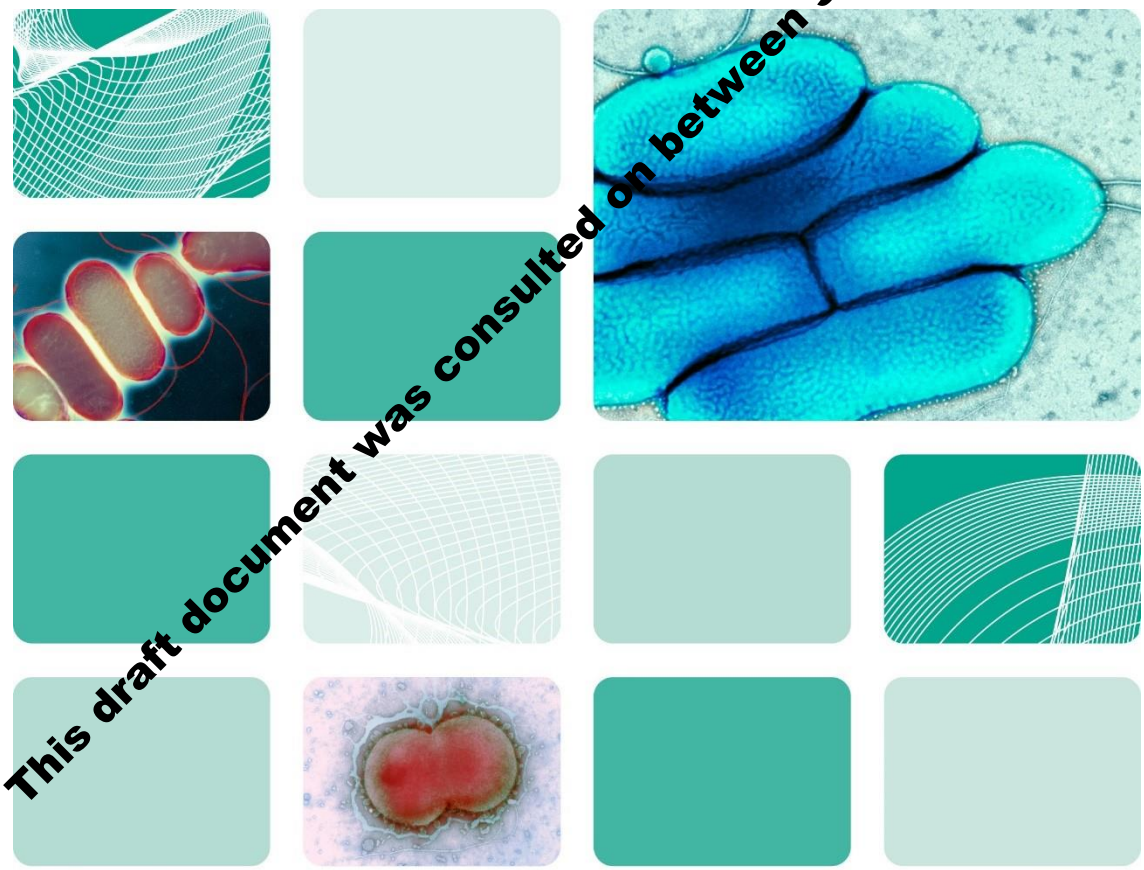
UK Health Security Agency

UK Standards for Microbiology Investigations

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.

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

Contents

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 (see the Steering Committee page on GOV.UK).

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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@phe.gov.uk.

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

Amendment number/date	x/dd.mm.yy
Issue number discarded	
Insert issue number	
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 SMI describes the identification to species level of *Corynebacterium diphtheriae*, *Corynebacterium ulcerans* and *Corynebacterium pseudotuberculosis*. These species are isolated from throat, skin and other sites in suspected cases of classical diphtheria, cutaneous diphtheria and very rarely from other clinical infections such as pharyngitis or chronic skin infections. The importance of toxin production by this specie in the pathogenesis of disease is emphasised.

The document also describes the identification of non-toxigenic species, *Corynebacterium jeikeium*, *Corynebacterium striatum* and other clinically significant species. 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.

