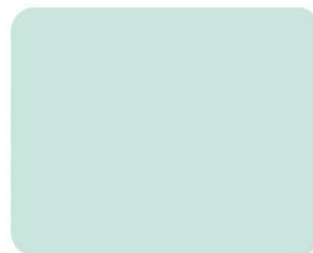
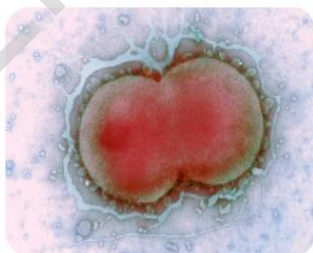
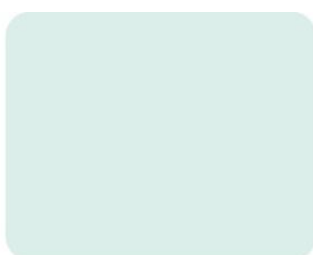




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UK Standards for Microbiology Investigations

Investigation of whooping cough



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Amendment table

The amendments since the previous version of this UK SMI document are listed in the amendments table below.

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

Amendment number/date	x/dd.mm.yy
Issue number discarded	
Insert issue number	
Anticipated next review date*	dd.mm.yy
Section(s) involved	Amendment

*Reviews can be extended up to 5 years where appropriate

1 General information

[View general information](#) related to UK SMIs.

2 Scientific information

[View scientific information](#) related to UK SMIs.

3 Scope of document

This UK Standards for Microbiology Investigation (UK SMI) document describes the investigation and confirmation of *Bordetella* species by Nucleic Acid Amplification Tests (NAAT), culture and serology.

Culture is still a valid method to confirm a suspected pertussis case. However, it's diagnostic use has decreased due to the higher sensitivity and rapidity of NAAT.

Aside from diagnosis, cultures are valuable for public health purposes, including antimicrobial resistance surveillance, typing for detecting transmission pathways, and monitoring the evolution of vaccine-related antigens.

In addition, this document does not provide the details for the identification of organisms causing pertussis. The identification section in this document only provides information on the level to which organisms should be identified to as part of the reporting process with direct links to the relevant identification documents for reference.

For the identification of *Bordetella* species, refer to [UK SMI ID 5: Identification of *Bordetella* species](#).

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

4 Introduction

Pertussis (whooping cough) is a respiratory infection caused primarily by the Gram-negative coccobacilli *Bordetella pertussis* and *Bordetella parapertussis*. Other *Bordetella* species, including *Bordetella holmesii* and *Bordetella bronchiseptica* may also be associated with pertussis-like illness in humans (1).

Symptoms typically begin with mild fever and malaise, followed by paroxysms of coughing and gasping with a characteristic “whoop” (2).

The infection is most severe in young children, particularly infants under six months, who are at highest risk of complications (2,3). Adolescents and adults may present with milder, non-specific symptoms followed by a prolonged cough (4,5).

Pertussis shows a cyclic pattern, with peaks every 3 to 5 years and has shown recent increases despite historically high vaccine coverage (6-9). Vaccination, including maternal vaccination, remains the most effective strategy for preventing disease and protecting infants; however immunity following infection or vaccination is not lifelong (10).

4.1 Diagnostic approach

The laboratory diagnosis of pertussis involves a combination of NAAT, culture, and serological methods, with the choice of test guided by the stage of illness and clinical presentation.

In addition to testing for pertussis, consideration should be given to investigation for other respiratory pathogens (e.g. viral and atypical bacterial infections) in accordance with local diagnostic pathways.

