UK Standards for Microbiology Investigations

Identification of *Corynebacterium* species

Issued by the Standards Unit, Microbiology Services, PHE

Bacteriology – Identification | ID 2 | Issue no: 4.1 | Issue date: 29.10.14 | Page: 1 of 24

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Identification of *Corynebacterium* species

Acknowledgments

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The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the Medical Editors for editing the medical content.

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UK Standards for Microbiology Investigations are produced in association with:

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Logos correct at time of publishing.

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NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation.
### Amendment Table

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

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

<table>
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<td>Whole document.</td>
<td>Hyperlinks updated to gov.uk.</td>
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<td>Updated logos added.</td>
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<tr>
<td><strong>Identification.</strong></td>
<td>Updates have been done on 3.4 and 3.5 to reflect standards in practice. Subsection 3.5 has been updated to include the Rapid Molecular Typing Methods.</td>
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<tr>
<td>Scope of document.</td>
<td>The title of SMI document ID 3 referred to in the text has been updated.</td>
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<tr>
<td>Introduction.</td>
<td>Taxonomy updated. More information has been added to the Characteristics section. Removal of two sentences on PCR from this section. Section on Principles of Identification has been updated to reflect the four preliminary tests used in identification of <em>Corynebacterium</em> species.</td>
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<td>Technical Information/Limitations.</td>
<td>Updated to include information regarding use of sheep blood agar media and variability of the nitrate test.</td>
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<td>Identification</td>
<td>guidelines laid out by the DH Green book.</td>
</tr>
<tr>
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<td>------------------------------------------</td>
</tr>
<tr>
<td>Identification.</td>
<td>Updates have been done on 3.3, 3.4 and 3.6 to reflect standards in practice. The table has also been updated with references and two more columns to include the catalase and pyrazinamidase tests. The footnote in the table has also been updated. - TP 36 Urease Test has been hyperlinked. - Variable test results for nitrate test in <em>C. pseudotuberculosis</em> have also been mentioned. - Referral of isolate to the Reference Laboratory has been updated. - References used for the table are listed in the footnote. Subsection 3.5 has been updated to include the Rapid Molecular Methods.</td>
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<tr>
<td>Identification Flowchart.</td>
<td>Addition of flowchart for identification of <em>Corynebacterium</em> species has been developed for guidance.</td>
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<td>Reporting.</td>
<td>Subsections 5.1, 5.2, 5.4, 5.5 and 5.6 have been updated to reflect reporting practice. The webpage link in section 5.4 and 5.5 has been removed as it is not accessible.</td>
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<td>Referral.</td>
<td>The address of the Reference Laboratory has been updated.</td>
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<td>Some references updated.</td>
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UK Standards for Microbiology Investigations#: Scope and Purpose

Users of SMIs
- SMIs are primarily intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK.
- SMIs provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests.
- SMIs provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs
SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages.

Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal Partnership Working
SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies.

The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process.

SMIs are developed, reviewed and updated through a wide consultation process.

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Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.
Quality Assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008.

SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development.

The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and Public Involvement

The SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information Governance and Equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions.

The development of SMIs are subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity. The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal Statement

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

SMIs are Crown copyright which should be acknowledged where appropriate.
Suggested Citation for this Document
Scope of Document

This SMI describes the identification to species level of *Corynebacterium diphtheriae*, *Corynebacterium ulcerans* and *Corynebacterium pseudotuberculosis* isolated from throat, skin and other sites. These organisms may be isolated from 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 species 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. *Arcanobacterium haemolyticum*, formerly known as *Corynebacterium haemolyticum* is covered in ID 3 - Identification of *Listeria* species and other Non-Sporing Gram Positive Rods (except *Corynebacterium*).

This SMI covers four tests for the preliminary identification of pathogenic *Corynebacterium* species and recommends that the organisms be sent to the Reference Laboratory for confirmation of identification and toxin testing if required.

This SMI should be used in conjunction with other SMIs.

Introduction

**Taxonomy**

There are currently 112 species and 11 subspecies in this genus. All *Corynebacterium* species that have genetic and chemotaxonomic features inconsistent with those currently attributed to this 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. Of these, 55 species are occasional or extremely rare causes of infection in humans or are transmitted to humans by zoonotic contact, with the remaining species having been recovered solely from animals or birds, the environment, water, foodstuffs or synthetic materials.

The potentially toxigenic corynebacteria comprise *C. diphtheriae*, *C. pseudotuberculosis* and *C. ulcerans*. *C. diphtheriae* consists of four biovars: *gravis*, *mitis*, *intermedius* and *belfanti*.

