IDENTIFICATION OF BORDETELLA PERTUSSIS AND BORDETELLA PARAPERTUSSIS FROM SELECTIVE AGAR

BSOP ID 5

Issued by Standards Unit, Evaluations and Standards Laboratory
Centre for Infections
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IDENTIFICATION OF BORDETELLA PERTUSSIS AND BORDETELLA PARAPERTUSSIS FROM SELECTIVE AGAR
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Reference no: BSOP ID 52.1
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IDENTIFICATION OF BORDETELLA PERTUSSIS AND BORDETELLA PARAPERTUSSIS FROM SELECTIVE AGAR

SCOPE OF DOCUMENT

This National Standard Method (NSM) describes the identification of *Bordetella pertussis* and *Bordetella parapertussis*, isolated from pernasal swabs on charcoal blood agar with cefalexin, to species level.

INTRODUCTION

Taxonomy

There are eight species in the genus *Bordetella*\(^2\). A further putative species has been reported\(^3\), but this has not yet been validly published.

Characteristics\(^4\)

*Bordetella* species are Gram-negative coccobacilli 0.2 - 0.5 x 0.5 - 2.0 µm. Microscopically they appear arranged singly or in pairs and rarely in chains\(^5\). *B. pertussis* may grow on Bordetella selective medium (charcoal blood agar with cefalexin) within three days, but normally five to seven days incubation is required for primary isolation. Plates should be incubated for 7 days before being discarded as negative\(^6\). Growth on subculture usually requires shorter incubation eg 3 days. Colonies are smooth, convex, pearly, glistening, greyish-white and butyrous. *B. pertussis* does not grow on nutrient agar and grows poorly on blood agar. Colonies of *B. parapertussis* are similar but larger, duller and become visible sooner.

Some species are motile, but *B. pertussis* and *B. parapertussis* are non-motile. They are strictly aerobic and the optimum temperature for growth is 35°C - 37°C.

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

The metabolism is 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 CO\(_2\).

Antigenic structure

Isolates of *B. pertussis* should be referred to the Respiratory and Systemic Infection Laboratory (RSIL) for typing. *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 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 90% of isolates\(^7\).

Principles of identification

Colonies isolated on Bordetella selective agar are preliminarily identified by colonial appearance, Gram stain and slide agglutination with polyvalent antiserum. Biochemical and other tests are used to distinguish between species of the genus *Bordetella* and to differentiate *Bordetella* from similar organisms. Presumptive isolates of *B. pertussis* and *B. parapertussis* should be referred to the Respiratory and Systemic Infection Laboratory. Serum, pernasal swabs or nasopharyngeal aspirates may also be referred to RSIL for investigation of pertussis infection. Contact the laboratory or see the following website for details: [http://www.hpa.org.uk/cfi/rsil/bordetella.htm](http://www.hpa.org.uk/cfi/rsil/bordetella.htm).
TECHNICAL INFORMATION

N/A
1 SAFETY CONSIDERATIONS

Hazard Group 2 organisms.

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

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

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

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.

Compliance with postal and transport regulations is essential.

2 TARGET ORGANISMS

*Bordetella* species reported to have caused pertussis

*Bordetella pertussis*

*Bordetella parapertussis*

3 IDENTIFICATION

3.1 MICROSCOPIC APPEARANCE

Gram stain (see BSOPTP 39 - Staining Procedures)

Gram-negative, thin coccobacilli. Occurring singly or in pairs, rarely in chains. Some strains may be capsulate.

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. Regular cleaning of surfaces with a disinfectant active against fungal spores helps prevent growth of moulds.

3.3 COLONIAL APPEARANCE

Colonies of *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 and longer on primary isolation. Colonies of *B. parapertussis* are similar but larger, duller and become visible within two days. On subculture to nutrient agar, *B. parapertussis* colonies produce a brown pigment, which diffuses into the medium. *B. pertussis* does not grow on nutrient agar.

3.4 TEST PROCEDURES

Oxidase test (see BSOPTP 26 - Oxidase Test)

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

Agglutination (slide) with specific antiserum

Follow manufacturer’s instructions. 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 to the Respiratory and Systemic Infection Laboratory.

Refer isolates of suspected *B. pertussis* and *B. parapertussis* to the Respiratory and Systemic Infection Laboratory for further characterisation.

3.5 FURTHER IDENTIFICATION

N/A
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 PERTUSSIS AND BORDETELLA PARAPERTUSSIS – FLOW CHART

Clinical specimens
Primary isolation plate

Butyrous glistening greyish-white colonies at 3-7 days (may be 2 days for B. parapertussis)

Charcoal blood agar with cefalexin

Gram stain of pure culture
Gram-negative thin cocobacilli

Oxidase test

Positive
B. pertussis

Negative
B. parapertussis or other Gram-negatives

Agglutination with specific antiserum on pure culture, and organisms giving the biochemical profile of Bordetella species B. pertussis and B. parapertussis

Positive agglutination

Strongly suspected to be Bordetella species based on colonial morphology or clinical details

Autoagglutination

Not strongly suspected to be Bordetella species based on colonial morphology or clinical details

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

Strongly suspected to be Bordetella species based on biotechnical or clinical details. Carry out additional tests

Not strongly suspected to be Bordetella species based on colonial morphology or clinical details. Report as Bordetella species not isolated
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(s).
Note: "Whooping cough" is a Notifiable disease.

5.4 CCDC
Refer to local Memorandum of Understanding.

5.5 CENTRE FOR INFECTIONS
Refer to current guidelines on CDSC and COSURV reporting

5.6 INFECTION CONTROL STAFF
Inform the hospital infection 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
For information on the tests offered, turn around times, transport procedure and the other requirements of the reference laboratory refer to: http://www.hpa.org.uk/cfi/rsil

Send to:
Respiratory and Systemic Infection Laboratory
Health Protection Agency
Centre for Infections
61 Colindale Avenue
London NW9 5HT

Contact Centre for Infections main switchboard: Tel. +44 (0) 20 8200 4400
ACKNOWLEDGMENTS AND CONTACTS

This National Standard Method has been developed, reviewed and revised by the National Standard Methods Working Group for Clinical Bacteriology (http://www.hpa-standardmethods.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.

The National Standard Methods are issued by Standards Unit, Evaluations and Standards Laboratory, Centre for Infections, Health Protection Agency London.

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exchanged between purchaser, vendor and installer and recommendations for installation.