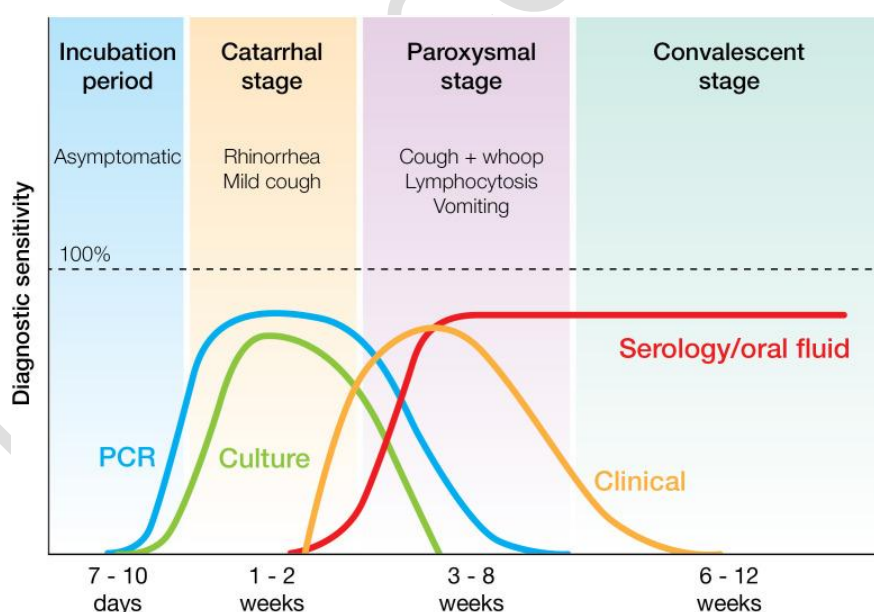


Figure 1. Schematic representation of the clinical stages of pertussis and the approximate period during which different diagnostic approaches may be informative. Reproduced from Fry et al 2021 (11).

Note: The figure is intended to illustrate the timing and overlap of diagnostic investigations during pertussis infection. The curves are illustrative only and do not represent the relative sensitivity or specificity of the different diagnostic methods.

4.1.1 Nucleic Acid Amplification Tests (NAAT)

NAAT is the preferred diagnostic test for suspected *Bordetella pertussis* infection because of its high sensitivity, rapid turnaround time, and ability to detect *Bordetella* organisms that may be difficult to culture or are no longer viable. NAAT is most informative when performed during the early stages of disease, typically within the first 2 to 3 weeks of cough onset and may remain useful beyond this period when culture sensitivity has declined.

Molecular assays for the detection of *Bordetella* species use a variety of genetic targets and target combinations. The genetic targets used, analytical performance characteristics and species differentiation capabilities vary between manufacturers and testing platforms.

Commonly employed targets include the insertion sequences (IS) IS481 and IS1001 which are present in high copy numbers within bacterial genomes and therefore provide high analytical sensitivity. However, the specificity of these targets is limited because they may be present in more than one *Bordetella* species. Due to this overlap, detection of a single insertion sequence target alone is insufficient for definitive species identification

Additional targets that may be used to improve species differentiation and confirmation are shown in Table 1. The targets listed are representative and not exhaustive as assay design varies between manufacturers and testing platforms, and some commercial assays utilise additional manufacturer-specific genetic markers (12,13).

Table 1. Examples of molecular targets used in *Bordetella* NAAT assays

This table is intended for information only and does not represent the target profile of any specific commercial assay

Target	Primary role
IS481	Highly sensitive screening target for <i>B. pertussis</i> ; also detected in <i>B. holmesii</i> and occasionally <i>B. bronchiseptica</i>
IS1001	Detection of <i>B. paraptussis</i> but may occur in some <i>B. holmesii</i> and <i>B. bronchiseptica</i> isolates
hIS1001	Differentiation of <i>B. holmesii</i> especially from <i>B. pertussis</i> in multiplex NAAT where IS481 is detected
IS1002	Supplementary IS target used alongside IS481 and/or IS1001 to support species differentiation and confirmation
ptxP	Promoter region of the pertussis toxin operon. A highly specific target for <i>B. pertussis</i> commonly used as a confirmatory marker to improve specificity of IS481-based detection
ptxA/ptxS1	Additional <i>B. pertussis</i> -specific confirmation targets

Target	Primary role
prn (pertactin)	Gene encoding pertactin, a <i>B. pertussis</i> virulence factor. Used as an additional confirmatory or supplementary target in some assays to support species identification

Optimal test performance depends on appropriate specimen collection, with nasopharyngeal swabs or nasopharyngeal aspirates (NPA) preferred over throat swabs or anterior nasal specimens, and on testing during the early phase of illness.

Some NAAT collection systems may permit downstream culture from NAAT positive samples. This approach may be useful to isolate cultures in outbreak settings and for national surveillance purposes (13,14).

4.1.2 Culture

Culture is less sensitive and more time consuming than NAAT for the diagnosis of pertussis. However, it is still a valid method for diagnosing pertussis and offers 100% specificity if positive.

