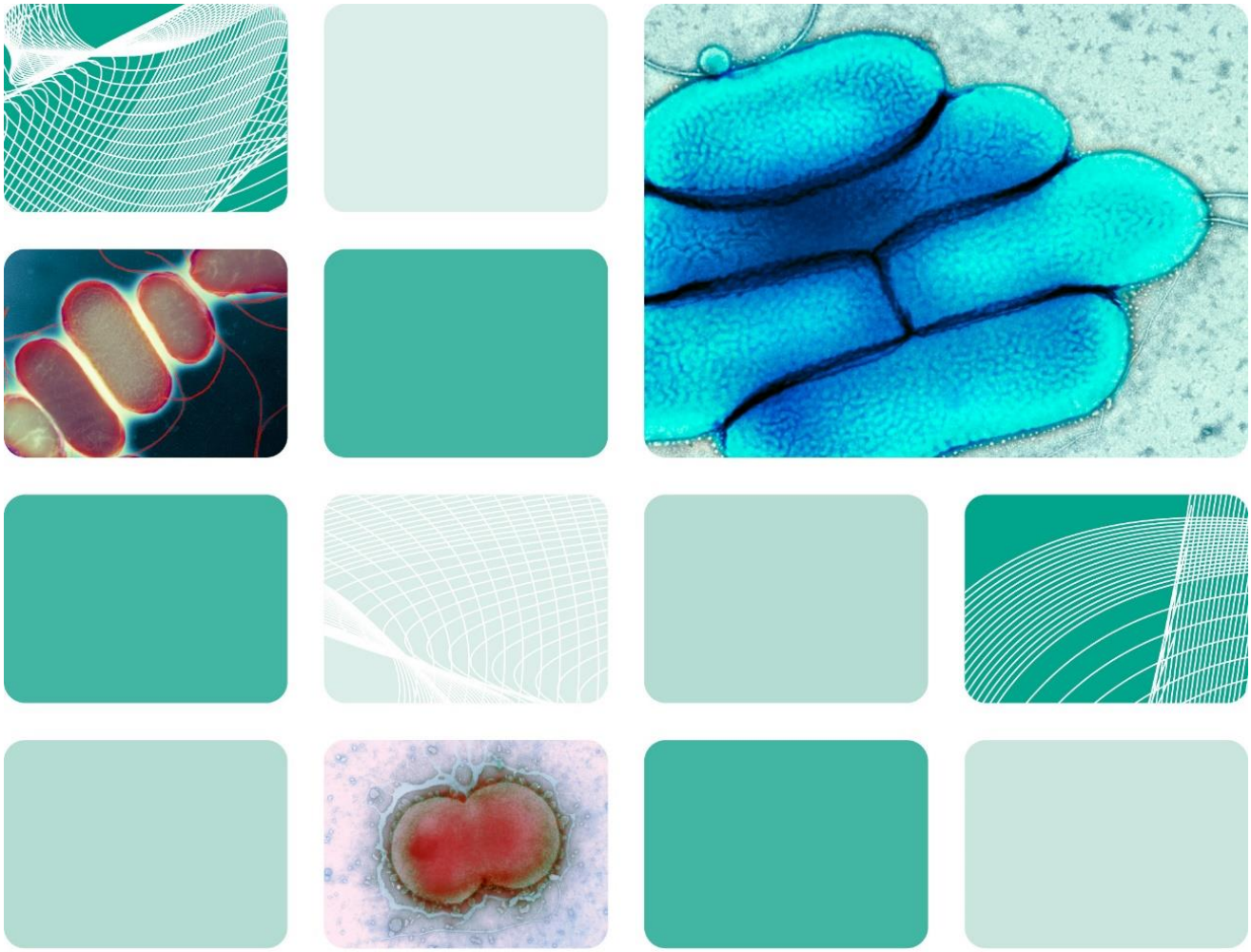
UK Health Security Agency

UK Standards for Microbiology Investigations

Identification of *Pasteurella* species and morphologically similar organisms



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Contents

Amendment Table

Each UK SMI document has an individual record of amendments. The amendments are listed on this page. The amendment history is available from standards@ukhsa.gov.uk.

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

Amendment number/date	10/17.07.25
Issue number discarded	3
Insert issue number	3.1
Section(s) involved	**Amendment**
Whole document.	**This is an administrative point change.** **The content of this UK SMI document has not changed.** **The last scientific and clinical review was conducted on 04/02/2015.** Hyperlinks throughout document updated to Royal College of Pathologists website. Public Health England replaced with UK Health Security Agency throughout the document, including the updated Royal Coat of Arms Partner organisation logos updated. Broken links to devolved administrations replaced. References to NICE accreditation removed. Scope and Purpose replaced with General and Scientific information to align with current UK SMI template. Section on public health responsibilities of diagnostic laboratories added

Amendment No/Date.	9/04.02.15
Issue no. discarded.	2.3
Insert Issue no.	3
Section(s) involved	**Amendment**
Whole document.	Hyperlinks updated to gov.uk.

