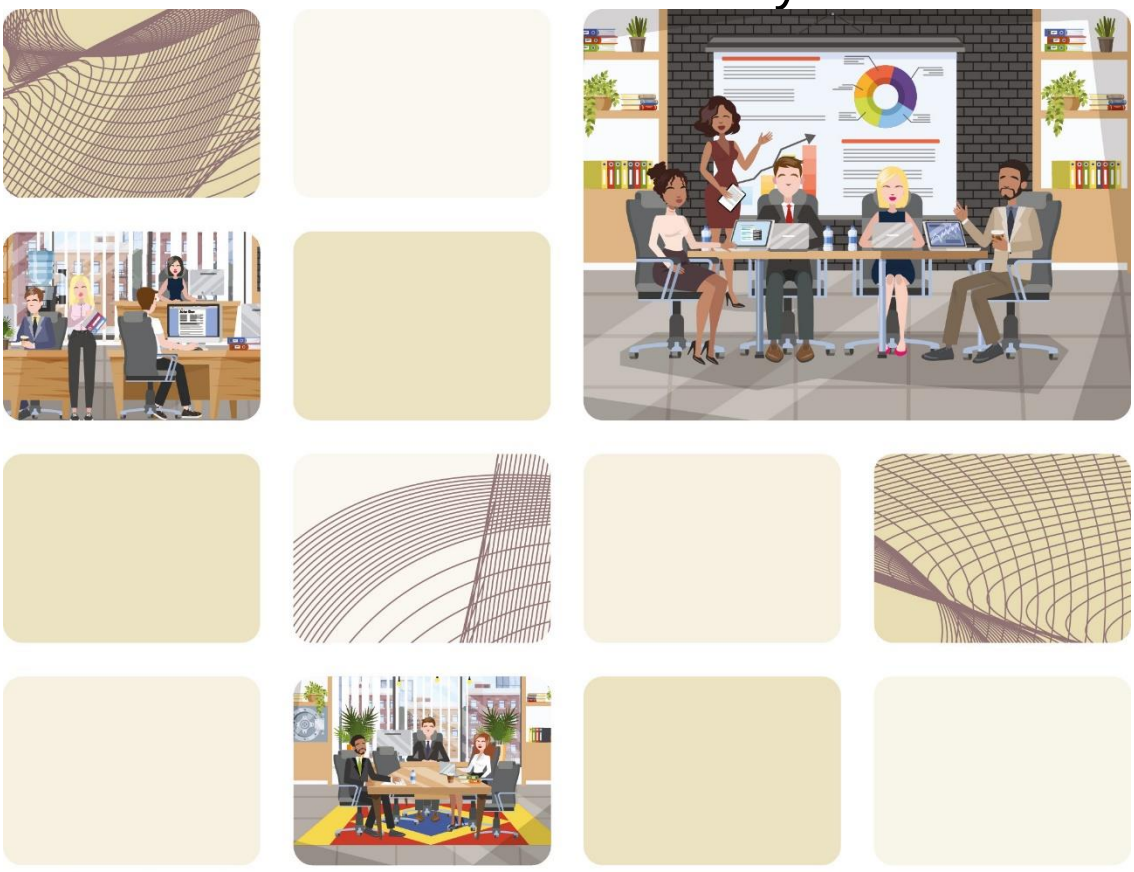
UK Health Security Agency

UK Standards for Microbiology Investigations

Review of users' comments received by Working group for bacteriological identification and test procedures

UK SMI ID 2 Identification of Corynebacterium species



National Institute for Health and Care Excellence (NICE) has renewed accreditation of the process used by the **UK Health Security Agency** to produce **UK Standards for Microbiology Investigations (UK SMIs)**. The renewed accreditation is valid until **30 June 2026** and applies to guidance produced using the processes described in '**UK Standards for Microbiology Investigations Development Process' (2021)**. The original accreditation term began on 1 July 2011.

This publication was created by UK Health Security Agency (UKHSA) in partnership with the partner organisations.

Recommendations are listed as ACCEPT/ PARTIAL ACCEPT/DEFER/ NONE or PENDING

Issued by the Standards Unit, Specialised Microbiology and Laboratories, UKHSA

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Consultation: 09/03/2022 – 23/03/2022
Version of document consulted on: do+

3 Scope of document

Comment number: 1

Date received: 16/03/2022
Laboratory or organisation name: Public Health England - now UK Health Security Agency

The importance of toxin production by this specie in the pathogenesis of disease is emphasised.
typo - Should say 'species'

Recommended action

ACCEPT: correction made.