Apart from diagnosis, cultures are valuable for public health purposes, including antimicrobial resistance surveillance, typing for detecting transmission pathways, and monitoring the evolution of vaccine-related antigens. Specimen requirements are summarised below; for culture methodology, refer to [UK SMI ID 5 - Identification of *Bordetella* species](#).

4.1.3 Immunological testing

Serology, specifically IgG against pertussis toxin (PT), is most useful ≥ 2 weeks after cough onset, with peak diagnostic value after week 3 when NAAT and culture sensitivity decline. However, recent pertussis vaccination or a previous infection can confound interpretation of positive results for up to 12 months.

Oral fluid testing for IgG against PT is available in the UK and provides a practical, non-invasive alternative to serology that can support pertussis surveillance where this service is available. However, recent pertussis vaccination or a previous infection can also confound interpretation for up to 12 months. Oral-fluid testing is available for children aged 2 - 16 years, and test kits can be requested from local UKHSA Health Protection Team. Testing is provided by the UKHSA national reference laboratory.

Atypical presentations and delayed recognition are common in vaccinated children (who may not exhibit a classic whoop) and in adolescents and adults presenting late with a prolonged cough meaning that many patients fall outside the optimal NAAT testing window

In such cases, serology (or oral-fluid testing in eligible children) may provide useful retrospective confirmation.

5 Safety considerations

The section covers specific safety considerations related to this UK SMI, and should be read in conjunction with the general [safety considerations](#).

Use aseptic technique.

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

Compliance with postal, transport and storage regulations is essential.

Specimen processing should occur in Containment Level 2.

It is recommended that all respiratory samples with the potential to generate aerosols during processing be handled within a microbiological safety cabinet (15). This should be done in accordance with a local risk assessment and the relevant guidance from the Advisory Committee on Dangerous Pathogens (ACDP) and the Health and Safety Executive (HSE), with additional precautions to minimise aerosol production.

Containment Level 3 precautions are particularly important unless it can be definitively ruled out that the specimen contains a Hazard Group 3 pathogen such as *Mycobacterium tuberculosis*, *Brucella* species, *Francisella* species, *Yersinia pestis*, *Burkholderia mallei*, *Burkholderia pseudomallei*, or certain endemic fungi (e.g., *Histoplasma* and *Blastomyces* species).

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

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

6 Pre-laboratory processes

6.1 Specimen type

The terms *pernasal swab* and *nasopharyngeal swab* are sometimes used interchangeably. However, nasopharyngeal swab is the preferred term throughout this document for consistency.

6.1.1 NAAT testing

- Nasopharyngeal swab

Dry swabs or swabs in a liquid universal or viral transport medium can be used according to local laboratory requirements and validation.

If attempting culture from NAAT positive samples, Amies or universal transport medium are preferred

Note: Charcoal swabs are not optimal for NAAT testing as they are more likely to cause inhibition.

- Nasopharyngeal aspirate (NPA)

Note: If a nasopharyngeal specimen cannot be obtained, a combined nose and throat swab may be used as an alternative; however, sensitivity may be lower than for nasopharyngeal specimens, with an increased likelihood of a false-negative result. Laboratories should refer to local protocols.

- Bronchoalveolar lavage fluid (BALF) or other lower respiratory tract specimens

These may be tested if locally validated and when clinically indicated.

6.1.2 Culture

- Nasopharyngeal swab

Charcoal gel transport medium is recommended (e.g. Regan Lowe charcoal medium or Amies charcoal medium).

Specimens collected in liquid transport media designed for NAAT testing are generally not recommended for culture. However, some have been shown to support *B. pertussis* viability for culturing (16,17).

- Nasopharyngeal aspirate (NPA)

6.1.3 Immunological testing

- Blood (serum)

Plasma may be acceptable for some immunoassays but only if validated by the manufacturer or locally by the laboratory.

- Oral fluid (where available and indicated).

Suitable for detection of anti-*Bordetella pertussis* toxin IgG antibodies through validated public health oral fluid testing programmes.

6.2 Specimen collection and handling

For safety considerations refer to Section 5.

For NAAT or culture, collect specimens as soon as possible after onset of symptoms, and, where possible, before antimicrobial therapy (18). Timely collection and appropriate specimen type are important to optimise diagnostic yield.

Culture is affected by several factors, as the organism is fastidious, fragile and susceptible to loss of viability through factors such as delays in processing and specimen quality (19).