**Characteristics**

*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. Their size is between 2-6µm in length and 0.5µm in diameter. They group together in a characteristic way, which has been described as the form of a "V", "palisades", or "Chinese letters". Metachromatic granules are usually present representing stored phosphate regions. They 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. Agar containing blood and potassium tellurite, such as Hoyle's 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.
C. diphtheriae grows as pinpoint grey/black colonies on Hoyle’s tellurite agar in 16-18hr and produces characteristic colonies after 48hr. Isolates of potentially toxigenic Corynebacterium species will also grow on blood agar. Colonial morphology varies among the species. C. ulcerans and 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. 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 eg 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.

Principles of Identification

Isolates from primary culture are identified by colonial appearance, Gram stain, and four preliminary tests (this includes nitrate, urease, catalase and pyrazinamidase tests) which permit the presumptive identification of the potentially toxigenic Corynebacterium species within 4hr. 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 the Streptococcus and Diphtheria Reference Unit (SDRU) for confirmation of identification and toxigenicity testing.

Use of Albert’s stain is not recommended in this 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 ≥10^4 cfu/mL in a pure culture from urine should be considered for identification to species level. 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.

Technical Information/Limitations

Corynebacterium pseudotuberculosis

C. pseudotuberculosis can give a variable nitrate test result. This is because it consists of two biovars: biovar equi (from horses or cattle) that reduces nitrate and the biovar ovis (from sheep or goats) that fails to do so.
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 Corynebacterium haemolyticum is far greater on sheep blood agar plate than most other corynebacteria.
1 Safety Considerations\textsuperscript{9-25}

\textit{C. diphtheriae, C. ulcerans and C. pseudotuberculosis} 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.

\textit{C. diphtheriae} and \textit{C. ulcerans} cause severe and sometimes fatal diseases. Laboratory acquired infections have been reported\textsuperscript{26,27}. The organism infects primarily by the respiratory route. Vaccination against diphtheria is available; guidance is given in the DH Green Book\textsuperscript{28}. 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\textsuperscript{26,29}.

Diphtheria antitoxin for the treatment of clinical cases is distributed by PHE 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 SMI.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet\textsuperscript{17}.

The above guidance should be supplemented with local COSHH and risk assessments.

Compliance with postal and transport regulations is essential.

2 Target Organisms

\textbf{Corynebacterium} species which are potentially toxigenic\textsuperscript{1}

\textit{Corynebacterium diphtheriae var belfanti, Corynebacterium diphtheriae var gravis, Corynebacterium diphtheriae var intermedius, Corynebacterium diphtheriae var mitis, Corynebacterium pseudotuberculosis, Corynebacterium ulcerans}

\textbf{Corynebacterium} species which are non-toxigenic\textsuperscript{6}

\textit{Corynebacterium diphtheriae, Corynebacterium pseudotuberculosis, Corynebacterium ulcerans, Corynebacterium jeikium, Corynebacterium striatum}

Other \textit{Corynebacterium} species have been known to cause human infection\textsuperscript{2}.

3 Identification

3.1 Microscopic Appearance

Gram stain \textbf{(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 (forming “Chinese letters”). Cells usually stain weakly and unevenly giving a beaded appearance.

### 3.2 Primary Isolation Media

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

Hoyle’s tellurite agar incubated in air at 35-37°C for 16-48hr.

### 3.3 Colonial Appearance

Appearance varies among species on blood agar plates. For more information, refer to the table in Section 3.4 Test Procedures below.

### 3.4 Test Procedures

#### 3.4.1 Biochemical tests

Rapid (4hr) 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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Culture media</th>
<th>Biochemical tests†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hoyle’s tellurite agar</td>
<td>Blood agar</td>
</tr>
<tr>
<td><em>C. diphtheriae</em> biotype</td>
<td>dull, grey/black, opaque colonies, 1.5-2.0mm in diameter, matt surface, friable, tending to break into small segments when touched with a straight wire</td>
<td>Non haemolytic</td>
</tr>
<tr>
<td>biotype biovar</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>gravis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. diphtheriae</em> biotype</td>
<td>grey/black, opaque colonies, 1.5 - 2.0mm in diameter, entire edge and glossy smooth surface; size variation is common</td>
<td>colonies exhibit a small zone of β-haemolysis</td>
</tr>
<tr>
<td><em>mitis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. diphtheriae</em></td>
<td>small, grey/black, shiny surface, discrete, colonies exhibit a small</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>zone</td>
<td></td>
</tr>
</tbody>
</table>
Identification of *Corynebacterium* species

