UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee).

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.

For further information please contact us at:

Standards Unit
Microbiology Services
Public Health England
61 Colindale Avenue
London NW9 5EQ
E-mail: standards@phe.gov.uk

Website: https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

UK Standards for Microbiology Investigations are produced in association with:

[Logos of various professional organisations]

Logos correct at time of publishing.
## Identification of *Bordetella* species

### Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>2</td>
</tr>
<tr>
<td>AMENDMENT TABLE</td>
<td>4</td>
</tr>
<tr>
<td>UK STANDARDS FOR MICROBIOLOGY INVESTIGATIONS: SCOPE AND PURPOSE</td>
<td>6</td>
</tr>
<tr>
<td>SCOPE OF DOCUMENT</td>
<td>9</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>9</td>
</tr>
<tr>
<td>TECHNICAL INFORMATION/LIMITATIONS</td>
<td>12</td>
</tr>
<tr>
<td>SAFETY CONSIDERATIONS</td>
<td>13</td>
</tr>
<tr>
<td>TARGET ORGANISMS</td>
<td>13</td>
</tr>
<tr>
<td>IDENTIFICATION</td>
<td>13</td>
</tr>
<tr>
<td>IDENTIFICATION OF <em>BORDETELLA</em> SPECIES</td>
<td>17</td>
</tr>
<tr>
<td>REPORTING</td>
<td>18</td>
</tr>
<tr>
<td>REFERRALS</td>
<td>18</td>
</tr>
<tr>
<td>NOTIFICATION TO PHE OR EQUIVALENT IN THE DEVOLVED ADMINISTRATIONS</td>
<td>19</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>20</td>
</tr>
</tbody>
</table>

For full details on our accreditation visit: [www.nice.org.uk/accreditation](http://www.nice.org.uk/accreditation)

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](http://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>
<thead>
<tr>
<th>Amendment No/Date.</th>
<th>10/14.04.15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Issue no. discarded.</td>
<td>3</td>
</tr>
<tr>
<td>Insert Issue no.</td>
<td>3.1</td>
</tr>
</tbody>
</table>

**Section(s) involved**

**Amendment**


<table>
<thead>
<tr>
<th>Amendment No/Date.</th>
<th>9/07.11.14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Issue no. discarded.</td>
<td>2.3</td>
</tr>
<tr>
<td>Insert Issue no.</td>
<td>3</td>
</tr>
</tbody>
</table>

**Section(s) involved**

**Amendment**

Whole document. Hyperlinks updated to gov.uk.

Page 2. Updated logos added.

Whole document. Title of document has been updated to include all *Bordetella* species.

Document presented in a new format.

Scope of document. The scope has been updated to include all *Bordetella* species isolated from clinical material but with more emphasis on the two species associated with pertussis in humans.

A webpage link for B 6 document has been added.

Introduction. The taxonomy of *Bordetella* species has been updated.

More information has been added to the Characteristics section. The medically important species are mentioned and their characteristics described.

Section on Principles of Identification has been updated to reflect the current name of the Reference Laboratory where presumptive
<table>
<thead>
<tr>
<th>Section</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Technical Information/Limitations.</td>
<td>Addition of information regarding agar media has been described and referenced.</td>
</tr>
<tr>
<td>Safety considerations.</td>
<td>This section has been updated on the necessity for laboratory staff to work in a safety cabinet and the references have also been included.</td>
</tr>
<tr>
<td>Target Organisms.</td>
<td>The section on the Target organisms has been updated and presented clearly. References have been updated.</td>
</tr>
<tr>
<td>Identification.</td>
<td>Updates have been done on 3.1, 3.2 and 3.4 to reflect standards in practice. Subsection 3.5 has been updated to include the Rapid Molecular Methods.</td>
</tr>
<tr>
<td>Identification Flowchart.</td>
<td>Modification of flowchart for identification of <em>Bordetella</em> species has been done for easy guidance.</td>
</tr>
<tr>
<td>Reporting.</td>
<td>Subsection 5.4 has been updated to reflect reporting practice.</td>
</tr>
<tr>
<td>Referral.</td>
<td>The address of the reference laboratory has been updated.</td>
</tr>
<tr>
<td>References.</td>
<td>Some references updated.</td>
</tr>
</tbody>
</table>
Identification of *Bordetella* species

**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.


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.