4.1 Taxonomy and characteristics

Comment number: 2

Date received: 16/03/2022
Laboratory or organisation name: Public Health England - now UK Health Security Agency

Paragraph 2 - These species may produce diptheria toxin and cause fatal disease.
typo - should say 'diphtheria'

Recommended action

ACCEPT: correction made.

Comment number: 3

Date received: 16/03/2022
Laboratory or organisation name: Public Health England - now UK Health Security Agency

Paragraph 3 - They are arranged together in a characteristic way, which has been described as the form of a 'V', 'palisades'. they can also be described as 'Chinese letter pattern or cuniform'

https://paramedicsworld.com/corynebacterium-diphtheriae/morphology-culture-characteristics-of-corynebacterium-diphtheriae-diphtheria-bacillus/medical-paramedical-studynotes#:~:text=Arrangement%20Of%20Cells%20%E2%80%93%20Corynebacteri

um%20diphtheriae%20is%20arranged,daughter%20cells%20after%20cell%20division%20%28by%20Binary%20fission%29

Recommended action

PARTIAL ACCEPT: text has been amended following working group discussion.

4.2 Principles of identification

Comment number: 4

Date received: 16/03/2022
Laboratory or organisation name: Public Health England - now UK Health Security Agency

Paragraph 3 - abscess or purulent sputum, from more than one blood culture set or present at greater than or equal to104 cfu/mL needs a space between 'to 104'

Recommended action

ACCEPT: correction made.

General Comments

Comment number: 5

Date received: 16/03/2022
Laboratory or organisation name: PHW Microbiology Cardiff

The SMI mentions urine- should C.urealyticum be included in the SMI

Recommended action

PARTIAL ACCEPT: *C. urealyticum* is not a clinically significant target organism to look for routinely, hence it has been added to the background and not as a target organism

Comment number: 6

Date received: 16/03/2022
Laboratory or organisation name: Reference laboratory UK Health Security Agency

Spelling mistake ‘specie’ also clearer if emphasize the three potentially toxigenic specie

Recommended action

ACCEPT: correction made.

Comment number: 7

Date received: 23/03/2022
Laboratory or organisation name: UK Health Security Agency

Currently: The importance of toxin production by this specie in the pathogenesis of disease is emphasised.

Propose: The importance of toxin production by these three species in the pathogenesis of disease is emphasised.

Recommended action

ACCEPT: Amendment made.

Comment number: 8

Date received: 23/03/2022
Laboratory or organisation name: UK Health Security Agency

Not clear what is meant by pathogenic Corynebacterium here ie which species, better to state that the 3 potentially toxigenic species must be set to the ref lab without dely.

Currently: This UK SMI covers 4 tests for the preliminary identification of pathogenic Corynebacterium species and recommends that the organism is sent to a reference laboratory for confirmation of identification and toxin testing if required.

Propose: This UK SMI covers 4 tests for the preliminary identification of pathogenic Corynebacterium species and recommends that putative C. diphtheriae, C. ulcerans and C. pseudotuberculosis isolates are sent promptly to the diphtheria reference laboratory for confirmation of identification and toxin testing.

Recommended action

ACCEPT: Amendment made.

Comment number: 9

Date received: 23/03/2022
Laboratory or organisation name: UK Health Security Agency

In what sense has pathogenic C. diphtheriae been isolated from cats and dogs? Where are the citations for this? Not aware of any toxigenic C.dip reports from companion animals.

Toxigenic C. diphtheriae has been isolated from horse wounds and there are citations for this eg,

Leggett BA, De Zoysa A, Abbott YE, Leonard N, Markey B, Efstratiou A. Toxigenic Corynebacterium diphtheriae isolated from a wound in a horse. Vet Rec. 2010 May 22;166(21):656-7. doi: 10.1136/vr.b4846. PMID: 20495169.
Henricson B, Segarra M, Garvin J, Burns J, Jenkins S, Kim C, et al. Toxigenic Corynebacterium diphtheriae associated with an equine wound infection. J Vet Diagn Invest. 2000;12:253–7.

