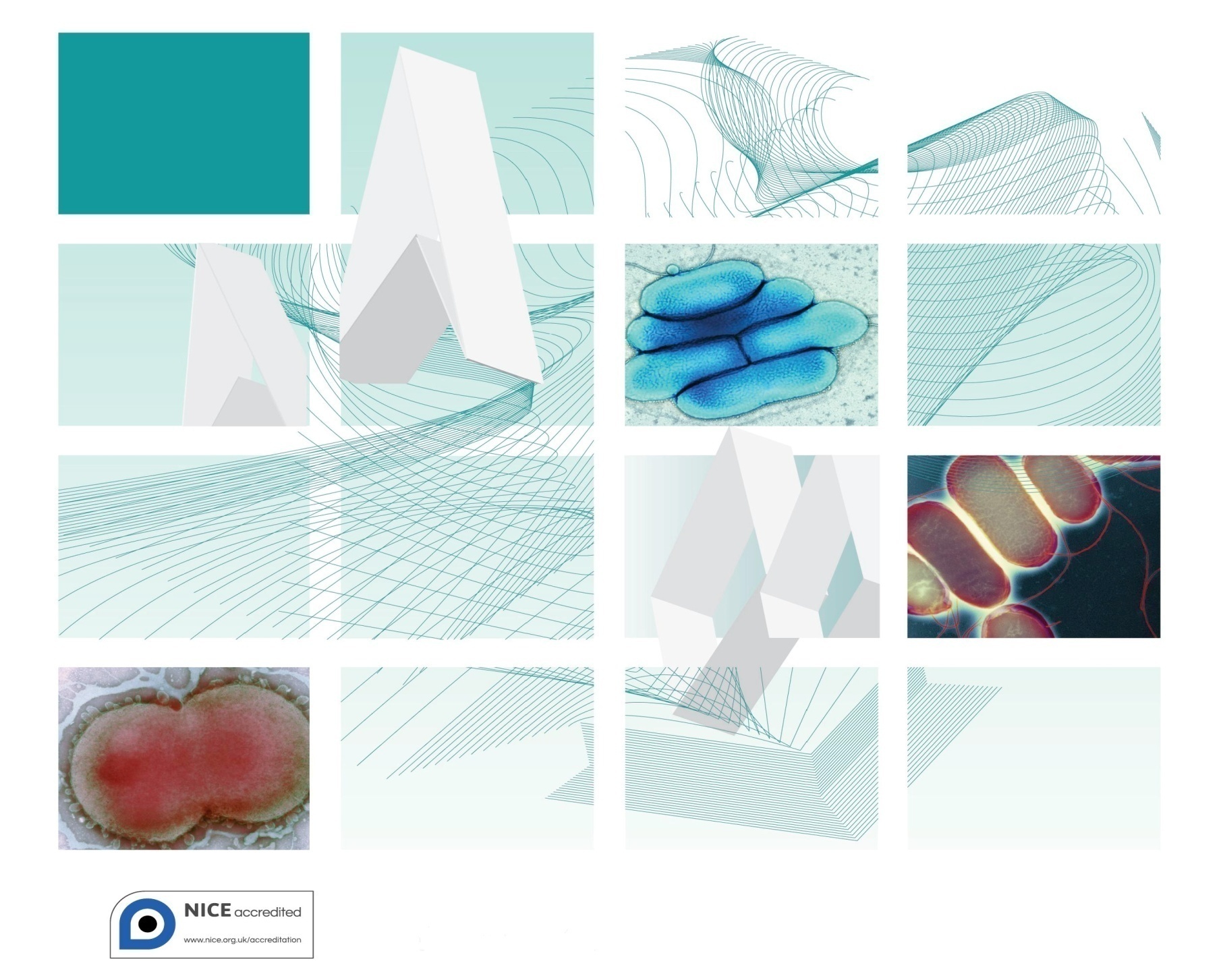
UK Standards for Microbiology Investigations

Culture of Specimens for *Bordetella pertussis* and *Bordetella parapertussis*



Acknowledgments

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.

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UK Standards for Microbiology Investigations are produced in association with:



Logos correct at time of publishing.

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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](mailto:standards@phe.gov.uk).