---

1 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.
Identification of *Bordetella* species

Suggested Citation for this Document
Identification of *Bordetella* species

**Scope of Document**

This SMI describes the identification of *Bordetella* species and importantly, the two associated with pertussis (whooping cough) in humans: *Bordetella pertussis* and *Bordetella parapertussis*, isolated from clinical specimens to species level. Refer to **B 6 - Investigation of Specimens for Bordetella pertussis and Bordetella parapertussis** for information.

This SMI should be used in conjunction with other SMIs.

**Introduction**

**Taxonomy**

There are currently eight validly named species in the genus *Bordetella*: *Bordetella pertussis*, *Bordetella parapertussis*, *Bordetella bronchiseptica*, *Bordetella hinzii*, *Bordetella holmesii*, *Bordetella trematum*, *Bordetella avium*, and *Bordetella petrii*. One species ‘*Bordetella anisorpii*’ is still awaiting formal description.

Of these nine species, all can potentially cause infections in humans, albeit rarely in some cases.

**Characteristics**

*Bordetella* species are Gram negative coccobacilli 0.2-0.5 x 0.5-2.0µm. Microscopically they appear arranged singly or in pairs and rarely in chains. They often exhibit bipolar staining. Cells may be motile or non-motile. They are strictly aerobic (except for one species, *B. petrii*) and the optimum temperature is 35-37°C. Colonies on plates appear smooth, convex, nearly glistening, nearly transparent and surrounded by a zone of haemolysis without definite periphery. The metabolism is respiratory and never fermentative. Species of *Bordetella* require nicotinamide, amino acids and organic sulphur eg cysteine. *Bordetella* species oxidatively utilise glutamic acid, proline, alanine, aspartic acid and serine with production of ammonia and CO₂.

*B. pertussis*

*B. pertussis* may grow on *Bordetella* selective medium (charcoal blood agar with cefalexin) within three days, but normally 5-7 days incubation is required for primary isolation. Plates should be incubated for 7 days before being discarded as negative. Growth on subculture usually requires shorter incubation (3 days). Colonies are smooth, convex, nearly glistening, greyish-white and have a butyrous consistency. *B. pertussis* does not grow on nutrient agar or MacConkey agar and grows poorly on blood agar. *B. pertussis* is weakly oxidase positive and is non-motile. They are also urinary negative and agglutinate to the *B. pertussis* polyvalent antiserum and weakly or not at all with *B. parapertussis* polyvalent antiserum, depending on how thoroughly it has been cross-absorbed.

Antibiotic susceptibility testing is not done routinely apart from two reports that suggest that there are erythromycin-resistant strains of *Bordetella pertussis*. Susceptibility testing of pertussis is complicated by the slow growth of the organism and poor growth on some media.

Isolates of *B. pertussis* should be referred to the Respiratory and Vaccine Preventable Bacteria Reference Unit for confirmation and serotyping. *B. pertussis* has three major surface agglutinogens (1, 2 and 3), which are detectable by bacterial agglutination with cross-absorbed antisera. There are three serotypes which can cause human infection.
disease 1,2, 1,3 and 1,2,3. Currently the least common is 1,2,3\(^6\). Type 1,3 remains the predominant type and accounts for most isolates\(^8\).

**Bordetella parapertussis**

Colonies of *B. parapertussis* are similar to *B. pertussis* but are larger, duller and become visible sooner. They grow rapidly and can appear on agar plates within 2-3 days. Unlike *B. pertussis*, it grows on nutrient agar giving a brown discoloration of the medium after several days.

*B. parapertussis* is non-motile, oxidase negative and urease positive. They are agglutinated by *B. parapertussis* polyvalent antiserum and slowly, if at all, by *B. pertussis* antiserum\(^6\).