A negative culture result cannot exclude infection.

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

6.2.1 Nasopharyngeal swabs

An ultrafine flexible 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 30 seconds or until the patient coughs, figure 2. In practice, it is more likely that a patient will only be able to tolerate this for a few seconds.

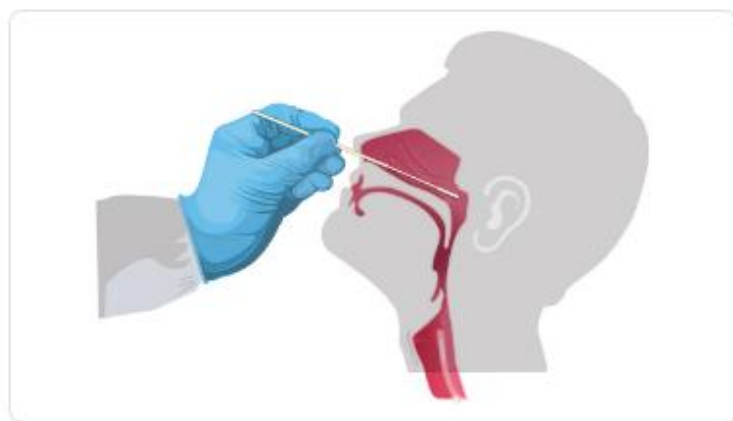


Figure 2. Nasopharyngeal specimen collection technique. Reproduced from the Centers for Disease Control and Prevention (CDC) (20).

6.2.2 Nasopharyngeal aspirates

Nasopharyngeal secretions collected by aspiration may be sampled when clinically indicated, particularly in infants or where swab collection is not feasible.

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

NPAs are obtained using a suction catheter (e.g. No. 8 French) inserted through the nose. The specimen is collected into a sterile plastic trap for transport to the laboratory, or into a sterile clear plastic universal container (30 mL or 60 mL, compliant with BS 5213).

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

Note: Cough plates are not recommended (21).

6.2.3 Serum

It is recommended that serum samples should not be taken until the patient has been coughing for at least 2 weeks, to allow sufficient time for them to mount an antibody response to infection. Antibody levels would be expected to remain elevated for many weeks following infection (13).

6.2.4 Oral fluid

Oral fluid specimens are typically self-collected by the patient, or by a parent/carer for a child, using an Oracol swab supplied as part of an oral fluid collection kit by the local UKHSA Health Protection Team. The absorbent swab is rubbed along the gum line, returned to the collection tube, and sent by post to the laboratory for testing.

6.3 Specimen transport and storage

For safety considerations refer to Section 5.

Specimens should be transported and processed as soon as possible (18).

It is recommended that samples for culturing are plated out within 48 hours of sampling. Swabs for culture must be transported in an appropriate medium designed to maintain the viability of the organisms (22-26).

If processing is delayed up to 24 hours, refrigeration or freezing is preferable to storage at ambient temperature. (13).

Samples should be retained in accordance with The Royal College of Pathologists guidelines 'The retention and storage of pathological records and specimens' (27).

7 Laboratory processes

7.1 Molecular testing

For optimal performance, a multi-target approach is recommended, combining:

- A high-sensitivity screening target
- species-specific or discriminatory confirmatory targets

This combined strategy improves both sensitivity and specificity and reduces the risk of misidentification. See Section 4.1.1 for further information on NAAT targets.

The choice of assay and target combination will depend on local service requirements, testing pathways, available platforms, and the level of species detection and differentiation required.

Extract nucleic acid from the swab or aspirate specimen and perform testing in accordance with locally validated and approved NAAT standard operating procedures.

Appropriate controls should be incorporated into all assays to ensure their validity and reliability. Refer to [UK SMI Q 1 - Evaluations, validations and verifications of diagnostic tests](#).

7.2 Serology

Perform serological testing in accordance with locally validated and approved procedures and manufacturer instructions. Methods may include enzyme-linked immunosorbent assays (ELISA) or other validated immunoassays.

Assays detecting IgG antibodies to pertussis toxin (PT) are recommended. Where available, results should be reported in International Units per millilitre (IU/mL) to support standardisation and comparability of results (13).

The choice of assay will depend on local service requirements, available platforms, and laboratory policy. Performance characteristics, reportable ranges and reporting thresholds may vary between assays and manufacturers.