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

|  |  |
| --- | --- |
| Amendment No/Date. | 11/10.11.14 |
| Issue no. discarded. | 7.2 |
| Insert Issue no. | 8 |
| **Section(s) involved** | **Amendment** |

|  |  |
| --- | --- |
| Whole document. | Hyperlinks updated to gov.uk. |
| Page 2. | Updated logos added. |
| Whole document. | The title changed from investigation to culture of specimens and therefore reference to serological investigation removed or minimised. The focus of the SMI is culture for Pertussis. |
| Type of Specimen. | Updated. |
| Scope. | Updated. |
| Introduction. | Updated to include PCR and MALDI-TOF and brought in line with the PHE Guidelines for Public Health Management of Pertussis 2012. |
| Technical Information/Limitations. | Updated and additional information added. |
| References. | Some references updated. |

UK SMI[[1]](#footnote-1)#: Scope and Purpose

Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also 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. The documents also 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.

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

Public Health England. (2014). Culture of Specimens for *Bordetella pertussis* and *Bordetella parapertussis*. UK Standards for Microbiology Investigations. B 6 Issue 8. <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>

Scope of Document

Type of Specimen

Pernasal swab, nasopharyngeal aspirate, nasopharyngeal swab

Scope

The SMI describes the culture and bacteriological investigation of pernasal swabs, nasopharyngeal aspirates and nasopharyngeal swabs for *Bordetella pertussis* and *Bordetella parapertussis*. Information about serological confirmation of pertussis is available from the [PHE website](https://www.gov.uk/rvpbru-reference-and-diagnostic-services).

This SMI should be used in conjunction with other SMIs.

Introduction

Pertussis, commonly known as whooping cough (“violent cough”) has been associated with high morbidity and mortality, particularly in infants. Whooping cough is a highly contagious disease that is caused by the fastidious Gram negative coccobacillus   
*B. pertussis* and *B. parapertussis* that colonises the respiratory tract1. The main symptoms include malaise, fever followed by long bursts of coughing and choking leaving the infected person gasping for breath with a characteristic whoop sound.

*B. pertussis* usually infects and causes severe respiratory disease in young children with infants under six months of age at most risk of severe complications. The infection can occur in adolescents and adults who exhibit milder symptoms of flu like illness followed by a prolonged cough2,3. The incubation period of pertussis is on average between 7–10 days (range 5–21days).

Despite a sustained period of high vaccine coverage, pertussis continues to display cyclical peaks in activity occurring every three to four years. An increase in pertussis activity in England and Wales was observed from the third quarter of 2011, predominantly in adolescents and adults. This increase continued into 2012 and extended into infants under three months who are at highest risk of severe complications, hospitalisation and death (<https://www.gov.uk/government/collections/pertussis-guidance-data-and-analysis>).

Diagnosis of pertussis is usually straight forward however, *formesfrustes* (abortive or atypical disease; disease stopped before it has run its full course) are known to occur, and may cause diagnostic difficulty. Consideration should be given to appropriate evaluation of patients with pertussis in whom infection with *B. pertussis* or   
*B. parapertussis* cannot be demonstrated. In addition to sampling for pertussis, it is recommended that consideration is given to testing the patient for respiratory viruses according to local procedures.

Laboratory confirmation of clinically suspected cases can be made by culture and isolation of the causative organisms *B. pertussis* and *B. parapertussis*, detection of its DNA (typically from nasopharyngeal swabs/pernasal swabs or nasopharyngeal aspirates) or serological tests (which usually only provide a late or retrospective diagnosis). Culture has sensitivity as low as 20-40% and can be affected by a number of factors as the organism is delicate including delays in processing and specimen quality4.

Culture is the gold standard for diagnosis of infection with *B. pertussis* and *B. parapertussis*. The method is highly specific but the sensitivity is low, declining with the duration of illness and with the age of the patients. However, polymerase chain reaction (PCR) is a specific, sensitive and rapid method for the diagnosis of pertussis in respiratory samples5. Developments in PCR have enabled the detection and differentiation of *B. pertussis* from other species of *Bordetella.* However, several amplification targets used for *Bordetella* are present in more than one *Bordetella* species and therefore PCR results should be interpreted with caution eg IS *481* is present in *B. pertussis, B. holmesii and B. bronchiseptica*6. qPCR is invaluable in pertussis confirmation in young infants and has shown to have improved sensitivity over culture7. qPCR is also valuable for epidemiological analysis however, culture techniques still remain necessary for antibiotic susceptibility8,9.

Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been shown to be a rapid and powerful identification 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 days10. However, there is currently very little scientific information published on use of MALDI-TOF MS for detection of *Bordetella* species11.

Early laboratory diagnosis is important for control and prevention of whooping cough. Isolation and typing of the organism is also important for the continued monitoring of the vaccine programme. Vaccination provides the most effective strategy for preventing pertussis transmission in the population, although protection afforded by vaccination or from past infection is not lifelong12.

In response to a significant increase in laboratory confirmed cases of pertussis and the high rates of disease in young infants, the Health Protection Agency (Public Health England since April 2013) declared a level 3 incident (national outbreak) in April 201213. On 28th September 2012, the Department of Health announced the introduction of a temporary programme to vaccinate pregnant women against pertussis14. This temporary programme, which is an outbreak control measure, aims to passively protect infants from birth before they reach the age of routine immunisation and during the period of greatest risk of complications and death12.

Technical Information/Limitations

Specimen Containers15,16

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”.

Selective Media17-19

The nature of selective media requires a balance between the performance characteristics and the costs of the tests. Selective media may not support the growth of all circulating strains of organisms. Refer to manufacturer’s instructions and recent evidence for limitations of growth.

The media should support the growth of *B. pertussis* and *B. parapertussis*, suppress nasopharyngeal flora and be stable during storage. There are several different types of medium available that contain blood or charcoal or both, along with selective antibiotic supplements - penicillin, cefalexin or meticillin.

Meticillin is the least inhibitory of these towards *B. pertussis*, but is also the least inhibitory towards nasopharyngeal flora. Cefalexin is the most inhibitory towards nasopharyngeal flora and is superior to penicillin. For these reasons it is the antibiotic of choice for selective media in this SMI19.

Primary isolation plates are incubated at 35-37°C, in an aerobic moist atmosphere maintained for 7 days18. A thickly poured plate is necessary to avoid desiccation on prolonged incubation.

Specimen Type

Current recommendation for specimen of choice is nasopharyngeal aspirates or nasopharyngeal swabs/pernasal swabs12. In addition to sampling for pertussis, it is recommended that consideration is given to testing the patient for respiratory viruses according to local procedures.

Cough plates are not recommended.

Pernasal Swabs

Dacron and rayon swabs are the swabs of choice for both PCR and culture. Both types of synthetic material performed well in studies with neither superior to the other20.

1 Safety Considerations15,16,21-35

1.1 Specimen Collection, Transport and Storage15,16,21-24

Use aseptic technique.

Collect specimens in appropriate transport medium in CE marked leak proof containers and transport in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen Processing15,16,21-35

Containment Level 2.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet27.

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

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

2 Specimen Collection

2.1 Type of Specimens

Pernasal swab, nasopharyngeal aspirate, nasopharyngeal swab

2.2 Optimal Time and Method of Collection36

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible36.

Swabs should be collected and transported in charcoal-based transport medium such as Regan-Lowe.

Pernasal swabs

A pernasal swab (Dacron or rayon with flexible ultrafine wire shaft) is inserted through a nostril and advanced along the floor of the nose until it reaches the nasopharynx. It has been suggested that the swab is held against the posterior nasopharynx for up to 30s or until the patient coughs. In practice, it is more likely that a patient will only be able to tolerate this for a few seconds.

Nasopharyngeal specimens

Sampling of nasopharyngeal secretions in patients with whooping cough may precipitate a paroxysm of coughing and cause obstruction of the airways. Resuscitation equipment must be available if whooping cough is suspected. The specimen collector should avoid exposure to direct coughs from the patient.

Nasopharyngeal exudate may be obtained using a suction catheter (No.8 French) inserted through the nose. The exudate is collected in a sterile plastic trap in which the specimen is transported to the laboratory, or in a sterile clear plastic universal container (30mL or 60mL, to BS 5213).