Note: Identification of *Arcanobacterium haemolyticum* is covered in ID 3: Identification of Listeria species and other non-sporing Gram Positive Rods (except Corynebacterium).

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

4 Introduction

4.1 Taxonomy and characteristics

There are currently 208 species and 14 subspecies in the genus at the time of writing (1). All *Corynebacterium* species that have genetic and chemotaxonomic features inconsistent with those currently attributed to the genus have been reassigned to other genera. Conversely, relevant taxa assigned to other genera and those with *Corynebacterium*-like features, have been added to the genus (2). Some species are

occasional or extremely rare causes of infection in humans or are transmitted to humans by zoonotic contact.

The potentially toxigenic corynebacteria comprise *C. diphtheriae*, *C. pseudotuberculosis* and *C. ulcerans*. These species may produce diptheria toxin and cause fatal disease. *C. diphtheriae* consists of 4 biovars: *gravis, mitis, intermedius and belfanti* (3). For many years *C. diptheriae* has been regarded as a human pathogen however it has been isolated from horses, cats and dogs.

Corynebacterium species are Gram positive non-motile rods, often with clubbed ends, occurring singly or in pairs. Some cells may stain unevenly giving a beaded appearance and their size is between 2 to 6µm in length and 0.5µm in diameter. They are arranged together in a characteristic way, which has been described as the form of a ‘V’, ‘palisades’. Metachromatic granules are usually present representing stored phosphate regions. The species are aerobic or facultatively anaerobic and exhibit a fermentative metabolism (carbohydrates to lactic acid) under certain conditions. They are fastidious organisms, growing slowly even on enriched medium (4).

All species are catalase positive and most are oxidase negative with the exception of *Corynebacterium bovis*, *Corynebacterium aurimucosum*, *Corynebacterium doosanense* and *Corynebacterium maris* (3).

Agar containing blood and potassium tellurite, such as blood tellurite medium, serves as a selective and differential medium. On blood agar, they form small greyish colonies with a granular appearance, mostly translucent, but with opaque centres, convex, with continuous borders. Their optimum growth temperature is 37°C (4).

Corynebacterium diphtheriae

C. diphtheriae is transmitted by respiratory droplets through person to person, with an incubation of 2 to 5 days. An individual person is infectious when virulent bacteria are present in respiratory secretions, usually 2 weeks without antibiotics (5).

Diphtheria is life threatening infection. But this can be prevented by administration of a vaccine. In England diphtheria is increasingly rare due to the mass immunisation in 1942, when the average annual number of cases was about 60,000 with 4,000 deaths. In England, 2020 there were no reports of toxigenic *C. diphtheriae* strains. But one non-toxigenic toxin-bearing strain was identified (5).

C. diphtheriae grows as grey or black colonies on blood tellurite agar in 16 to 18 hours and produces characteristic colonies after 48 hours. Colony morphology of isolates of will vary in size and appearance but are generally appear 1 to 3mm at 24 hours on blood agar (except for intermedius). 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.

Corynebacterium ulcerans

Only one case of toxigenic *C. ulcerans* was identified in England in 2020 (5). Transmissions to humans occurs through contact with farm animals or their milk.

On Tinsdale medium colonies appear brown with halos with the production of cystinase and do not produce pyrazinamidase. Colonies may be slightly β—haemolytic on blood agar.

Corynebacterium pseudotuberculosis

C. pseudotuberculosis colonies may be slightly β—haemolytic on blood agar.

C. diphtheriae, *C. ulcerans* and *C. pseudotuberculosis* are facultatively anaerobic, non-sporing, non-capsulated and non-acid-fast. These organisms are non-motile and catalase positive.

C. ulcerans and *C. pseudotuberculosis* are both urease positive which may be used to distinguish them presumptively from *C. diphtheriae*.

Strains of these species can all harbour the phage borne diphtheria tox gene, which is required for the production of toxin (6). Toxigenic strains may cause diphtheria or diphtheria-like illness. Possible toxigenic strains of *Corynebacterium* species should be referred to the Reference Laboratory for detection of toxin production as soon as possible.