There is currently no internationally accepted serological correlate of protection or universally agreed diagnostic cut-off for recent pertussis infection. Individual assay-specific thresholds should therefore be applied in accordance with the manufacturer's instructions and local validation data.

Validated serological assays for the routine diagnosis of *Bordetella parapertussis* infection are not currently available. Serological testing is therefore primarily directed towards the detection of antibodies to *Bordetella pertussis*.

7.3 Culture

7.3.1 Nasopharyngeal swabs

Inoculate a *Bordetella*-selective agar plate (e.g. charcoal blood agar with cefalexin) with the swab as soon as possible after collection. Incubate the plates immediately according to Table 2.

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

For more detail, refer to [UK SMI Q 5 - Inoculation of culture media for bacteriology](#).

7.3.2 Nasopharyngeal aspirate

Mix the specimen thoroughly. Using a sterile loop, select a representative portion of the specimen, including any visible cellular or mucoid material where present, and inoculate each agar plate. For more detail, refer [UK SMI Q 5 - Inoculation of culture media for bacteriology](#).

For the isolation of individual colonies, spread the inoculum using a sterile loop with a standard streaking technique. The volume of inoculum need not be standardised using a calibrated loop, as culture is qualitative rather than quantitative. Centrifugation or concentration of nasopharyngeal aspirates is not routinely required prior to culture unless supported by local validation or specific laboratory protocols.

7.4 Identification

Refer to [UK SMI ID 5 - Identification of *Bordetella* species](#).

Table 2. Culture media, conditions, and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temperature °C	Atmosphere	Time		
Pertussis or whooping cough	Nasopharyngeal swab Nasopharyngeal aspirate,	Charcoal blood agar with cefalexin	35-37	air, moist chamber	7d	4d and 7d	<i>B. pertussis</i> <i>B. parapertussis</i>

8 Post-laboratory processes

8.1 Reporting procedure

8.1.1 Reporting molecular results

NAAT results should be interpreted in accordance with the manufacturer's instructions, local validation data, and laboratory reporting procedures.

The genetic targets employed and species differentiation capabilities vary between assays and manufacturers (see section 4.1.1).

Table 3 provides examples of interpretation for assays incorporating these commonly used targets. It is intended for illustrative purposes only and does not represent the target profile or reporting algorithm of any specific commercial assay.

Table 3. Example interpretation of selected *Bordetella* NAAT target combinations

IS481	<i>ptxP</i>	<i>hIS1001</i>	IS1001	Interpretation	Example interpretive comment
+	-	-	-	<i>B. pertussis</i> DNA detected ^a	This result is consistent with <i>B. pertussis</i> infection; however, due to an absence of species-specific targets infection with <i>B. holmesii</i> and <i>B. bronchiseptica</i> cannot be excluded. Correlation with clinical and epidemiological information is recommended.
+	+	-	-	<i>B. pertussis</i> DNA detected	Species detection was confirmed by the presence of a <i>Bordetella pertussis</i> -specific target.

-	+	-	+	<i>B. parapertussis</i> DNA detected	Although uncommon, some <i>B. bronchiseptica</i> isolates may produce a similar result.
+	-	+	-	<i>B. holmesii</i> DNA detected	Species detection was confirmed by the presence of a <i>B. holmesii</i> -specific target.
+	+	-	+	<i>B. pertussis</i> and <i>B. parapertussis</i> DNA detected	Mixed Bordetella infection is uncommon. Correlation with clinical and epidemiological information is recommended.
+	+	+	-	<i>B. pertussis</i> and <i>B. holmesii</i> DNA detected	Mixed Bordetella infection is uncommon. Correlation with clinical and epidemiological information is recommended.

Footnotes

a. Detection of IS481 alone may occur when the concentration of *B. pertussis* DNA is very low because IS481 is present in multiple copies within the genome, whereas confirmatory targets such as ptxP are present in fewer copies and may fall below the assay limit of detection.

Note: Interpretation of target combinations should be undertaken only in the context of the specific assay design, as different commercial and laboratory-developed assays utilise different targets, algorithms and reporting criteria

8.1.2 Reporting immunological results

Pertussis serology results should be interpreted according to the manufacturer's guidance. A case of pertussis caused by *B. pertussis* may be serologically confirmed when the anti-PT IgG concentration exceeds a defined cutoff. There is no internationally agreed cutoff to denote a positive result; thresholds of 60, 70 and 125 IU/mL have been used in different assays and studies as evidence of recent pertussis infection, generally within the previous year (13,28,29). Concentrations may be indicative of infection occurring more than one year previously (13,28).

