

NATIONAL STANDARD METHOD

INVESTIGATION OF SPECIMENS FOR *BORDETELLA PERTUSSIS* AND *BORDETELLA* *PARAPERTUSSIS*

BSOP 6

Issued by Standards Unit, Department for Evaluations, Standards and Training

Centre for Infections



Association of Medical Microbiologists
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The reader is informed that all taxonomy in this document was correct at time of issue.

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AMENDMENT PROCEDURE

Controlled document reference	BSOP 6
Controlled document title	Investigation of specimens for <i>Bordetella pertussis</i> and <i>Bordetella parapertussis</i>

Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@hpa.org.uk.

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
8/ 06.11.08	6.1	7	1	Front Page	CMN and NIMAG logos added
			2	Status	Taxonomy sentence inserted
			All	All	PDF links amended to title of reference document
			9	4.5.2	Link to user manuals added
			10	7	Relevant National Standard Methods section inserted
			12	Appendix	Flowchart added
			13	References	Reviewed and updated
			All	All	Department name changed from Evaluations and Standards Laboratory to Department for Evaluations, Standards and Training

INVESTIGATION OF SPECIMENS FOR *BORDETELLA PERTUSSIS* AND *BORDETELLA PARAPERTUSSIS*

Types of specimens: Pernalasal swab
Nasopharyngeal aspirate
Nasopharyngeal swab
Blood

SCOPE OF DOCUMENT

This National Standard Method (NSM) describes the processing and bacteriological investigation of specimens for *Bordetella* species.

INTRODUCTION

The fully developed syndrome of whooping cough (pertussis, “violent cough”) is not difficult to diagnose, but *formes frustes* (abortive or atypical disease, disease stopped before it has run its full course) are known to occur, and may cause diagnostic difficulty. Whooping cough syndrome is a highly contagious disease that is caused by the fastidious Gram-negative coccobacillus *Bordetella pertussis*². In some cases it may also be caused by *Mycoplasma pneumoniae*, and by viruses such as adenoviruses and enteroviruses³. Consideration should be given to appropriate evaluation of patients with pertussis syndrome in whom infection with *B. pertussis* cannot be demonstrated. It is advisable to take two pernasal swabs: one for the culture of *Bordetella* species and the other for viral culture.

Developments in PCR have enabled the detection and differentiation of *B. pertussis* from other species of *Bordetella*⁴. Although culture is still regarded as the gold standard, PCR is a specific sensitive and rapid method for the diagnosis of *B. pertussis* in respiratory samples⁵. It is believed that using a PCR approach would reduce the number of missed diagnoses that could lead to nosocomial outbreaks. However culture techniques still remain vital for epidemiologic analysis and testing for antibiotic susceptibility⁶.

B. pertussis usually infects and causes severe respiratory disease in young children, although it does cause disease in adults^{7,8}. Figures show that there has been an increased incidence of the disease in adolescents and adults since the millennium². It presents a particular problem to patients with acquired immune deficiency syndrome (AIDS)⁹. Symptoms can be more virus infection-like in adults and older children. The disease is severe in young children causing choking and cyanosis. Treatment in the early stages reduces the severity of the illness and further spread of disease. Early laboratory diagnosis is vital for control and prevention of whooping cough. Isolation and typing of the organism is vital for the continued monitoring of the vaccine programme.

Bordetella parapertussis causes a mild form of infection although it can cause severe infection in patients who are immunocompromised^{10,11}.

TECHNICAL INFORMATION/LIMITATIONS

Media – Must support the growth of *B. pertussis*, 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 meticillin¹²⁻¹⁴.

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 NSM¹⁴.

Primary isolation plates are incubated at 35-37°C, in an aerobic moist atmosphere maintained for 7 days¹⁵. A thickly poured plate is necessary.

Specimen type – Current recommendations for specimen collection advocate the use of nasopharyngeal aspirate. However, when this type of specimen cannot be collected or is not the usual practice, pernasal swabs and nasopharyngeal swabs are taken¹⁵. It is advisable to take two pernasal swabs: one for the culture of *Bordetella* species and the other for viral culture. There is considerable debate as to whether nasopharyngeal aspirates or pernasal swabs are the preferred specimen to give a sensitive diagnosis¹⁶⁻¹⁸.

Pernasal swabs – The only swab fibre recommended for diagnosis of whooping cough is Dacron™. *B. pertussis* has a stronger affinity for Dacron™ than for plain cotton wool or for treated cotton wool and its use improves recovery of the organism¹⁹. It is also less inhibitory for PCR techniques²⁰.

Blood specimens – They are taken for serological testing from patients that have been coughing ≥2 weeks

Although this NSM does not recommend the use of cough plates, they are used by some paediatricians.

1 SAFETY CONSIDERATIONS²¹⁻³²

1.1 SPECIMEN COLLECTION

Pernasal and nasopharyngeal swabs

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.