Non-toxigenic strains of corynebacteria for example, *C. ulcerans*, *C. jeikeium*, *C. striatum* and non-toxigenic *C. diphtheriae* are also known to cause infections in humans including pulmonary infection, leukaemia and endocarditis. Both *C. jeikeium* and *C. striatum* are non-haemolytic, urease negative and catalase positive (7).

4.2 Principles of identification

Isolates from primary culture are identified by colonial appearance, Gram stain, and 4 preliminary tests (this includes nitrate, urease, catalase and pyrazinamidase tests) which permit the presumptive identification of the potentially toxigenic *Corynebacterium* species within 4 hours. Additional identification may be made using a commercial identification kit in conjunction with toxin testing. It is advisable that suspected toxigenic cultures are sent promptly to a Diphtheria Reference Laboratory for confirmation of identification and toxigenicity testing.

Use of Albert's stain is not recommended in this UK SMI, as metachromatic granules are not specific to *C. diphtheriae* or any of the potentially toxigenic corynebacteria.

The interpretation of the clinical significance of *Corynebacterium* isolated from microbiological samples can be problematic. *Corynebacterium* isolated as a predominant organism from a specimen from a normally sterile site, wound, abscess or purulent sputum, from more than one blood culture set or present at greater than or equal to104 cfu/mL in a

pure culture from urine should be considered for identification to species level (4). The clinical significance is strengthened when isolating *Corynebacterium* species from multiple samples or when they are seen in a Gram stained smear as the predominant organism or associated with a significant leucocyte response (8).

Identification to species level is recommended especially if the organism is isolated from normally sterile body sites, from adequately collected clinical material if the *Corynebacterium* species is the predominant organism, and if recovered from urine specimens.

5 Technical information and limitations

Corynebacterium pseudotuberculosis

C. pseudotuberculosis can give a variable nitrate test result. This is because it consists of 2 biovars: biovar *equi* (from horses or cattle) that reduces nitrate and the biovar *ovis* (from sheep or goats) that fails to do so (7).

Agar media

The classic colonial morphology apparently develops better on media containing sheep blood rather than horse in some Corynebacterium species. For example, the degree of haemolysis in *Arcanobacterium haemolyticum*, formerly known as *C. haemolyticum* is far greater on sheep blood agar plate than most other corynebacteria (9).

6 Safety considerations

The section covers specific safety considerations (10-31) related to this UK SMI, and should be read in conjunction with the general safety considerations on GOV.UK.

C. diphtheriae, *C. ulcerans* and *C. pseudotubercolosis* are Hazard Group 2 organisms, and in some cases the nature of the work may dictate full Containment Level 3 conditions. All laboratories should handle specimens as if potentially high risk.

All suspected isolates of potentially toxigenic corynebacteria should always be handled in a microbiological safety cabinet. For the urease test, a urea slope is considered safer than a liquid medium.

C. diphtheriae and *C. ulcerans* cause severe and sometimes fatal diseases. Laboratory acquired infections have been reported (32,33). The organism infects primarily by the respiratory route. Vaccination against diphtheria is available; guidance is given in the DH Green Book (34). In addition, all staff that may be exposed to diphtheria in the course of their

work should be protected by immunisation and exceptions to this recommendation are those who have had a booster within the last 10 years or have had an adverse reaction to immunisation (34,35).

Diphtheria antitoxin for the treatment of clinical cases is distributed by UKHSA Immunisation Department and should be given without waiting for bacteriological confirmation.

Refer to current guidance on the safe handling of all Hazard Group 2 organisms documented in this UK SMI.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet (21).

The above guidance should be supplemented with local COSHH and risk assessments and read in conjunction with the general safety considerations on GOV.UK.

Compliance with postal and transport regulations is essential.

7 Target organisms

Corynebacterium species that are potentially toxigenic

Corynebacterium diphtheriae var belfanti, *Corynebacterium diphtheriae var gravis*, *Corynebacterium diphtheriae var intermedius*, *Corynebacterium diphtheriae var mitis*, *Corynebacterium pseudotuberculosis*, *Corynebacterium ulcerans* (4).

