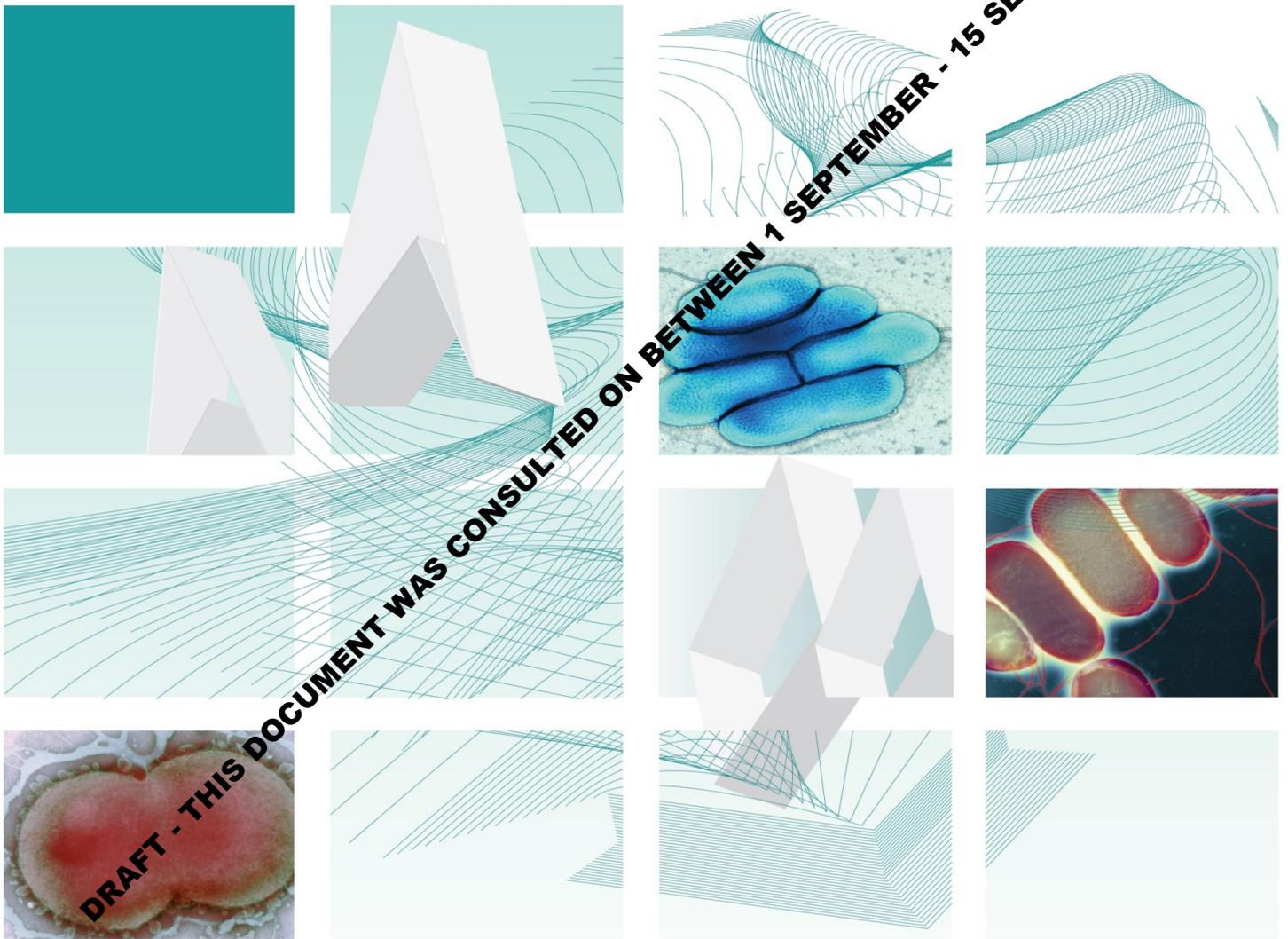




UK Standards for Microbiology Investigations

Investigation of *Bordetella pertussis* and *Bordetella parapertussis*



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Acknowledgments

UK Standards for Microbiology Investigations (UK 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>. UK 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
National Infection Service
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>

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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.