Page 2.	Updated logos added.
Whole document.	Document presented in a new format. Reorganisation of some text. Edited for clarity. Test procedures updated. Updated contact details of Reference Laboratories.
Scope of document.	The scope has been edited for clarity.
Introduction.	The taxonomy of *Pasteurella* species and other similar organisms has been updated. More information has been added to the Characteristics section. The medically important species have been grouped and their characteristics described. Use of up-to-date references.
Technical Information/Limitations.	Addition of information regarding commercial identification systems has been described and referenced.
Safety considerations.	Reference added. Text re-organised. Update on Laboratory-acquired infections.
Target Organisms.	The section on the Target organisms has been updated and presented clearly. References have been updated.
Identification.	Amendments and updates have been done on 3.1, 3.2, 3.3 and 3.4 have been updated to reflect standards in practice. Addition of a table (in 3.3) to explain the different species and their colonial morphology. Section 3.4.3 and 3.4.4 have been updated to include MALDI-TOF MS and NAATs with references. Subsection 3.5 has been updated to include the Rapid Molecular Methods.
Identification Flowchart.	Modification of flowchart for identification of species has been made for easy guidance.
Referral.	The contact detail of the reference laboratory has been updated.

References.	Some references updated.

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 SMI describes the identification of *Pasteurella* species and distinguishes these from those species which are morphologically similar.

This SMI should be used in conjunction with other UK SMIs.

4 Introduction/Background

4.1 Taxonomy

The genera *Pasteurella, Actinobacillus, Aggregatibacter, Avibacterium, Basfia, Bibersteinia, Bisgaardia, Chelonobacter, Gallibacterium, Haemophilus, Histophilus, Lonepinella, Mannheimia, Necropsobacter, Nicoletella, Otariodibacter, Phocoenobacter* and *Volucribacter* currently belong to the family Pasteurellaceae[1].

Currently, there are 22 validly published species in the genus *Pasteurella,* 8 of which have been reclassified to other genera[1]. The taxonomy of the *Pasteurella* genus has been under constant revision. *Pasteurella ureae* was transferred to the genus *Actinobacillus* as *Actinobacillus ureae,* while *Pasteurella haemolytica, Pasteurella granulomatis,* and *Pasteurella anatis* were, respectively, assigned to the new genera *Mannheimia* (*Mannheimia haemolytica* and *Mannheimia granulomatis*) and *Gallibacterium* (*Gallibacterium anatis*). *Pasteurella trehalosi* has been assigned to the genus *Bibersteinia* as *Bibersteinia trehalosi.* The species *Pasteurella gallicida* has been rejected from the genus because it has the same type strain as *P. multocida* on the Approved Lists 1980 and is therefore a homotypic synonym.

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.

4.2 Characteristics

Pasteurella species are spherical, ovoid or rod-shaped cells 0.3-1.0µm in diameter and 1.0-2.0µm 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. Acid is not produced from L-sorbose, L-rhamnose, m-inositol, adonitol, or salicin. 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 resembling *Haemophilus influenzae*. On chocolate agar, colonies are round, greyish or yellowish, and nearly 2mm in diameter after 48hr. Rough, irregular colonies may also occur. There is no haemolysis on blood agar.

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.

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.

Pasteurella species are generally susceptible to chloramphenicol, penicillin, tetracycline, and the macrolides.

Pasteurella species have been isolated from infected bite wounds and abscesses, pus, bronchial secretion, CSF, and blood.

4.3 Medically important Pasteurella species

Most commonly encountered

P. canis[3]

Strains are V-factor independent. They are positive for the ornithine decarboxylation test, and negative for acid production from L-arabinose, raffinose, D-lactose, maltose, mannitol, sorbitol, or dulcitol. The urease test is also negative; variable reactions are obtained for acid production from trehalose and D-xylose. There are 2 biotypes: Biotype 1 strains exhibit positive reactions for indole, biotype 2 strains exhibit negative reactions for indole.

P. canis biotype 1 is found in the oral cavities of dogs and is often isolated from injuries in humans resulting from dog bites. Biotype 2 strains have been isolated from calves.

They have been isolated from wound sites after dog/cat bite and synovial fluid.

P. multocida[3]

This is divided into the following three subspecies: *P. multocida* subsp. *multocida, P. multocida* subsp*. septica* and *P. multocida* subsp*. gallicida.* In addition to the features that are consistent with all members of the genus, the biochemical reactions are also common to the 3 subspecies. However, the main characteristics used for differentiation of the three subspecies are fermentation of sorbitol and dulcitol. *P. multocida* subsp*. multocida* strains ferment sorbitol but do not ferment dulcitol; *P. multocida* subsp. *septica* strains exhibit negative reactions for both sorbitol and dulcitol fermentation, whereas the *P. multocida* subsp. *gallicida* strains ferment both sorbitol and dulcitol[3].