But I am not aware of any citations for isolation of toxigenic (ie diphtheria toxin expressing) C. diphtheriae from cats or dogs.
I am aware of the isolation of a non-toxigenic toxin gene-bearing (NTTB) C. diphtheriae cat Belgium
Zakikhany K, Neal S, Efstratiou A. Emergence and molecular characterisation of non-toxigenic tox gene-bearing Corynebacterium diphtheriae biovar mitis in the United Kingdom, 2003-2012. Euro Surveill. 2014 Jun 5;19(22):20819. doi: 10.2807/1560-7917.es2014.19.22.20819. PMID: 24925458.

And this but this is also non-toxigenic
Hall AJ, Cassiday PK, Bernard KA, Bolt F, Steigerwalt AG, Bixler D, Pawloski LC, Whitney AM, Iwaki M, Baldwin A, Dowson CG, Komiya T, Takahashi M, Hinrikson HP, Tondella ML. Novel Corynebacterium diphtheriae in domestic cats. Emerg Infect Dis. 2010 Apr;16(4):688-91. doi: 10.3201/eid1604.091107. PMID: 20350389; PMCID: PMC3321947.

Recommended action

ACCEPT: Line removed.

Comment number: 10

Date received: 23/03/2022
Laboratory or organisation name: UK Health Security Agency

The following sentence just repeats itself - this would be an opportunity to mention the most widely used such media ie Hoyle’s medium

Currently: Agar containing blood and potassium tellurite, such as blood tellurite medium

Propose: Agar containing blood and potassium tellurite, such as Hoyle’s medium

Recommended action

ACCEPT: amendment made.

Comment number: 11

Date received: 23/03/2022
Laboratory or organisation name: UK Health Security Agency

Currently: Colonies on modified Tinsdale agar are 1 to 2 mm, black or charcoal grey and have a brown-black halo visible in the agar. This is because the organism produces cysteinas, which reacts with the cysteine in the medium
Propose: Modified Tinsdale agar is another selective and differential medium that contains both tellurite selective, L-Cystine and sodium thiosulphate. Colonies on modified Tinsdale agar are 1 to 2 mm, black or charcoal grey and have a brown-black halo visible in the agar due to cysteinase activity.

Recommended action

ACCEPT: amendment made.

Comment number: 12

Date received: 23/03/2022
Laboratory or organisation name: UK Health Security Agency

Only *C. diphtheriae*, *C. ulcerans* and *C. pseudotuberculosis* give black colonies with distinctly outlined halo. Other corynebacteria, staphylococci, and some streptococci may also reduce tellurite, although halos around the colonies are not generally present.
The SMI should reference the latest/recent WHO laboratory manual for the diagnosis of diphtheria and other related infections. Now online in IRIS at:
https://apps.who.int/iris/bitstream/handle/10665/352275/9789240038059-eng.pdf
For Biblio (item description):
https://apps.who.int/iris/handle/10665/352275
World Health Organization. (2021). WHO laboratory manual for the diagnosis of diphtheria and other related infections. World Health Organization. https://apps.who.int/iris/handle/10665/352275. License: CC BY-NC-SA 3.0 IGO

Recommended action

ACCEPT: document updated, references now include WHO laboratory manual.

Comment number: 13

Date received: 23/03/2022
Laboratory or organisation name: UK Health Security Agency

In the taxonomy section the recently proposals for strains belonging to biovar belfanti be considered a new species (Corynebacterium belfantii sp. nov.) due to their differences from C. diphtheriae biovar mitis and gravis (Dazas et al. 2018).

Corynebacterium belfantii sp. nov. appears synonymous with Corynebacterium rouxii sp. nov. described by another group (Schlez et al 2021)

Dazas M, Badell E, Carmi-Leroy A, Criscuolo A, Brisse S. Taxonomic status of Corynebacterium diphtheriae biovar Belfanti and proposal of Corynebacterium belfantii

sp. nov. Int J Syst Evol Microbiol. 2018 Dec;68(12):3826-3831. doi: 10.1099/ijsem.0.003069. Epub 2018 Oct 24. PMID: 30355399.

Schlez K, Eisenberg T, Rau J, Dubielzig S, Kornmayer M, Wolf G, Berger A, Dangel A, Hoffmann C, Ewers C, Sing A. Corynebacterium rouxii, a recently described member of the C. diphtheriae group isolated from three dogs with ulcerative skin lesions. Antonie Van Leeuwenhoek. 2021 Sep;114(9):1361-1371. doi: 10.1007/s10482-021-01605-8. Epub 2021 Jun 25. PMID: 34170418; PMCID: PMC8379122.