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

Amendment number/date	
Issue number discarded	
Insert issue number	
Anticipated next review date*	
Section(s) involved	Amendment

*Reviews can be extended up to five years subject to resources available.

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UK SMI[#]: scope and purpose

Users of UK SMIs

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

UK 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. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of UK 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

UK 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 UK SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing UK SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop UK 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. UK SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the UK SMI working groups to produce UK SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of UK SMIs is certified to ISO 9001:2008. UK SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. UK SMIs are NICE

[#] 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.

accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using UK SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. UK SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. UK SMIs also provide a reference point for method development. The performance of UK 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 UK SMI working groups are committed to patient and public involvement in the development of UK SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting UK 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 UK SMIs is subject to PHE Equality objectives <https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity>.

The UK 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

While every care has been taken in the preparation of UK SMIs, PHE and the partner organisations, 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 UK SMI or any information contained therein. If alterations are made by an end user to an UK SMI for local use, it must be made clear where in the document the alterations have been made and by whom such alterations have been made and also acknowledged that PHE and the partner organisations shall bear no liability for such alterations. For the further avoidance of doubt, as UK SMIs have been developed for application within the UK, any application outside the UK shall be at the user's risk.

The evidence base and microbial taxonomy for the UK SMI is as complete as possible at the date 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.

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Suggested citation for this document

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Scope of document

Type of specimen

Pernasal swab, nasopharyngeal aspirate, nasopharyngeal swab

The UK 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](#).

This UK SMI should be used in conjunction with other UK SMIs.

Introduction

Pertussis, commonly known as whooping cough (“violent cough”) has been associated with high morbidity and mortality, particularly in infants¹. 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 tract². 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 sound³.

B. pertussis usually infects and causes severe respiratory disease in young children with infants under six months of age at most risk of severe complications³. The infection can occur in adolescents and adults who exhibit milder symptoms of flu like illness followed by a prolonged cough^{4,5}. The incubation period of pertussis is on average between 7–10 days (range 5–21 days)².

Despite a sustained period of high vaccine coverage, pertussis continues to display cyclical peaks in activity occurring every three to four years⁶. 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, *forms frustes* (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)⁷.

Culture is conventionally performed to confirm infection with *B. pertussis* and *B. parapertussis*. The method is highly specific but sensitivity is low 20-40% and can be affected by a number of factors as the organism is delicate including delays in processing and specimen quality⁸. Culture is also more likely to be unsuccessful the

longer the time since the onset of illness. Diagnostic sensitivity can be maximised by supplementing culture with polymerase chain reaction (PCR) methods and serology. PCR is more sensitive than culture as it does not require organisms to be viable. Serology is particularly useful in diagnosing infection in patients who have been coughing for some weeks, when both culture and PCR would be anticipated to be unhelpful^{4,9-19}.

Developments in PCR have enabled the detection and differentiation of *B. pertussis* from other species of *Bordetella*. However amplification targets used for *Bordetella* are present in more than one *Bordetella* species eg IS 481 is present in *B. pertussis* and *B. holmesii*. Although there is evidence that IS481 detection of *B. pertussis* should not be confounded as other *Bordetella* species are not among the causative agents of pertussis-like symptoms, in the available qPCR assays, two regions of the *B. pertussis* genome are targeted to ensure a correct interpretation: the pertussis toxin S1 promoter region (ptxA-pr), and the insertion element IS481²⁰⁻²⁵.

Recommended interpretation is as follows:

IS481	ptxP	Final reported result
+	+	<i>B. pertussis</i> DNA detected by PCR
+	-	<i>Bordetella</i> spp. DNA detected by PCR

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 days²⁶. Increasingly MALDI-TOF is being used to identify bacteria (including *Bordetella* species) in hospital microbiology laboratories²⁷, however, there is currently very little scientific information published on use of MALDI-TOF MS for detection of *Bordetella* species²⁸.