**Bordetella bronchiseptica**

Colony morphology of this organism ranges from smooth to rough when grown on agar plates. On agar media containing blood, it exhibits glistening β-haemolytic colonies and develops an average diameter of 2.0mm in 1 to 2 days. They equally grow well on MacConkey agar. They are oxidase positive and motile by peritrichous flagella. They are also nitrate and urease positive (usually within 4hr) which is a distinguishing factor from *B. pertussis*\(^9\).

**Bordetella ansorpii**

They grow on both blood and MacConkey agar. They are negative for oxidase, urease, nitrate reduction, esculinase, mannitol, and arginine dihydrolase but positive for citrate, adipate, malate, gelatinase activity and motility\(^1\).

**Bordetella trematum**

*B. trematum* cells are motile by means of peritrichous flagella. Motility does not differ significantly when cells are grown at 25, 30, or 37°C. In 16-24hr cultures on blood agar, the average cell is 0.5 to 0.6µm wide and 1 to 1.8µm long; the longest rods are up to 2.4µm long. They produce convex, circular, and greyish cream white colonies with entire edges on blood agar. They do not require special growth factors and grow on conventional media. Growth is not inhibited at an incubation temperature of 42°C, but is reduced markedly at 25°C. Strains grow microaerobically, but not anaerobically. Colonies grown for 16 to 24hr on transparent Diagnostic Sensitivity Test agar at 37°C exhibit greenish yellow to yellow-red iridescence in obliquely transmitted light under a stereomicroscope \(^10\).

They are negative for oxidase, urease activity, glucose fermentation, but give variable results when tested for nitrate reduction and this depends on the strain\(^10\).

**Bordetella holmesii**

They are small coccoid and short rods, with medium-width longer rods occasionally observed. On blood agar, colonies are punctuate, semiopaque, convex, and round with complete edges. A zone of browning or greening of the media is observed. They are oxidase negative, non-motile, asaccharolytic, fastidious and they produce a brown soluble pigment. They do not grow on Simmons Citrate agar but grow on MacConkey agar plates at 3-7 days after incubation of 35°C\(^11\). They are negative for motility, aerobic growth at 25°C and at 42°C, urease activity, glucose fermentation but positive for arginine, praline and leucyl glycine\(^10\).
**Bordetella hinzii**

The cells are motile by means of peritrichous flagella. Two distinct colony types occur. Some strains on blood agar plates show round, convex, glistening, greyish colonies about 1 to 2mm in diameter after 24-48hr of incubation at 37°C in air containing 5% CO₂. Under the same conditions, other strains produce flat, dry, crinkled colonies that are up to 5mm in diameter. *Bordetella hinzii* also grows on MacConkey agar, and are positive for catalase, oxidase and assimilation of citrate adipate, L-malate and phenylacetate. They give variable results for urease production and do not reduce nitrates. They are also negative for glucose fermentation and they grow aerobically at 25°C and 42°C.

**Bordetella petrii**

These are characterized by an ability to grow in aerobic, microaerophilic and anaerobic conditions. Cells possess fimbriae of different diameters. The organism can be cultured on MacConkey agar and appears as creamy white non-haemolytic colonies on blood agar. They are asaccharolytic, non-fermenting bacteria. They are positive for oxidase and tetrazolium reduction tests; and have negative reaction for urease production, nitrate reduction and motility. They can assimilate, citrate, adipate, L-malate and D-Gluconate.

They are susceptible to erythromycin, gentamicin, ceftriaxone, and piperacillin/tazobactam and are resistant to amoxicillin, co-amoxiclav, tetracycline, clindamycin, ciprofloxacin and metronidazole.

**Bordetella avium**

These are non-lactose fermenting, small rods that are characterized by the ability to grow in aerobic conditions. They can grow on trypticase soy agar supplemented with 5% sheep blood, chocolate agar and MacConkey agar incubated at 35°C in 5% CO₂. They appear as non-haemolytic colonies on blood agar. They are positive for motility, oxidase, catalase and tetrazolium reduction tests; and negative for nitrate reduction and urease production. They can assimilate L-malate, adipate and phenylacetate although some strains may exhibit a weak reaction for L-malate and adipate.