Recommended action

PARTIAL ACCEPT: the proposal to designated biovar Belfanti as a novel species of *Corynebacterium* has been mentioned in the document

Comment number: 14

Date received: 23/03/2022
Laboratory or organisation name: UK Health Security Agency

In the section on C. diphtheriae would be worth mentioning transmission is rare but has been reported in UK in 2017.

Edwards D, Kent D, Lester C, Brown CS, Murphy ME, Brown NM, Sule O, Itani A, Chand M, Trindall A, Pearson C, Roddick I, Fry NK, Hoffmann J, Iyanger N, Kemp L, White J, Javid B, Ramsay ID, Zenner D, Ahmed A, Amirthalingam G, Salimee S, Litt D, Reacher M. Transmission of toxigenic Corynebacterium diphtheriae by a fully immunised resident returning from a visit to West Africa, United Kingdom, 2017. Euro Surveill. 2018 Sep;23(39):1700681. doi: 10.2807/1560-7917.ES.2018.23.39.1700681. Erratum in: Euro Surveill. 2018 Oct;23(42): PMID: 30280689; PMCID: PMC6169202.

Recommended action

NONE: This is effectively covered by recent (2022 and 2023) references from UKHSA that have now been added to the document

Comment number: 15

Date received: 23/03/2022
Laboratory or organisation name: UK Health Security Agency

The epidemiology of C. dip and C. ulc has changed in the UK

It would be good to cite this reference

Gower CM, Scobie A, Fry NK, Litt DJ, Cameron JC, Chand MA, Brown CS, Collins S, White JM, Ramsay ME, Amirthalingam G. The changing epidemiology of diphtheria in the United Kingdom, 2009 to 2017. Euro Surveill. 2020 Mar;25(11):1900462. doi:

10.2807/1560-7917.ES.2020.25.11.1900462. PMID: 32209165; PMCID: PMC7096772.

And also to note that there is now a national incident declared for diphtheria by UKHSA due to rise in cases Briefing Note and Updated UKHSA Diphtheria guidance due to come out in next weeks.

Recommended action

PARTIAL ACCEPT: reference to recent epidemiology and the national incident is effectively covered by recent (2022 and 2023) references from UKHSA that have now been added to the document

Comment number: 16

Date received: 23/03/2022
Laboratory or organisation name: UK Health Security Agency

It would be helpful to state the risk factors for toxigenic C. diphtheriae /C.ulcerans/ (C. pseudotuberculosis)

- C.dip: travel to endemic country
- C.dip/C.ulc/C.pseudo: absent/incomplete/unknown immunisation
- Laboratory worker (healthcare scientist)
- C.ulc: raw milk/ products; rural contacts; cattle; horses; companion animals (dogs and cats)

Recommended action

PARTIAL ACCEPT: Risk factors are briefly mentioned in the Reporting section under “Infection Specialist”. See also UK SMI B 9 Investigation of throat swabs

Comment number: 17

Date received: 23/03/2022
Laboratory or organisation name: UK Health Security Agency

Currently: Possible toxigenic strains of Corynebacterium species should be referred to the Reference Laboratory for detection of toxin production as soon as possilbe.

Suggest: Potentially toxigenic strains from the three species C. diphtheriae, C. ulcerans and C. pseudotuberculosis should be referred to the Diphtheria Reference Laboratory for detection of toxigenicity test as soon as possible
(and provide link to guidance)
https://www.gov.uk/government/collections/diphtheria-guidance-data-and-analysis

Recommended action

ACCEPT: amendment made.

Comment number: 18

Date received: 23/03/2022
Laboratory or organisation name: UK Health Security Agency

Safety considerations should refer specifically to immunisation of laboratory and pathology staff as recommended by the Green book and reference the latest version of Chapter 12 of the Green Book (Immunisation of healthcare and laboratory staff)

https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/147882/Green-Book-Chapter-12.pdf

the exceptions should be checked/updated with the UKHSA Immunisation Lead or deleted.

Recommended action

ACCEPT: Reference has been added and wording adopted directly from the Green Book.

