NATIONAL STANDARD METHOD

IDENTIFICATION OF PASTEURELLA SPECIES

BSOP ID 13

Issued by Standards Unit, Evaluations and Standards Laboratory
Specialist and Reference Microbiology Division
STATUS OF NATIONAL STANDARD METHODS

National Standard Methods, which include standard operating procedures (SOPs), algorithms and guidance notes, promote high quality practices and help to assure the comparability of diagnostic information obtained in different laboratories. This in turn facilitates standardisation of surveillance underpinned by research, development and audit and promotes public health and patient confidence in their healthcare services. The methods are well referenced and represent a good minimum standard for clinical and public health microbiology. However, in using National Standard Methods, laboratories should take account of local requirements and may need to undertake additional investigations. The methods also provide a reference point for method development.

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AMENDMENT PROCEDURE

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

Scope of document

This SOP describes the procedure for the identification of Pasteurella species and distinguishes these from morphologically similar species.

Taxonomy

Currently some 20 species are included in the genus Pasteurella. Not all of these are true members. DNA-DNA hybridisation indicates that some of the species are more closely related to the genus Actinobacillus1.

Pasteurella multocida is the type species of the genus

Characteristics of Pasteurella species2

Pasteurella species are spherical, ovoid or rod-shaped cells 0.3-1.0 μm in diameter and 1.0-2.0 μm in length. Cells are Gram-negative, and occur singly, or in pairs or short chains. Bipolar staining is may be seen. Capsules may be present. Pasteurella species are non-motile, and are facultatively anaerobic.

Pasteurella species have both an oxidative and fermentative metabolism. The optimum growth temperature is 37°C. Glucose and other carbohydrates are catabolised with the production of acid but no gas. Most species are catalase-positive and oxidase-positive; nitrates are reduced to nitrites by almost all species.

Colonies of Pasteurella species are usually grey and viscous, with a strong mucinous odour. Rough, irregular colonies may also occur. Freshly isolated strains of Pasteurella haemolytica produce clear zones of β-haemolysis on blood agar – this organism is a cause of mastitis and septicaemia in some peridomestic animals, but is very rarely the cause of human infection.

Pasteurella and Actinobacillus species are so similar, that no single phenotypic feature reliably distinguishes between the two genera. In clinical practice, however, an organism with characteristics corresponding to the genus Pasteurella, is highly likely to be so, if recovered from clinical specimens in association with a bite from a cat or dog.

The genus Actinobacillus now includes Actinobacillus ureae – formerly Pasteurella ureae. A. ureae is thought to a commensal or opportunist pathogen of human beings, and is principally reported in connection with disease of the respiratory tract (eg cases of pneumonia, lung abscess). Occasionally, invasive infections (septicaemia, meningitis) are also reported.

As the name suggests, A. ureae is urease positive. Most species of Pasteurella are urease negative (including P. multocida). Thus, a Pasteurella – like organism, urease positive, recovered in association with human respiratory tract disease, is likely to be A. ureae.

Phenotypically, Pasteurella species may resemble Haemophilus species – but Pasteurella species will not regularly exhibit satellitism around colonies of Staphylococcus species, nor are they regularly auxotrophic for X or V factors; growth is not especially enhanced by use of chocolatised blood agar.
Principles of identification

Colonies on blood agar are identified by colonial morphology, Gram stain, oxidase test and catalase production. Additional tests are needed for confirmation and/or isolates should be referred to the Reference Laboratory.

1.0 SAFETY CONSIDERATIONS

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

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

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

Compliance with postal and transport regulations is essential

2.0 TARGET ORGANISMS

2.1 Pasteurella species reported to have caused human infection

P. aerogenes
P. bettayae
P. canis
P. dagmatis
P. gallinarum
P. haemolytica (Biotype A)
P. multocida subspecies gallicida
P. multocida subspecies multocida
P. multocida subspecies septica
P. pneumotropica
P. stomatis
3.0 IDENTIFICATION

3.1 Primary isolation media

Blood agar 16-48h incubation in 5-10% CO₂ at 35-37°C

3.2 Colonial appearance

Colonies are grey and viscous but rough irregular colonies occur frequently. Freshly isolated strains of *P. haemolytica* produce clear zones of ß-haemolysis on blood agar.

