NATIONAL STANDARD METHOD

IDENTIFICATION OF *PASTEURELLA* SPECIES AND MORPHOLOGICALLY SIMILAR BACTERIA

BSOP ID 13

Issued by Standards Unit, Evaluations and Standards Laboratory
Centre for Infections

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AMENDMENT PROCEDURE

Controlled document reference	**BSOP ID 13**
Controlled document title	**Identification of *Pasteurella* species and morphologically similar bacteria**

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
6/ 14/07/2007	2	2.1	1	**Front Page**	Northern Ireland logo added
			10	**4 Flow chart**	Title changed and flowchart put in to visio format. Contents of flow chart updated.
			11	**5.3 Medical Microbiologist**	Section amended to make it more informative in the work place.
			11	**6 Referrals**	Links to reference laboratory user manuals inserted.
			12	**References**	References reviewed and updated
			All	**All**	PDF links inserted to cross- reference NSM documents

IDENTIFICATION OF *PASTEURELLA* SPECIES AND MORPHOLOGICALLY SIMILAR BACTERIA

SCOPE OF DOCUMENT

This National Standard Method (NSM) describes the procedure for the phenotypic identification of *Pasteurella* species and distinguishes these from morphologically similar species.

INTRODUCTION

Taxonomy

Currently some 15 - 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 Actinobacillus[2].

Pasteurella multocida is the type species of the genus.

Characteristics[3]

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 may be seen and capsules may be present. All 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 ß-haemolysis on blood agar – this organism is a cause of mastitis and septicaemia in some peridomestic animals, but very rarely infects humans.

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 be a commensal or occasionally an opportunist pathogen of human beings, and has principally been reported in connection with disease of the respiratory tract (eg cases of pneumonia, lung abscess). Occasionally, invasive infections (bacteraemia, meningitis) have also been 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 auxotropic for X or V factors; growth is not especially enhanced by use of chocolate 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.

TECHNICAL INFORMATION

N/A

1 SAFETY CONSIDERATIONS[4-14]

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 COSSH and task specific risk assessments.

Compliance with postal and transport regulations is essential.

2 TARGET ORGANISMS

***Pasteurella* species reported to have caused human infection[2]**
P. aerogenes
P. bettyae
P. canis
P. dagmatis
P. multocida subspecies *gallicida*
P. multocida subspecies *multocida*
P. multocida subspecies *septica*
P. pneumotropica
P. stomatis
P. trehalosi (previously *P. haemolytica* biotype T)
Avibacterium gallinarum (formerly *P. gallinarum*)
Mannheimia haemolytica (formerly *P. haemolytica* (Biotype A))

3 IDENTIFICATION

3.1 MICROSCOPIC APPEARANCE

Gram stain (see BSOPTP 29 - Staining Procedures)

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.

3.2 PRIMARY ISOLATION MEDIA

Blood agar 16 – 48 h incubation in 5 - 10% CO_2 at 35°C - 37°C.

3.3 COLONIAL APPEARANCE

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

3.4 TEST PROCEDURES

Oxidase test (see BSOPTP 26 - Oxidase Test)
Positive (almost always)

Catalase test (see BSOPTP 8 - Catalase Test)
Positive

Growth on CLED or MacConkey
No growth *(P. multocida)* on MacConkey but can grow poorly on some CLED agars.
Sensitivity to penicillin
A zone of inhibition around a 1U penicillin disc may aid differentiation from other Gram-negative bacilli.