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 lifelong²⁹.

In 2012, 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)³⁰. On 28th September 2012, the Department of Health announced the introduction of a temporary programme to vaccinate pregnant women against pertussis³¹. This temporary programme, which is an outbreak control measure, aimed to passively protect infants from birth before they reach the age of routine immunisation and during the period of greatest risk of complications and death²⁹. From the 1st April 2016, Public Health England suggests that pertussis containing vaccine should be offered to pregnant women from 16 weeks gestation, ideally after their foetal anomaly scan (usually at around 20 weeks)³².

Technical information/limitations

Specimen containers^{33,34}

UK 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 media³⁵⁻³⁷

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. parapertussis*, 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 SMI³⁷.

Primary isolation plates are incubated at 35-37°C, in an aerobic moist atmosphere maintained for 7 days³⁶. 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 swabs²⁹. 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 other³⁸.

1 Safety considerations^{33,34,39-52}

1.1 Specimen collection, transport and storage^{33,34,39-42,53}

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 processing^{33,34,39-52}

Containment Level 2.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet⁴⁵.

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

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

2 Specimen collection

2.1 Type of specimens

Pernasal swab, nasopharyngeal aspirate, nasopharyngeal swab

2.2 Optimal time and method of collection⁵⁴

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible⁵⁴.

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 medium⁵⁵⁻⁵⁹.

2.3 Adequate quantity and appropriate number of specimens⁵⁴

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

3 Specimen transport, storage and retention^{33,34}

3.1 Optimal transport and storage conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible.

If processing is delayed, refrigeration is preferable to storage at ambient temperature⁵⁴.

Samples should be retained in accordance with The Royal College of Pathologists guidelines 'The retention and storage of pathological records and specimens'⁶⁰.

4 Specimen processing/procedure^{33,34}

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](#)).

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](#)).

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	<i>B. pertussis</i> <i>B. parapertussis</i>

4.6 Identification

Refer to individual UK SMIs for organism identification.

4.6.1 Minimum level of identification in the laboratory

Bordetella species	"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](#).

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](#).

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 PHE^{61,62}, or equivalent in the devolved administrations⁶³⁻⁶⁶

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.

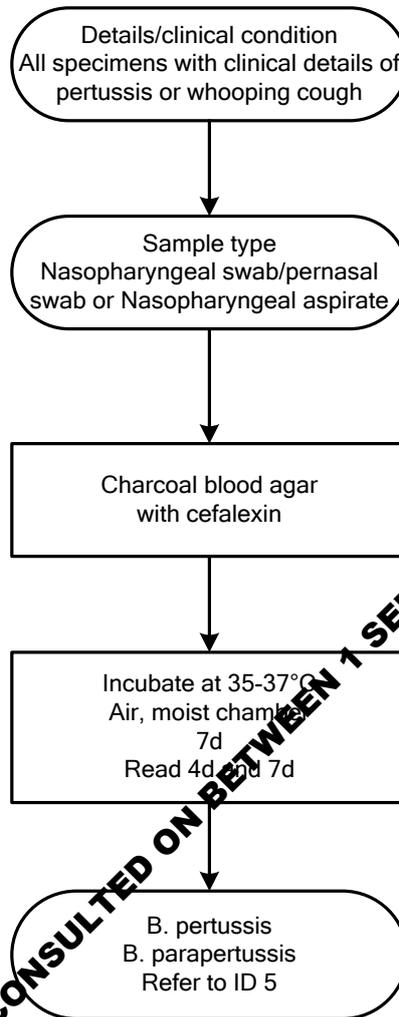
Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health Protection Teams have agreements with local 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 (HCAs) 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](#)^{63,64}, [Wales](#)⁶⁵ and [Northern Ireland](#)⁶⁶.

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



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