3.3 Microscopic appearance

**Gram stain** (BSOP SP 8)
Spherical, ovoid or rod-shaped Gram-negative cells which occur singly or in pairs or short chains. Bipolar staining is common. Capsules may be present

3.4 Identification tests

**Oxidase test** (BSOP TP 26)
Positive (almost always)

**Catalase test** (BSOP TP 8)
Positive

**Growth on CLED**
No growth (*P. multocida*)

**Sensitivity to penicillin**
Sensitive

**Commercial identification kit**

3.5 Storage and referral

If required save pure isolate on a blood agar slope for referral to the Reference Laboratory
4.0 IDENTIFICATION OF PASTEURELLA SPECIES - SUMMARY

Clinical specimens
Primary isolation plate (blood agar)

* Pasteurella species are grey, viscous, rough, irregular, non-haemolytic colonies on blood agar
  * P. haemolytica are β-haemolytic on blood agar
  * No growth on CLED (P. multocida)

Gram stain
Gram-negative rods or coccobacilli
If there is a different Gram-stain appearance refer to the appropriate SOP

Oxidase*

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<th>Negative</th>
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<td>Not Pasteurella species†</td>
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Catalase*

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<td>Possible Pasteurella species</td>
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Sensitivity to Penicillin

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<tr>
<td>Possible Pasteurella species</td>
<td>Not Pasteurella species</td>
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</table>

Commercial identification kit
(If clinically indicated)

* All oxidase and catalase reactions may be weak
† P. bettyae is oxidase negative
‡ P. haemolytica Biotype T is catalase negative
If required, save pure isolate on a blood agar slope for referral to the Reference Laboratory

This flowchart is for guidance only

* All oxidase and catalase reactions may be weak
† P. bettyae is oxidase negative
‡ P. haemolytica Biotype T is catalase negative
If required, save pure isolate on a blood agar slope for referral to the Reference Laboratory
5.0 RESULTS AND REPORTING

5.1 Presumptive identification may be made:

If appropriate growth characteristics, colonial appearance, Gram stain of the culture, oxidase and catalase test results are demonstrated

5.2 Confirmation of identification may be made:

Following use of a commercial characterisation kit and/or referral to a Reference Laboratory

5.3 To medical microbiologist

The medical microbiologist should be informed of presumptive or confirmed Pasteurella species when the request card bears relevant information eg

- animal bite
- wound infection or septic arthritis
- septicaemia
- meningoencephalitis
- pneumonia, empyema thoracis or lung abscess
- history of farming or veterinary work

Follow local protocols for reporting to clinician

5.4 CCDC

Refer to local Memorandum of Understanding

5.5 CDSC^14

Refer to current guidelines on CDSC and COSURV reporting
6.0 REFERRALS

6.1 Examination required

All clinically important isolates requiring confirmation of identification should be sent to the Reference Laboratory

6.2 Transport procedure

Cultures should be sent on blood agar slopes

Compliance with postal and transport regulations is essential

6.3 Other requirements

Always notify the Reference Laboratory when sending urgent cultures

Do not delay submission

6.4 Reference Laboratory

Gram Negative Reference Laboratory
Specialist and Reference Microbiology Division
Health Protection Agency
61 Colindale Avenue
London
NW9 5HT
Contact SRMD main switchboard: Tel. +44 (0) 20 8200 4400
REFERENCES


3 Advisory Committee on Dangerous Pathogens. Categorisation of biological agents according to hazard and categories of containment, 4th ed. Suffolk: HSE books; 1995 (with supplements 1, 1998 and 2, 2000)


6 Health and Safety Executive. 5 steps to risk assessment: a step-by-step guide to a safer and healthier workplace, IND (G) 163 (REVL). Suffolk: HSE Books; April 2002

7 Health and Safety Executive. A guide to risk assessment requirements: common provisions in health and safety law, IND (G) 218 (L). Suffolk: HSE Books; March 2002

8 Health Services Advisory Committee. Safety in Health Service laboratories. Safe working and the prevention of infection in clinical laboratories and similar facilities. 2nd ed. Suffolk: HSE Books; 2003


13 Advisory Committee on Dangerous Pathogens. The management, design and operation of microbiological containment laboratories. Suffolk: HSE Books; 2001

14 PHLS CDSC. Reporting to the PHLS Communicable Disease Surveillance Centre: a reference for laboratories. May 2001