**Principles of Identification**

Colonies isolated on Bordetella selective agar are identified preliminarily by colonial appearance, Gram stain and slide agglutination with polyvalent antiserum. Biochemical and other additional tests are used to distinguish between species of the genus *Bordetella* and to differentiate *Bordetella* from similar organisms.

Presumptive and confirmed positive isolates of *B. pertussis* and *B. parapertussis* should be referred to the Respiratory and Vaccine Preventable Bacteria Reference Unit.

Full molecular identification using for example, PCR and Matrix Assisted Laser Desorption Ionisation Time-of-Flight Mass Spectrometry (MALDI-TOF MS) can be used to identify isolates to species level.

All PHE laboratories offering *B. pertussis* PCR testing should refer all positive samples to PHE Colindale for surveillance purposes.

For information, contact the laboratory or see the following website for details: [https://www.gov.uk/rvpbru-reference-and-diagnostic-services](https://www.gov.uk/rvpbru-reference-and-diagnostic-services).
Technical Information/Limitations

Agar Media

Plates should be incubated aerobically in a moist chamber for 5 to 7 days at 35 to 37°C. Do not incubate in an aerobic atmosphere enriched with carbon dioxide.

Cefalexin is included into the Charcoal media as an inhibitor of many Gram positive and certain Gram negative bacteria present in the normal throat flora, but is not completely inhibitory to all organisms. Growth of *B. pertussis* is slightly retarded on cefalexin-containing media. Some strains of *B. pertussis* are said to be inhibited by cefalexin; therefore, the use of both selective and non-selective media has been advocated\(^{16}\).

Another selective media, modified Cyclodextrin Solid Medium (MCS) with cefdinir can be used. It has shown to improve the selective isolation of *B. pertussis* from clinical specimens, exhibit higher sensitivity and greater inhibition of nasopharyngeal flora than the media with cefalexin. A long shelf life is another benefit of this medium as most clinical microbiology laboratories are infrequently required to culture specimens from pertussis patients\(^ {17}\). The cost of the MCS medium is similar to that of other media used.
1 Safety Considerations

Hazard Group 2 organisms.

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

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet\textsuperscript{26}.

If a swab is used to harvest growth from a plate and emulsify it in saline for agglutination tests, a risk of infection may result, and should be included in local risk assessments.

In the case of sputa, or other lower tract respiratory material, where the risk is that these samples may contain viable \textit{Mycobacterium tuberculosis} (MTB); all work must be carried out in a Containment Level 3 facility.

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

Compliance with postal and transport regulations is essential.

2 Target Organisms

\textit{Bordetella} species reported to have caused pertussis\textsuperscript{4} - \textit{Bordetella pertussis}, \textit{Bordetella parapertussis}

Other \textit{Bordetella} species reported to have caused infections in humans - \textit{Bordetella bronchiseptica}\textsuperscript{35}, \textit{Bordetella trematum}\textsuperscript{10}, \textit{Bordetella hinzii}\textsuperscript{12}, \textit{Bordetella holmesii}\textsuperscript{11}, ‘\textit{Bordetella ansorpii}’, \textit{Bordetella petrii}\textsuperscript{14}, \textit{Bordetella avium}\textsuperscript{15}.

3 Identification

3.1 Microscopic Appearance

\textbf{Gram stain (TP 39 - Staining Procedures)}

Gram negative, thin coccobacilli occurring singly or in pairs, rarely in chains. Some strains may be capsulated.

3.2 Primary Isolation Media

Charcoal selective agar, incubated aerobically with high humidity and good circulation of air, for 7 days at 35°C-37°C is used for primary isolation. However, extending plate incubation up to 12 days has shown improved recovery of \textit{Bordetella} species from clinical specimens\textsuperscript{36}.