**Note:** Cough plates are not recommended.

Collect specimens other than swabs into appropriate CE marked leak proof containers and place in sealed plastic bags.

Unless otherwise stated, swabs for bacterial and fungal culture should then be placed in appropriate transport medium37-41.

2.3 Adequate Quantity and Appropriate Number of Specimens36

Numbers and frequency of specimen collection are dependent on clinical condition of patient.

3 Specimen Transport and Storage15,16

3.1 Optimal Transport and Storage Conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible36.

If processing is delayed, refrigeration is preferable to storage at ambient temperature36.

4 Specimen Processing/Procedure15,16

4.1 Test Selection

N/A

4.2 Appearance

N/A

4.3 Sample Preparation

For safety considerations refer to Section 1.2.

4.4 Microscopy

N/A

4.5 Culture and Investigation

Pernasal and nasopharyngeal swabs

Inoculate each agar plate with swab (refer to [Q 5 - Inoculation of Culture Media for Bacteriology)](https://www.gov.uk/government/collections/standards-for-microbiology-investigations-smi#quality-related-guidance).

For the isolation of individual colonies, spread inoculum with a sterile loop.

Nasopharyngeal aspirate

With a sterile loop select a representative portion of specimen and inoculate a loopful to each agar plate (refer to [Q 5 - Inoculation of Culture Media for Bacteriology)](https://www.gov.uk/government/collections/standards-for-microbiology-investigations-smi#quality-related-guidance).

For the isolation of individual colonies, spread inoculum with a sterile loop.

4.5.1 Culture media, conditions and organisms

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Clinical details/**  **conditions** | **Specimen** | **Standard media** | **Incubation** | | | **Cultures read** | **Target organism(s)** |
| **Temp °C** | **Atmos** | **Time** |
| Pertussis or whooping cough | Pernasal swab, nasopharyngeal aspirate, nasopharyngeal swab | Charcoal blood agar with cefalexin | 35-37 | air,  moist chamber | 7d | 4d and  7d | *B. pertussis*  *B. parapertussis* |

4.6 Identification

Refer to individual SMIs for organism identification.

4.6.1 Minimum level of identification in the laboratory

|  |  |
| --- | --- |
| [*Bordetella* species](https://www.gov.uk/government/collections/standards-for-microbiology-investigations-smi#identification) | "Species" level |

4.7 Antimicrobial Susceptibility Testing

N/A

4.8 Referral for Outbreak Investigations

N/A

4.9 Referral to Reference Laboratories

For information on the tests offered, turn around times, transport procedure and the other requirements of the reference laboratory [click here for user manuals and request forms](https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services).

For the investigation of suspected clusters or outbreaks of pertussis, please contact the Respiratory and Vaccine Preventable Bacteria Reference Unit, Colindale for the most appropriate test.

Information regarding specialist and reference laboratories is available via the following website: [PHE - Specialist and Reference Microbiology Tests and Services](https://www.gov.uk/rvpbru-reference-and-diagnostic-services).

Organisms with unusual or unexpected resistance, and whenever there is a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

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

England and Wales

<https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services>

Scotland

<http://www.hps.scot.nhs.uk/reflab/index.aspx>

Northern Ireland

<http://www.publichealth.hscni.net/directorate-public-health/health-protection>

5 Reporting Procedure

5.1 Microscopy

N/A

5.2 Culture

Negatives

"*Bordetella pertussis* NOT isolated".

Positives

"*Bordetella pertussis* isolated" or

"*Bordetella parapertussis* isolated".

5.3 Antimicrobial Susceptibility Testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

6 Notification to PHE42,43 or Equivalent in the Devolved Administrations44-47

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’.

<https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010>

Other arrangements exist in [Scotland](http://www.scotland.gov.uk/Topics/Health/Policy/Public-Health-Act/Implementation/Guidance/Guidance-Part2)44,45, [Wales](http://www.wales.nhs.uk/sites3/page.cfm?orgid=457&pid=48544)46 and [Northern Ireland](http://www.publichealth.hscni.net/directorate-public-health/health-protection)47.

Appendix: Culture of Specimens for *Bordetella pertussis* and *Bordetella parapertussis*



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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. [↑](#footnote-ref-1)