Corynebacterium species that are non-toxigenic

Corynebacterium diphtheriae, *Corynebacterium pseudotuberculosis*, *Corynebacterium ulcerans*, *Corynebacterium jeikeium*, *Corynebacterium striatum*.

Other Corynebacterium species have been known to cause human infection (7),(36).

8 Identification

8.1 Microscopic appearance

Gram stain TP 39 – Staining procedures

Gram positive rods, pleomorphic, slightly curved with tapered or clubbed ends.

Cells may occur singly or in pairs, often in a ‘V’ formation.

Cells usually stain weakly and unevenly giving a beaded appearance.

8.2 Primary isolation media

Blood agar – skin swabs incubated in 5 to 10% CO2 at 35 to 37°C for 40 to 48hour and throat swabs incubated anaerobically at 35 to 37°C for 16 to 24hour. β-haemolytic streptococci may also be present, particularly in throat swabs.

Blood tellurite agar incubated in air at 35 to 37°C for 16 to 48hour.

8.3 Colonial appearance

Appearance varies among species on blood agar plates. For more information, refer to the table below.

Strain	Culture media	
	Blood tellurite agar	**Blood agar**
C. diphtheriae biotype biovar *gravis* (37)	Dull, grey or black, opaque colonies, 1.5 to 2.0mm in diameter, matt surface, friable, tending to break into small segments when touched with a straight wire	Non-haemolytic
C. diphtheriae biotype biovar *mitis* (37)	Grey or black, opaque colonies, 1.5 to 2.0mm in diameter, entire edge and glossy smooth surface; size variation is common	Colonies exhibit a small zone of β-haemolysis
C. diphtheriae biotype biovar *intermedius* (37)	Small, grey or black, shiny surface, discrete, translucent colonies, 0.5 to 1.0mm in diameter	Colonies exhibit a small zone of β-haemolysis
C. diphtheriae biotype biovar *belfanti* (37)	Grey or black, opaque colonies, 1.5 to 2.0mm in diameter, entire edge and glossy smooth surface; size variation is common	Colonies exhibit a small zone of β-haemolysis
C. ulcerans (37)	Grey or black, very dry opaque colonies	Colonies exhibit a small zone of β-haemolysis
C. pseudo-tuberculosis (4,7,38)	Grey or black, very dry opaque colonies	Colonies exhibit a small zone of β-haemolysis

C. striatum (4,36,38)	Grey or black, colonies	Non-haemolytic White moist smooth colonies greater than 2mm after 24hour
C. jeikeium	Grey or black, colonies	Non—haemolytic Grey or white low convex colonies

8.4 Test procedures

8.4.1 Biochemical tests

Rapid 4hour tests should be performed for urease, pyrazinamidase, catalase and nitrate reduction.

Catalase test TP 8 – Catalase test

All potentially toxigenic corynebacteria are catalase positive and for non-toxigenic *Corynebacterium* species, the catalase test results are varied.

Pyrazinamidase test

All potentially toxigenic corynebacteria (*C. diphtheriae, C. ulcerans* and *C. pseudotuberculosis*) are pyrazinamidase negative while other corynebacteria are positive.

Urease test TP 36 – Urease test

The urease test is used to determine the ability of an organism to split urea, through the production of the enzyme urease.

C. ulcerans and *C. pseudotuberculosis* are urease positive.

Nitrate reduction test

See table below.

Strain	Biochemical tests†			
	Nitrate	Urease*	Catalase	Pyrazinamidase
C. diphtheriae biotype biovar *gravis* (37)	Positive	Negative	Positive	Negative

C. diphtheriae biotype biovar *mitis* (37)	Positive	Negative	Positive	Negative
C. diphtheriae biotype biovar *intermedius* (37)	Positive	Negative	Positive	Negative
C. diphtheriae biotype biovar *belfanti* (37)	Negative	Negative	Positive	Negative
C. ulcerans (37)	Negative	Positive	Positive	Negative
C. pseudo-tuberculosis (4,7,38)	Positive or Negative	Positive	Positive	Negative
C. striatum (4,36,38)	Positive or Negative	Negative	Positive	Positive
C. jeikeium	Negative	Negative	Positive	Positive

† Refer to TP 36 – Urease Test

*If results of these 4hour tests indicate *Corynebacterium* species, immediately inform medical microbiologist and refer isolate to the Reference Laboratory. *C. xerosis* can be used as a positive control for this test.