3.3 Colonial Appearance

Colonies of \textit{B. pertussis} on charcoal blood agar with cefalexin are smooth, convex, pearly and glistening, greyish-white and butyrous and appear in 3 days on subculture, longer on primary isolation. Colonies of \textit{B. parapertussis} are similar but larger, duller and become visible within two days. On subculture to nutrient agar, \textit{B. parapertussis} colonies produce a brown pigment, which diffuses into the medium. \textit{B. pertussis} does not grow on nutrient agar.
3.4 Test Procedures

3.4.1 Biochemical tests

Oxidase test (TP 26 - Oxidase Test)

*B. parapertussis* is oxidase negative, *B. pertussis* is oxidase positive.

Agglutination (slide) with specific antiserum

Follow manufacturer’s instructions and kits should be validated and be shown to be fit for purpose prior to use. A suspension of the suspect colony should be prepared in saline on a microscope slide. Specific *B. pertussis* antiserum, *B. parapertussis* antiserum or saline should be added to the suspensions and mixed.

A positive result is indicated by agglutination with one specific antiserum and no agglutination with saline. If the agglutination result is equivocal, refer the isolate.

Refer isolates of suspected *B. pertussis* and *B. parapertussis* to the Respiratory and Vaccine Preventable Bacteria Reference Unit for further characterisation.

3.4.2 Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS)

This has been shown to be a rapid and powerful tool because of its reproducibility, speed and sensitivity of analysis. The advantage of MALDI-TOF as compared with other identification methods is that the results of the analysis are available within a few hours rather than several days. The speed and the simplicity of sample preparation and result acquisition associated with minimal consumable costs make this method well suited for routine and high throughput use.

This new and less time consuming technique has been used to produce reliable species-level identification for the non-classical *Bordetella* species, as in the case of endocarditis on a prosthetic homograft aortic valve caused by *Bordetella holmesii* where routine laboratory testing initially misidentified the strain as *Acinetobacter* specie but 16S rRNA gene and outer membrane protein A (*ompA*) gene sequencing and identification by MALDI-TOF MS were all consistent with *B. holmesii*.

3.4.3 Nucleic Acid Amplification Tests (NAATs)

PCR is usually considered to be a good method as it is simple, 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.

This is an invaluable tool both for enhanced epidemiological surveillance and for the provision of a rapid diagnosis of Pertussis where results can affect patient (and contact) management. This has been used successfully in the identification of *Bordetella pertussis* and *B. holmesii*. This service (by the Respiratory and Vaccine Preventable Bacteria Reference Unit) is used for infants aged ≤6months admitted to a paediatric unit with respiratory illness compatible with pertussis.

3.5 Further Identification

Rapid Molecular Methods

Molecular methods have had an enormous impact on the taxonomy of *Bordetella*. Analysis of gene sequences has increased understanding of the phylogenetic
relationships of *Bordetella* species and this has resulted in the recognition of new species. Molecular techniques have made identification of many species more rapid and precise than is possible with phenotypic techniques.

A variety of rapid typing methods have been developed for isolates from clinical samples; these include molecular techniques such as Pulsed Field Gel Electrophoresis (PFGE), Multiple-locus Variable-Number Tandem Repeat Analysis (MLVA) and 16S rRNA gene 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 a clinical laboratory.

**Pulsed Field Gel Electrophoresis (PFGE)**

PFGE detects genetic variation between strains using rare-cutting restriction endonucleases, followed by separation of the resulting large genomic fragments on an agarose gel. PFGE is known to be highly discriminatory and is frequently used technique for outbreak investigations and has gained broad application in characterising epidemiologically related isolates. However, the stability of PFGE may be insufficient for reliable application in long-term epidemiological studies. However, due to its time consuming nature (30hr or longer to perform) and its requirement for special equipment, PFGE is not used widely outside the reference laboratories.

Molecular characterisation of strains is important in identifying pertussis epidemiology. This method was used initially but was found to be laborious and results were difficult to compare between laboratories.