It is recommended that a comment should be included stating that vaccination against pertussis or a previous pertussis infection can elicit elevated anti-PT IgG antibodies and that patients who have been vaccinated or previously infected within the last 12 months may produce a false positive result in this test.

In addition, the timing of sample collection should be considered. Samples obtained within the first two weeks following cough onset may yield false negative results due to insufficient antibody production at this early stage of infection.

8.1.3 Reporting culture results

Positive report:

"*Bordetella pertussis* isolated" or "*Bordetella parapertussis* isolated".

Negative report:

"*Bordetella pertussis* NOT isolated" or "*Bordetella parapertussis* NOT isolated"

Notes:

An appropriate comment should be added to negative culture reports to state:

"The sensitivity of culture for Bordetella species is sub-optimal therefore negative results cannot conclusively rule out Bordetella infection, NAAT is the most sensitive method for diagnosing Bordetella infection".

The sensitivity of *Bordetella* culture is influenced by specimen quality, transport conditions and delays in processing. Negative culture results, particularly where processing has been delayed, do not exclude *Bordetella* infection.

8.1.4 Reporting time

If interim or preliminary results are required as per local policy, these should be issued as soon as actionable findings are available (significant microscopical results or initial culture growth), unless alternative arrangements have been agreed with the requestor.

Final reports should be issued as soon as all investigations are complete and authorised by competent staff.

Pertussis is a [notifiable disease](#) in England and Wales. Suspected and confirmed cases should be notified to the relevant body in accordance with local and national public health reporting requirements.

9 Antimicrobial susceptibility testing

Monitoring and reporting of antimicrobial resistance in *B. pertussis* is important for surveillance and public health purposes (13). In particular, monitoring for macrolide resistance is important because macrolides remain the recommended first-line treatment for pertussis. Antimicrobial resistance testing is not routinely undertaken and is generally performed only when clinically or epidemiologically indicated either locally where capability exists or through referral to a specialist or reference laboratory.

NAAT-based assays have been developed to detect the most common mutation associated with macrolide resistance and may be applied to cultured isolates or NAAT-positive clinical specimens. These assays may be useful in cases of suspected treatment failure during macrolide therapy and during investigation of pertussis clusters or outbreaks and may be available through specialist or reference laboratories (13,30)

Phenotypic susceptibility testing may be undertaken by specialist laboratories using validated methods. As EUCAST does not currently provide clinical breakpoints for *Bordetella* species, interpretation should be undertaken by reference laboratories with appropriate expertise (13,30). Where investigation of antimicrobial resistance is indicated, isolates or suitable clinical specimens should be referred, with the clinical indication clearly stated. Indications for referral may include suspected treatment failure, pertussis clusters or outbreaks, surveillance or epidemiological investigations, or severe disease in infants

9.1 Reporting of antimicrobial susceptibility testing

Report susceptibilities as clinically indicated and in accordance with local laboratory procedures. Antimicrobial therapy should be guided by local and national antimicrobial stewardship principles and relevant clinical guidelines.

10 Referral to reference or specialist testing laboratories

When sending away isolates, NAAT-positive samples or sera to reference or specialist testing laboratories for processing, ensure that the specimen is placed in the appropriate package and transported accordingly. Follow local regulations and instructions provided by the reference or specialist testing laboratories for sending isolates.

Contact the appropriate reference laboratory (refer to the links provided below) for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission.

[England](#)

[Wales](#)

[Scotland](#)

[Northern Ireland](#)

11 Public health responsibilities of diagnostic laboratories

Diagnostic laboratories have public health responsibility as part of their duties. Amongst these are additional local testing, or referral to further characterise the organism as required, primarily for public health purposes e.g., routine *Cryptosporidium* detection; serotyping or microbial subtyping; and a duty to refer appropriate specimens and isolates of public health importance to a reference laboratory.