If these preliminary tests do not indicate *Corynebacterium* species then consider further identification tests if clinically indicated.

Result for the nitrate test can be variable for *C. pseudotuberculosis*. This is because it consists of 2 biovars: biovar *equi* (from horses or cattle) that reduces nitrate and the biovar *ovis* (from sheep or goats) that fails to do so.

Use commercial identification kit and refer isolate to the Reference Laboratory if clinically indicated.

Note: Fresh culture of control organism is advisable.

These test results are consistent with taxonomy from widely published systems.

It is important that a preliminary identification of possible colonies of *C. diphtheriae* or other potentially toxigenic *Corynebacterium* species is made as rapidly as possible with the use of 4hour tests. The preliminary tests provide an indication of the likely presence or absence of *C. diphtheriae, C. ulcerans* or *C. pseudotuberculosis*. The results should be considered together with the clinical details.

All suspected isolates of *C. diphtheriae* or other potentially toxigenic *Corynebacterium* species should be sub—cultured to a blood agar plate for purity and to a blood agar slope (preferably) or Loeffler's media (for possible referral to a reference laboratory) at the time that the tests are set up.

8.4.2 Commercial identification systems

Laboratories should follow manufacturer's instructions and rapid tests and kits should be validated and be shown to be fit for purpose prior to use.

8.4.3 Matrix-Assisted Laser Desorption Ionization – Time of Flight Mass Spectrometry (MALDI-TOF)

MALDI-TOF MS has been used successfully to identify potentially toxigenic *Corynebacterium* species at the species level in clinical isolates within 15 minutes (39,40). This technology is used as a rapid screening method helping to decide whether suspicious colonies should be analysed for the presence of the *tox* gene by real-time PCR. It can also discriminate *C. aurimucosum* from *C. minutissimum*, 2 closely related *Corynebacterium* species previously considered difficult to differentiate (41).

Refer to UK SMI TP39: Matrix-Assisted Laser Desorption Ionization – Time of Flight Mass Spectrometry (MALDI-TOF) test procedure for more information on the use of this technique.

8.4.4 Nucleic Acid Amplification Tests (NAATs)

PCR for *Corynebacterium diphtheriae* is rapid and completed within 4hour of receipt of the strain, although toxin production must always be verified by the phenotypic test for toxigenicity (42). A PCR directed at the A subunit of the diphtheria toxin gene can also be used to detect the *tox* gene, the structural gene for diphtheria toxin, although it does not confirm toxin production (35). 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).

8.5 Further identification

Other rapid typing methods

A variety of rapid typing methods have been developed for isolates from clinical samples; these include molecular techniques such as 16S rRNA gene (rDNA) sequence analysis, Multi-locus Sequence typing (MLST) and Whole Genome Sequencing. These approaches enable subtyping of unrelated strains, but do so with different accuracy, discriminatory power, and reproducibility.

However, some of these methods remain accessible to reference laboratories only and are difficult to implement for routine bacterial identification in clinical laboratories.

Whole genome sequencing (WGS)

Whole genome sequencing determines the complete DNA sequence of an organism's genome at a single time. This entails sequencing an entire organism's chromosomal DNA as well as DNA contained in the mitochondria.

Several Corynebacterium species have had complete genomes sequenced (2). Genome sequences are available in the public database for *C. glutamicum*, *C. efficiens*, *C. diphtheriae*, *C. jeikeium*, *C. pseudotuberculosis* and *C. ulcerans*. This has also aided in the identification of Corynebacterium species.