1.2 SPECIMEN TRANSPORT AND STORAGE

Pernasal and nasopharyngeal swabs

Sealed plastic bag

Nasopharyngeal aspirates

Sterile leakproof container in a sealed plastic bag

1.3 SPECIMEN PROCESSING

Containment Level 2

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

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

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

Compliance with postal and transport regulations is essential

2 SPECIMEN COLLECTION

2.1 OPTIMAL TIME FOR SPECIMEN COLLECTION

At onset or presentation of symptoms

Before antimicrobial therapy where possible

2.2 CORRECT SPECIMEN TYPE AND METHOD OF COLLECTION

A pernasal swab (Dacron™ with flexible 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 be held against the posterior nasopharynx for up to 30 seconds 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 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 (30 mL or 60 mL, to BS 5213).

Note : Cough plates are not recommended

2.3 ADEQUATE QUANTITY AND APPROPRIATE NUMBER OF SPECIMENS

N/A

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3 SPECIMEN TRANSPORT AND STORAGE

3.1 TIME BETWEEN SPECIMEN COLLECTION AND PROCESSING

Specimens should be transported and processed as soon as possible^{33,34}

Culture plates may be inoculated at the bedside

3.2 SPECIAL CONSIDERATIONS TO MINIMISE DETERIORATION

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

4 SPECIMEN PROCESSING

4.1 TEST SELECTION

N/A

4.2 APPEARANCE

N/A

4.3 MICROSCOPY

N/A

4.4 CULTURE AND INVESTIGATION

4.4.1 PRE-TREATMENT

N/A

4.4.2 SPECIMEN PROCESSING

Pernasal and nasopharyngeal swabs

Inoculate each agar plate with swab (see [QSOP 52 - Inoculation of Culture Media](#))

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 (see [QSOP 52 - Inoculation of Culture Media](#))

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

4.4.3 CULTURE MEDIA, CONDITIONS AND ORGANISMS FOR SWABS AND ASPIRATES^{15,33}:

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

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4.5 IDENTIFICATION

4.5.1 MINIMUM LEVEL OF IDENTIFICATION IN THE LABORATORY

[Bordetella](#) species level

4.5.2 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](#).

Isolates associated with outbreaks, where epidemiologically indicated, 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.

4.6 ANTIMICROBIAL SUSCEPTIBILITY TESTING

N/A

5 REPORTING PROCEDURE

5.1 MICROSCOPY

N/A

5.1.1 MICROSCOPY REPORTING TIME

N/A

5.2 CULTURE

Negatives

"*Bordetella pertussis* NOT isolated"

Positives

"*Bordetella pertussis* isolated" or

"*Bordetella parapertussis* isolated"

5.2.1 CULTURE REPORTING TIME

Clinically urgent culture results to be telephoned or sent electronically

Written report, up to 7 days

5.3 ANTIMICROBIAL SUSCEPTIBILITY TESTING

N/A

6 REPORTING TO THE HPA³⁵ (LOCAL AND REGIONAL SERVICES AND CENTRE FOR INFECTIONS)

Refer to the following:

Individual NSMs on organism identification

Health Protection Agency publications:

"Laboratory reporting to the Health Protection Agency. Guide for diagnostic laboratories"

"Hospital infection control: Guidance on the control of infection in hospitals"

Local Memorandum of Understanding

Local guidelines

7 RELEVANT NATIONAL STANDARD METHODS

For additional details on specific areas of diagnosis refer to the relevant NSMs available through the Department for Evaluations, Standards and Training web page (www.hpa-standardmethods.org.uk).

Other documents that may be of relevance to this NSM are:

[QSOP 52 - Inoculation of Culture Media](#)

8 ACKNOWLEDGEMENTS AND CONTACTS

This National Standard Method has been developed, reviewed and revised by the National Standard Methods Working Group for Clinical Bacteriology (http://www.hpastandardmethods.org.uk/wg_bacteriology.asp). The contributions of many individuals in clinical bacteriology laboratories and specialist organisations who have provided information and comment during the development of this document, and final editing by the Medical Editor are acknowledged.

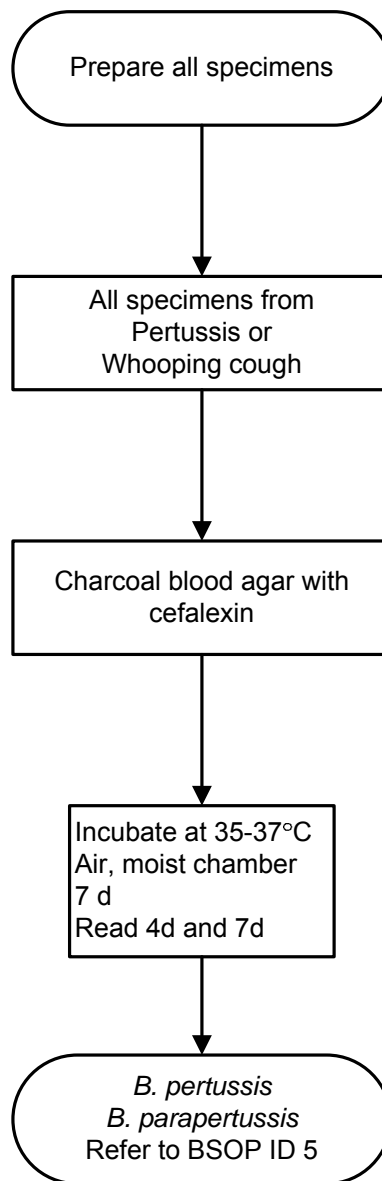
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APPENDIX



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