Commercial identification kit

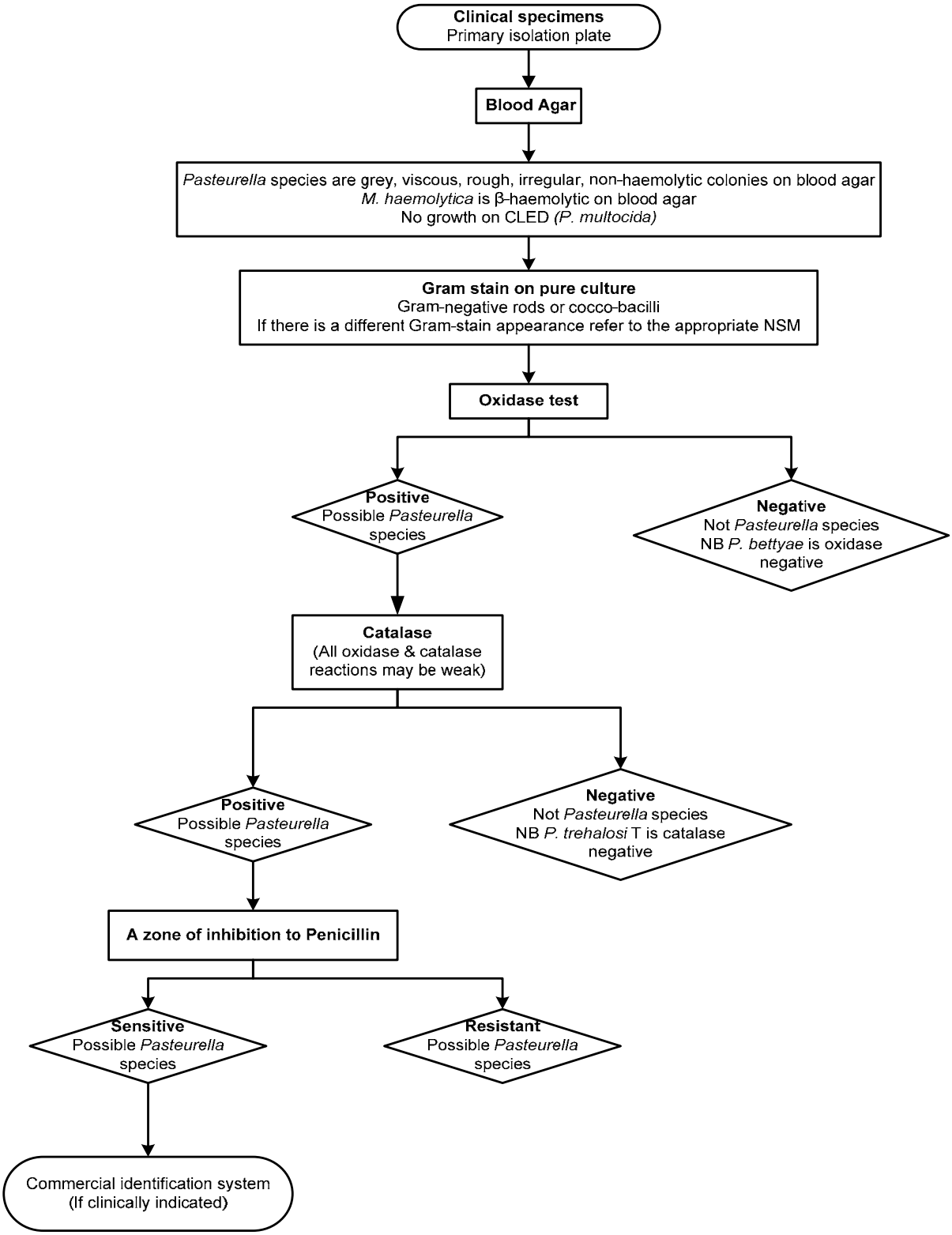
3.5 FURTHER IDENTIFICATION

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

3.6 STORAGE AND REFERRAL

If required save pure isolate on a blood agar slope for referral to the Reference Laboratory.

4 IDENTIFICATION OF *PASTEURELLA* SPECIES AND MORPHOLOGICALLY SIMILAR BACTERIA - FLOWCHART



5 RESULTS

5.1 PRESUMPTIVE IDENTIFICATION

If appropriate growth characteristics, colonial appearance and Gram stain of the culture are demonstrated.

5.2 CONFIRMATION OF IDENTIFICATION

N/A

5.3 MEDICAL MICROBIOLOGIST

The medical microbiologist should be informed of presumptive or confirmed *Pasteurella* species if isolated from a specimen from a normally sterile site or from other specimens in accordance with local protocols.

Follow local protocols for reporting to clinician.

5.4 CCDC

Refer to local Memorandum of Understanding.

5.5 CENTRE FOR INFECTIONS[15]

Refer to current guidelines on CDSC and COSURV reporting.

5.6 INFECTION CONTROL STAFF

N/A

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/lhcai/default.htm

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7 ACKNOWLEDGMENTS AND CONTACTS

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

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