Amplified fragment length polymorphism (AFLP)

Amplified Fragment Length Polymorphism is a high-resolution whole genome methodology used as a tool for rapid and cost-effective analysis of genetic diversity within bacterial genomes. It is useful for identification and subtyping of microorganisms from clinical samples, for identification of outbreak genotypes, for studies of micro and macro-variation, and for population genetics (44,45).

This gel-based method can also be used for further identification and has been successful in the discrimination and differentiation of *C. diphtheriae* isolates. This has been evaluated as a quicker, more affordable method to ribotyping, which is the preferred gold standard for typing of *C. diphtheriae*. This method is more adaptable especially in laboratories that have limited funding and equipment (46,47).

16S rRNA gene (rDNA) sequence analysis

A genotypic identification method, 16S rRNA gene sequencing is used for phylogenetic studies and has subsequently been found to be capable of re—classifying bacteria into completely new species, or even genera. It has also been used to describe new species that have never been successfully cultured.

The use of molecular genetic methods such as 16S rRNA gene (rDNA) sequence analysis has facilitated a much tighter circumscription of the genus *Corynebacterium*, and the availability of comparative 16S rRNA gene sequence data with improved phenotypic data has resulted in much improved and more reliable species identification; however, *rpoB* gene sequences are used as they are more polymorphic than the 16S rDNA and can ensure reliable phylogenetic studies(41,48). The only drawback with using the *rpoB* gene sequencing is that it is a time-consuming process which requires training staff to a competent level (39).

8.6 Storage and referral

Refer the presumptive *C. diphtheriae, C. ulcerans* or *C. pseudotuberculosis* isolate on a Loeffler or blood agar slope immediately to a reference laboratory.

9 Reporting

9.1 Infection specialist

Inform the laboratory associated infection specialist of presumptive and confirmed *C. diphtheriae, C. ulcerans* or *C. pseudotuberculosis* species. The infection specialist should also be informed if the request bears relevant information, for example:

- membranous or pseudomembranous pharyngitis or tonsillitis
- contact with a confirmed case within the last 10 days
- travel abroad to high risk area within the last 10 days
- contact with someone who has been to a high risk area within the last 10 days
- contact with any animals (including household pets, visiting a farm or petting zoo) within the last 10 days
- recent consumption of any type of unpasteurised milk or dairy products
- the patient works in a clinical microbiology laboratory, or similar occupation, where *Corynebacterium* species may be handled

For presumptive and confirmed non-toxigenic *Corynebacterium* species, the infection specialist should be informed when the request bears relevant information for example:

- cases of suspected endocarditis associated with appropriate specimen
- infection of indwelling medical devices (prosthetic valves, pacemakers, peritoneal and vascular catheters, CSF shunts)
- history of substance abuse, alcoholism, immunodeficiency or other serious underlying disorder such as cancer, or patients receiving treatment for cancer, inducing neutropenia or mucositis

Follow local protocols for reporting to the clinician.

9.2 Preliminary identification

Presumptive identification may be made if appropriate growth characteristics, colonial appearance, Gram stain of the culture, 4 hour test results and rapid methods are demonstrated.

9.3 Confirmation of identification

For confirmation and identification please see Specialist and reference microbiology: laboratory tests and services page on GOV.UK for reference laboratory user manuals and request forms.

9.4 Health Protection Team (HPT)

Refer to local agreements in devolved administrations.

9.5 UK Health Security Agency

Refer to current guidelines on Second Generation Surveillance System (SGSS) reporting (26).

As diphtheria is a notifiable disease in the UK, and so for public health management of cases, contacts and outbreaks, all suspected cases should be notified immediately to the local UK Health Security Agency Laboratories.

All clinically significant isolates should be notified by the diagnostic laboratories to ensure urgent initiation of proper procedures and all such isolates should be referred to the national reference laboratory for toxigenicity testing.

9.6 Infection prevention and control team

Inform the infection prevention and control team of presumptive and confirmed isolates of *C. diphtheriae* according to local protocols.

10 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.