<table>
<thead>
<tr>
<th>Biotype</th>
<th>Description</th>
<th>Zone of β-Haemolysis</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. diphtheriae</em> biotype biovar [intermedius][30]</td>
<td>Grey/black, opaque colonies, 1.5-2.0mm in diameter, entire edge and glossy smooth surface; size variation is common</td>
<td>Negative</td>
<td>Positive; Negative</td>
</tr>
<tr>
<td><em>C. ulcerans</em> [30]</td>
<td>Grey/black, very dry opaque colonies</td>
<td>Negative</td>
<td>Positive; Positive; Positive; Negative</td>
</tr>
<tr>
<td><em>C. pseudotuberculosis</em> [1,6,31]</td>
<td>Grey/black, very dry opaque colonies</td>
<td>Positive/ Negative</td>
<td>Positive; Positive; Positive; Negative</td>
</tr>
<tr>
<td><em>C. striatum</em> [1,2,31]</td>
<td>Grey/black, colonies</td>
<td>Positive/ Negative</td>
<td>Negative; Positive; Positive; Negative</td>
</tr>
<tr>
<td><em>C. jeikeium</em></td>
<td>Grey/black, colonies</td>
<td>Negative</td>
<td>Negative; Negative; Positive; Positive</td>
</tr>
</tbody>
</table>

† Refer to TP 36 - Urease Test

*If results of these 4hr 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 two 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[1,2,6,30,31].

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 4hr 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 the Reference Laboratory) at the time that the tests are set up.
3.4.2 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.

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

MALDI-TOF MS analysis is a routinely used tool in many microbiology laboratories, whereby proteins liberated from bacteria are ionized and detected by a mass spectrometer (MS), the spectrum is analysed, and its pattern is compared to entries found in a database, giving rise to a degree of match. This technology is touted as being revolutionary, because it does not require extensive training or expertise in mass spectrometry or chemistry to use. The cost for consumables is described as relatively low per sample, excluding the cost of the MALDI-TOF instrument.

MALDI-TOF MS has been used successfully to identify potentially toxigenic Corynebacterium species at the species level in clinical isolates within 15 minutes. Thus, this technology could be 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.

MALDI-TOF can also discriminate Corynebacterium aurimucosum from Corynebacterium minutissimum, two closely related Corynebacterium species previously considered difficult to differentiate.

3.4.4 Nucleic Acid Amplification Tests (NAATs)

PCR is usually considered to be a good method for bacterial detection as it is simple, rapid, sensitive and specific. The basis for PCR diagnostic applications in microbiology is the detection of infectious agents and the discrimination of non-pathogenic from pathogenic strains by virtue of specific genes. However, it does have limitations. Although the 16S rRNA gene is generally targeted for the design of species-specific PCR primers for identification, designing primers is difficult when the sequences of the homologous genes have high similarity.

PCR for Corynebacterium diphtheriae is rapid and can be completed within 4hr of receipt of the strain, although toxin production must always be verified by the phenotypic test for toxigenicity. 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. 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.

3.5 Further Identification

Rapid Molecular Methods

A variety of rapid typing methods have been developed for isolates from clinical samples; these include molecular techniques such as Amplified Fragment Length Polymorphism (AFLP), 16S rRNA gene (rDNA) sequence analysis, Multi-locus Sequence typing (MLST) and Whole Genome Sequencing. All of 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 (also known as full genome sequencing, complete genome sequencing, or entire genome sequencing), is a laboratory process that determines the complete DNA sequence of an organism's genome at a single time. This entails sequencing all of an organism's chromosomal DNA as well as DNA contained in the mitochondria.

A number of *Corynebacterium* species have had complete genomes sequenced\(^4\). 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.

Multi-locus Sequence Typing (MLST)

MLST measures the DNA sequence variations in a set of housekeeping genes directly and characterizes strains by their unique allelic profiles. The principle of MLST is simple: the technique involves PCR amplification followed by DNA sequencing. Nucleotide differences between strains can be checked at a variable number of genes depending on the degree of discrimination desired. The technique is highly discriminatory, as it detects all the nucleotide polymorphisms within a gene rather than just those non-synonymous changes that alter the electrophoretic mobility of the protein product. One of the advantages of MLST over other molecular typing methods is that sequence data are portable between laboratories and have led to the creation of global databases that allow for exchange of molecular typing data via the internet\(^37\).

MLST has been used successfully for characterisation of *Corynebacterium diphtheriae* as has been evaluated in various reports and it has also been found to provide a good understanding of the diversity of the pathogen\(^38\)-\(^40\).

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 a broad range of applications such as identification and subtyping of microorganisms from clinical samples, for identification of outbreak genotypes, for studies of micro and macro-variation, and for population genetics\(^41\),\(^42\).

Amplified Fragment Length Polymorphism (AFLP), a 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 current gold standard for typing of *C. diphtheriae*). This method is more adaptable especially in laboratories that have limited funding and equipment\(^43\),\(^44\).

