEPTED: word 'slide' removed

Comment number: 13

Date received: 13/05/2021
Laboratory/organisation name: IBMS

a) Diagnostic tests - If the SMI is directed at identification, then the inclusion of other antigen and antibody detection tests is required.

Recommended action

a) ACCEPTED: this has been updated in the document

8. Section for comments: 8.7 Rapid identification methods

Comment number: 14

Date received: 06/05/2021
Laboratory/organisation name: UKCMN

a) MALDI-TOF MS: replace fungi with fungal morphology
b) Cryptococcus, Saccharomyces and Trichosporon: Genera and species must be in italic throughout
c) Note: If a commercial yeast identification kit provides a biochemical profile but also specifies examination of key morphological features to obtain a numerical profile it is not sufficient to identify the organism based only on the biochemical profile. Comment: This needs to be re-written. It's not clear.
d) pulsed-field gel electrophoresis, Multi-locus: These are typing tools and not used for identification.
e) sequence typing, multiple-locus variable-number tandem-repeat analysis also known as VNTR, 16S rDNA sequencing and 18S rRNA sequencing: 18S rRNA sequencing? Does this refer to ITS and D1/2 for identification? The first sentence

refers to molecular methods refers to detection and then real-time PCR. We have to be clear about these terms: panfungal PCR, 18S PCR, real-time PCR, etc.
f) When combined with reverse transcription (RT) real-time PCR is the preferred method also for the detection and quantification of RNA. Benefits of this procedure over conventional methods include sensitivity, enhanced specificity, and the potential for high throughout as well as accurate quantification: What is the relevance of this? Reverse transcriptase for bacterial/fungal detection?
g) intergenic: internal
h) method: methods

Recommended action

a) ACCEPTED: this has been updated in the document
b) ACCEPTED: this has been updated in the document
c) ACCEPTED: this has been updated in the document
d) ACCEPTED: this has been updated in the document
e) ACCEPTED: this has been updated in the document
f) ACCEPTED: information on reverse transcription real-time PCR removed
g) ACCEPTED: this has been updated in the document
h) ACCEPTED: plural of method added

Comment number: 15

Date received: 13/05/2021
Laboratory/organisation name: IBMS

a) These improve turnaround times, not increase them as indicated in document.
b) Line 6 compare should be compared.
c) Paragraphs 1 and 2 should be checked for grammar as there are a number of apparent errors.
d) Second paragraph loses some coherence.
e) Whole Genome Sequencing (WGS) page 17 - This may benefit from using some of the newer and more UK centric references.
f) Appendix 5 Characterisation of fungi - Yeasts: Consensus opinion is that after Yeast (unicellular), the line should go to three boxes showing a systematic way of identifying yeast species morphologically. (Germ tube could be an off shoot): True Hyphae - Pseudo Hyphae - No Hyphae-Arthrospore -Chlamydospores- arrangement of blastospores-capsule
g) Moulds: there is no mention of the HG3 NON dimorphic species as a warning: e.g. Cladophialophora bantiana, Rhinocladiella mackenzii. Other HG3 species could be included.

Recommended action

a) ACCEPTED: this has been updated in the document
b) ACCEPTED: this has been updated in the document
c) ACCEPTED: this has been updated in the document
d) ACCEPTED: this has been updated in the document
e) ACCEPTED: this has been updated in the document
f) NONE: Appendix 5 flowchart has been significantly revised
g) ACCEPTED: some example of HG3 non dimorphic species added.

9. Section for comments: 9.9 Appendix 5: Characteristics of fungi

Comment number: 16

Date received: 06/05/2021
Laboratory/organisation name: UKCMN

a) Appendix 5: Can Trichosporon, Geotrichum and Saprochaete classified as 'unicellular'?
b) Minute colonies: Many species do not grow on SAB agar
c)Aseptate: or pauci-septate
d) Pseudohyphae or: budding yeast cells and pseudohyphae can be present simultaneously therefore, OR is not technically correct: and/or should be used.
e) Cryptococcus species: Putting Crypto with Candida and Saccharomyces will cause problem. Although urease test was mentioned in the test, it needs to be included in this chart too.
f) Comment: Geotrichum?

Recommended action

a) NONE: Appendix 5 flowchart has been significantly revised
b) ACCEPTED: box for the 'minute colonies' removed
c) ACCEPTED: this has been updated in the document
d) ACCEPTED: this has been updated in the document
e) NONE: Appendix 5 flowchart has been significantly revised
f) NONE: Appendix 5 flowchart has been significantly revised

10. General comments

Comment number: 17

Date received: 13/05/2021
Laboratory/organisation name: IBMS

a) Some of the references may require updating as a number of them are greater than eight years old.
b) A decision should be taken whether to use the new names for the fungi along with their previous names to keep the document up to date.
c) It can be confusing to try and write about bacteria and fungi together; it is recommended that they should have their own sections.
d) The way in which moulds are identified is different from that of yeasts resulting in the text being disjointed in places.

Recommended action

a) ACCEPTED: references updated where necessary.
b) Ask BWG members
c) NONE: for detailed microscopical, cultural morphology and tests both fungi and bacteria have their own sections.

d) NONE: identification of yeast and moulds are very different from one another hence why some text seems disjointed.

Section for comments: Financial barriers

Respondents were asked "are there any potential organisational and financial barriers in applying the recommendations or conflict of interest?".

Comment number: 18

Date received: 03/05/2021
Laboratory/organisation name: Nursing and Midwifery Council UK

None

Comment number: 19

Date received: 06/05/2021
Laboratory/organisation name: UKCMN

No response given

Comment number: 20

Date received: 13/05/2021
Laboratory/organisation name: IBMS

No response given

Section for comments: Health benefits

Respondents were asked "are you aware of any health benefits, side effects and risks that might affect the development of this UK SMI?".

Comment number: 21

Date received: 03/05/2021
Laboratory/organisation name: Nursing and Midwifery Council UK

None

Comment number: 22

Date received: 06/05/2021
Laboratory/organisation name: UKCMN

No response given

Comment number: 23

Date received: 13/05/2021
Laboratory/organisation name: IBMS

No response given

Section for comments: Interested parties

Respondents were asked “are you aware of any interested parties we should consider consulting with on the development of this document

Comment number: 24

Date received: 03/05/2021
Laboratory/organisation name: Nursing and Midwifery Council UK

None

Comment number: 25

Date received: 06/05/2021
Laboratory/organisation name: UKCMN

No response given

Comment number: 26

Date received: 13/05/2021
Laboratory/organisation name: IBMS

No response given

Respondents indicating they were happy with the contents of the document

Overall number of comments: 1

Date received: 13/05/2021
Laboratory/organisation name: IBMS

Date received	03/05/2021	**Lab name/Professional body (delete as applicable)**	Nursing and Midwifery Council UK