Comment number: 19

Date received: 23/03/2022
Laboratory or organisation name: UK Health Security Agency

Target organisms: it would be better to just state the three species here and omit mention of the C. dip biovars. The evidence is that biovar belfanti is non-toxigenic but we do not wish to prevent labs sending any C.diphtheriae to the ref lab for checking.

Recommended action

ACCEPT: document has been updated in line with this comment

Comment number: 20

Date received: 23/03/2022
Laboratory or organisation name: UK Health Security Agency

8.4.3 MALDI-TOF ID: it would be worth checking with Julie Logan at BIDS UKHSA Colindale about current interpretation/guidance for C.dip/C.ulc/C.pseudo. We much prefer MALDI-TOF ID to biochemical. I believe >2.3 is good ID; but for C.ulc/C.pseudo I do not believe the current MALDI Bruker can differentiate between these two species. Whilst highly likely to be C.ulc and C.pseudo rare they should be sent to ref lab and its useful for us if they quote their MALDI score. However, there are other platforms with different scoring eg I believe biomerieux use %.

Recommended action

PARTIAL ACCEPT: use of MALDI-TOF has been briefly mentioned in the document scope.

Comment number: 21

Date received: 23/03/2022
Laboratory or organisation name: UK Health Security Agency

8.4.4 This section needs updating and its not just C.dip

De Zoysa A, Efstratiou A, Mann G, Harrison TG, Fry NK. Development, validation and implementation of a quadruplex real-time PCR assay for identification of potentially toxigenic corynebacteria. J Med Microbiol. 2016 Dec;65(12):1521-1527. doi: 10.1099/jmm.0.000382. Epub 2016 Nov 1. PMID: 27902437.

There is no evidence of revision from NTTB to toxigenicity (we have looked over several years. Fry NK, unpublished data). It is considered highly unlikely.

Recommended action

ACCEPT: Amendment made

Comment number: 22

Date received: 23/03/2022
Laboratory or organisation name: UK Health Security Agency

The following sentence will be at variance to the forthcoming UKHSA guidance and should be deleted: Molecular characterization based on polymerase chain reaction (PCR) of some of the non-toxigenic strains has demonstrated that the bacteria often contain functional dtxR proteins, which could potentially produce toxin (43).
(please delete)

Recommended action

ACCEPT: Amendment made.

Comment number: 23

Date received: 23/03/2022
Laboratory or organisation name: UK Health Security Agency

8.5 16S rRNA gene sequence analysis is not a typing method and mention of this should be deleted. This section should mention only MLST, core gene MLST, WGS and should refer to the Institut Pasteur BIGSdb-Pasteur databases at https://bigsdb.pasteur.fr/diphtheria/

Recommended action

PARTIAL ACCEPT: 16 rRNA is now covered in its own section, rather than under the typing section.

Comment number: 24

Date received: 23/03/2022
Laboratory or organisation name: UK Health Security Agency

The AFLP section should be deleted - this is not performed any more.

Recommended action

ACCEPT: Amendment made.

Comment number: 25

Date received: 23/03/2022
Laboratory or organisation name: UK Health Security Agency

The 16S rRNA gene analysis section should be deleted As above this is not typing. This section is now outdated due to WGS.

Recommended action

NONE: 16S rRNA gene analysis is now covered in its own section, rather than under the typing section.

Comment number: 26

Date received: 23/03/2022
Laboratory or organisation name: UK Health Security Agency

8.6 to the diphtheria reference laboratory

Recommended action

ACCEPT: Amendment made

Financial barriers

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

Comment number: 27

Date received: 16/03/2022
Laboratory or organisation name: Public Health England - now UK Health Security Agency

No

Recommended action

NONE

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: 28

Date received: 16/03/2022
Laboratory or organisation name: Public Health England - now UK Health Security Agency

No

Recommended action

NONE

Comment number: 29

Date received: 16/03/2022
Laboratory or organisation name: PHW Microbiology Cardiff

No

Recommended action

NONE

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: 30

Date received: 16/03/2022
Laboratory or organisation name: Public Health England - now UK Health Security Agency
No

Recommended action

NONE

Comment number: 31

Date received: 16/03/2022
Laboratory or organisation name: PHW Microbiology Cardiff

No

Recommended action

NONE

Respondents indicating they were happy with the contents of the document

Overall number of comments:			
Date received	22/03/2022	**Lab name/Professional body (delete as applicable)**	BSAC
Health benefits			