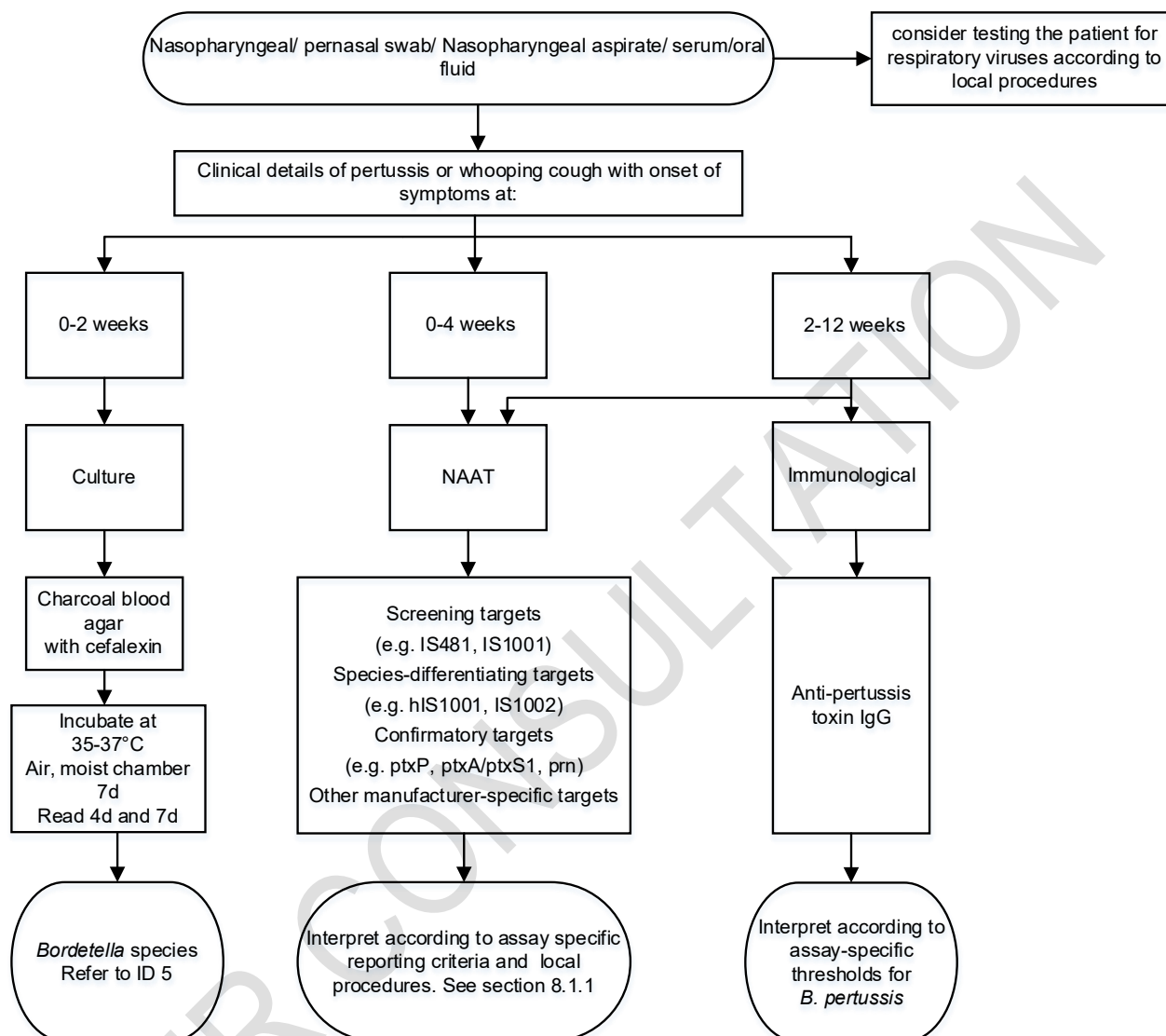
Diagnostic laboratory outputs inform public health intervention, and surveillance data is required to develop policy and guidance forming an essential component of

healthcare. It is recognised that additional testing and referral of samples may entail some costs that has to be borne by the laboratory but in certain jurisdictions these costs are covered centrally.

Diagnostic laboratories should be mindful of the impact of laboratory investigations on public health and consider requests from the reference laboratories for specimen referral or enhanced information.

UNDER CONSULTATION

Algorithm: Investigation of whooping cough



References

An explanation of the reference assessment used is available in the [scientific information section on the UK SMI website](#).

For suggested citation of UK SMIs, refer to [UK SMI Development](#).

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