**Multiple-locus Variable-Number Tandem Repeat Analysis (MLVA)**

MLVA is a robust, simple, and portable method which can be used to create strain profiles that are easily electronically exchanged. MLVA has been successfully used to type several different bacterial species and proven to be an excellent method with high resolution, particularly useful for organisms with a low level of sequence diversity.

This new approach, MLVA Typing was introduced and this is used to analyse the number of tandem repeat sequences in the *B. pertussis* genome. This technique does not require culturing and can be applied directly to nasal or pharyngeal swabs. Variable-number tandem repeat (VNTR) analysis has revealed considerable heterogeneity of the *B. pertussis* genome and clonal expansion during epidemic periods.

**16S rRNA gene sequencing**

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.

This method has been used recently for the accurate identification of the non-classical *Bordetella* species (ie not including *B. pertussis*, *B. parapertussis* or *B. bronchiseptica*) as in the first case of fatal septicemia caused by *Bordetella hinzii*.

The greater mutational variation of the *Bordetella* outer membrane protein A gene (*ompA*) gene compared to the 16S rRNA gene allows unambiguous identification of the non-classical *Bordetella* species. However, it should be noted that the 16S rRNA...
gene sequences of *B. pertussis*, *B. parapertussis* and *B. bronchiseptica* are identical so this method cannot be used to provide a species identification within these 3 species. This is also the case for the *ompA* sequence of these 3 species\(^4\).  

### 3.6 Storage and Referral  

Save pure isolates on a charcoal blood agar slope for referral to the Reference Laboratory. The slope may require several days incubation before adequate growth is achieved.
4 Identification of *Bordetella* species

Clinical specimens
Primary isolation plate

Charcoal blood agar with cefalexin
Butyrous glistening greyish-white colonies at 3-7 days (may be 2 days for *B. parapertussis*)

Gram stain of pure culture
Gram negative thin coccobacilli

Oxidase test

Positive
- *B. pertussis*
- *B. bronchiseptica*
- *B. hinzii*
- *B. petrii*
- *B. avium* (*

Negative
- *B. parapertussis*
- *B. ansorpii*
- *B. trematum*
- *B. holmesii*
- *B. avium* (*

Agglutination with specific antiserum on pure culture and organisms giving the biochemical profile of *Bordetella* species

Positive agglutination
- Strongly suspected to be *Bordetella* species based on colonial morphology or clinical details

Negative agglutination
- Not strongly suspected to be *Bordetella* species based on colonial morphology or clinical details
  - Carry out additional tests
  - Report as *Bordetella* species not isolated

Further identification if clinically indicated
Refer to the Reference Laboratory
If required, save the pure isolate on to a charcoal blood agar slope

* *B. avium* is positive when Kovac’s oxidase reagent is used and negative when Gaby and Hadley reagents are used.

The flowchart is for guidance only.
5 Reporting

5.1 Presumptive Identification
If appropriate growth characteristics, colonial appearance, Gram stain of the culture, oxidase and serological results are demonstrated.

5.2 Confirmation of Identification
Following the Reference Laboratory report.

5.3 Medical Microbiologist
Inform the medical microbiologist of presumptive and confirmed *B. pertussis* or *B. parapertussis* isolates in accordance with local protocols.
Follow local protocols for reporting to the patient's clinician.

5.4 CCDC
Refer to local Memorandum of Understanding.

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

“Whooping cough” is a Notifiable disease, for public health management of cases, contacts and outbreaks, all suspected cases should be immediately notified 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.

5.6 Infection Prevention and Control Team
Inform the hospital infection prevention and control team of presumptive and confirmed *B. pertussis* or *B. parapertussis* isolates from hospital inpatients. Other isolates should be reported to the relevant Infection Control Staff in accordance with local protocols, notably if an outbreak is suspected.

6 Referrals

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

Respiratory and Vaccine Preventable Bacteria Reference Unit
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{47,48} or Equivalent in the Devolved Administrations\textsuperscript{49-52}

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.

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’.


Other arrangements exist in Scotland\textsuperscript{49,50}, Wales\textsuperscript{51} and Northern Ireland\textsuperscript{52}.
References


18. 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".