Contact appropriate reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

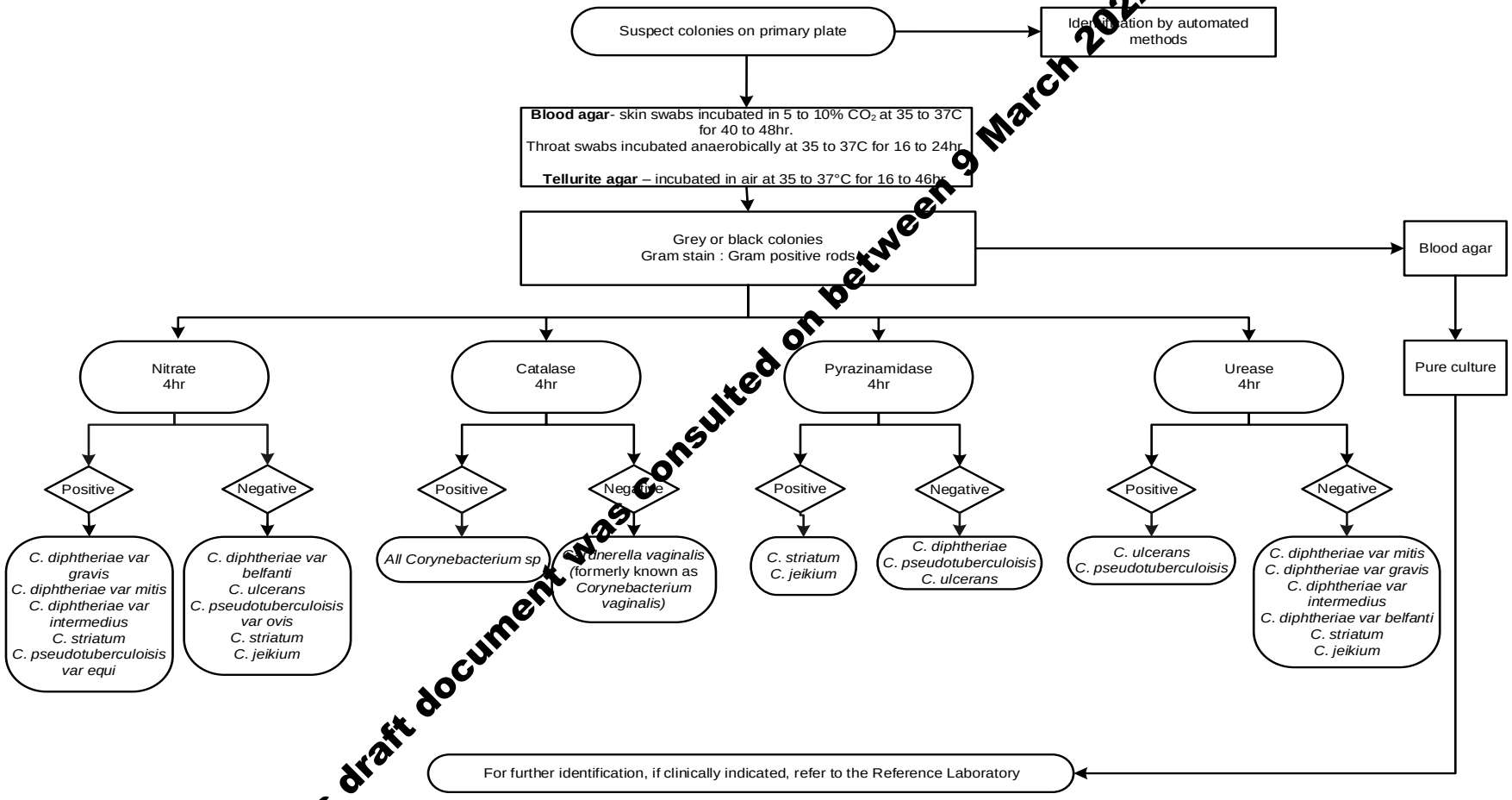
- England
- Wales
- Scotland
- Northern Ireland

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

View scientific information for details on notification to UKHSA or equivalent in the devolved administrations.

Algorithm: Identification of Corynebacterium species

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



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An explanation of the reference assessment used is available in the scientific information section on the UK SMI website.

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