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\(^34,45\). 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\(^33\).

### 3.6 Storage and Referral

Refer the presumptive *C. diphtheriae, C. ulcerans* or *C. pseudotuberculosis* isolate on a Loeffler or blood agar slope immediately to the Reference Laboratory.
4 Identification of *Corynebacterium* species

Clinical Specimen

Screening, diagnosis/investigation of cases, contacts/carriers

Blood agar- skin swabs incubated in 5-10% CO₂ at 35-37°C for 40-48hr and throat swabs incubated anaerobically at 35-37°C for 16-24hr.

Tellurite agar - incubated in air at 35-37°C for 16-46hr.

Grey/black colonies
Gram stain : Gram positive rods

Blood agar

Nitrate 4hr

Positive
C. diphtheriae var gravis
C. diphtheriae var mitis
C. diphtheriae var intermedius
C. striatum
C. pseudotuberculosis var equi

Negative
C. diphtheriae var belfanti
C. ulcerans
C. pseudotuberculosis var ovis
C. striatum
C. jeikium

Catalase 4hr

Positive
All *Corynebacterium* sp

Negative
Gardnerella vaginalis
(formerly known as *Corynebacterium vaginalis*)

Pyrazinamidase 4hr

Positive
C. striatum
C. jeikium

Negative
C. diphtheriae
C. pseudotuberculosis
C. ulcerans

Urease 4hr

Positive
C. ulcerans
C. pseudotuberculosis

Negative
C. diphtheriae var mitis
C. diphtheriae var gravis
C. diphtheriae var intermedius
C. diphtheriae var belfanti
C. striatum
C. jeikium

For further identification, if clinically indicated, refer to the Reference Laboratory

Pure culture
5 Reporting

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

5.2 Confirmation of Identification
Confirmation of identification and toxigenicity are undertaken only by the Respiratory and Vaccine Preventable Bacteria Reference Unit (RVPBRU) PHE Colindale.

5.3 Medical Microbiologist
Inform the medical microbiologist of presumptive and confirmed C. diphtheriae, C. ulcerans or C. pseudotuberculosis species. The medical microbiologist should also be informed if the request bears relevant information eg:

- Suspected case of contact with diphtheria or foreign travel
- Membranous/Pseudomembranous tonsillitis
- Ulcerating skin lesions acquired overseas. The medical microbiologist should be aware of possible factors from overseas protocols that could influence results
- Any of the above, with neurological or cardiological manifestations
- History of farming or veterinary work
- Any foreign travel to a high risk area, particularly Indian subcontinent, South-East Asia, Africa, South America, former Soviet States and Eastern Europe

For presumptive and confirmed non-toxigenic Corynebacterium species the medical microbiologist should be informed when the request bears relevant information eg:

- 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 and/or mucositis

Follow local protocols for reporting to the clinician.

5.4 CCDC
Refer to local Memorandum of Understanding.

5.5 Public Health England
Refer to current guidelines on CIDSC and COSURV reporting.

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

5.6 Infection Prevention and Control Teams

Inform the infection prevention and control team of presumptive and confirmed isolates of \textit{C. diphtheriae} according to local protocols.

6 Referrals

6.1 Reference Laboratory

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

\textbf{Potentially toxigenic corynebacteria (\textit{C. diphtheriae, C. ulcerans, C. pseudotuberculosis})}

Streptococcus and Diphtheria Reference Section
WHO Global Collaborating Centre for Streptococcal and Diphtheria Infections
Respiratory and Vaccine Preventable Bacteria Reference Unit
Microbiology Services
Public Health England
61 Colindale Avenue
London
NW9 5EQ

\textbf{Other \textit{Corynebacterium} species}

Antimicrobial Resistance and Healthcare Associated Infections Reference Unit (AMRHAI)
Microbiology Services
Public Health England
61 Colindale Avenue
London
NW9 5EQ

Contact PHE’s main switchboard: Tel. +44 (0) 20 8200 4400

England and Wales

Scotland

Northern Ireland
http://www.belfasttrust.hscni.net/Laboratory-MortuaryServices.htm
7 Notification to PHE\textsuperscript{46,47} or Equivalent in the Devolved Administrations\textsuperscript{48-51}

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

\textbf{Note:} The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under ‘Notification Duties of Registered Medical Practitioners’: it is not noted under ‘Notification Duties of Diagnostic Laboratories’.

\url{https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010}

Other arrangements exist in Scotland\textsuperscript{48,49}, Wales\textsuperscript{50} and Northern Ireland\textsuperscript{51}.
References


9. European Parliament. UK Standards for Microbiology Investigations (SMIs) use the term "CE marked leak proof container" to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: "The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes".


Identification of Corynebacterium species