They have been isolated from wound infections after cat/dog inflicted bites, sputum, blood, middle ear fluid and cerebrospinal fluid.

P. pneumotropica

Cells are coccobacilli and show bipolar staining. Colonies on blood agar at 18-24hr are non-haemolytic, greyish with a smooth surface, convex, and round with an entire edge and are 1mm in diameter. They have a strong odour resembling that of *Haemophilus influenzae*. They grow poorly in nutrient agar and show no growth at all on MacConkey agar.

They have been isolated from rat/guinea pig bite wounds and tracheal aspirates.

Less commonly encountered:

P. aerogenes[4]

Cells on blood agar are 0.5–1.0µm wide and 1.1–2.0µm long with filaments seen, especially in older cultures. After 24hr incubation on bovine blood agar, colonies are circular, smooth, convex, regular and greyish, 0.5–1.0mm in diameter. Haemolysis is not observed on bovine blood agar. Growth on MacConkey is positive. Pigment is not formed. Cells do not show motility at 22 or 37°C. They are positive for catalase reaction, Hugh & Leifson fermentation test with D-glucose within 3-14 days, porphyrin test, indole, urease and alanine aminopeptidase tests and negative for arginine dehydrolase, lysine decarboxylase and phenylalanine deaminase, symbiotic growth (NAD requirement), growth on Simmons' citrate agar, Tween 20 or 80 hydrolysis and acid production from mucate.

They have been isolated from pig bite wound in humans.

P. bettyae[5]

Cells are small non-motile bacilli and coccobacilli that are often <1µm long. Endospores are not formed. Growth is aerobic and facultatively anaerobic, mesophilic. Surface colonies grown aerobically on sheep blood agar are round, greyish, semi-transparent, about 2mm in diameter after 48hr at 37°C, and without haemolysis but sometimes with greening of the erythrocytes. They are notable for fermenting carbohydrates slowly and weakly, for not acidifying galactose, and for giving negative oxidase, catalase and urease reactions.

They have been isolated from human Bartholin gland abscesses and human finger infections as well as urine, gastric aspirate and urethral discharge.

P. dagmatis[3]

Cells are small, and coccoid to rod shaped. They produce small amounts of gas from D-glucose; positive reactions are obtained for urease, indole, acid production from maltose and, with few exceptions, acid production from trehalose. They are negative for acid production from D-xylose, L-arabinose, raffinose, mannitol, sorbitol, or dulcitol and ornithine is not decarboxylated. Gelatin may be liquefied after more than 14 days of incubation.

They have been isolated from local and systemic infections resulting from animal bites, blood, tracheal aspirate and middle ear fluid.

P. stomatis[3]

The features are consistent with the genus *Pasteurella*, in addition, they are V-factor independent and are negative for ornithine decarboxylase and urease tests. No acid is produced from L-arabinose, D-xylose, raffinose, D-lactose, maltose, trehalose, mannitol, sorbitol, or dulcitol. Indole and acid are produced from trehalose.

They have been isolated from wound infections (dog bite).

P. caballi[6]

Cells are non-acid-fast, rod-shaped (0.8-1.0µm x 1.3-1.9µm), and are bipolar stained. They are arranged singly or in pairs with occasional swollen, curved, or filamentous forms observed. They are facultatively anaerobic. Endospores are not formed. Colonies are 1.0-1.5mm in diameter, non-haemolytic, smooth, slightly raised, and greyish yellow on blood agar after 24hr of incubation. Colonies grow at room temperature and at 37°C but not at 4°C. They do not grow on MacConkey agar.

They are usually oxidase positive and also positive for nitrate reduction, phosphatase, β-galactosidase and acid production from D - Glucose fermentation. They are negative for catalase, indole, urease, Voges-Proskauer and methyl red tests. Acid is produced from D-fructose, D-galactose, lactose, D-mannose, and sucrose. Fermentation of lactose is delayed. Acid is not produced from adonitol, L-arabinose, cellobiose, dulcitol, inulin, salicin, starch, or trehalose.

They have been isolated from equine infections, including endocarditis, wounds, abscesses and genital infections and from humans[7-9].

P. oralis (was previously known as *Pasteurella* species B)[10]

Cells are coccobacilli to rod-shaped and are non-motile at both 22 and 37°C. On blood agar, colonies are circular, slightly raised and regular with an entire margin. The surface of the colonies is smooth, shiny and opaque with a greyish tinge. Colonies demonstrate a diameter of approximately 1.5mm after aerobic incubation for 24hr at 37°C. The consistency of the colonies is unguent-like and colonies do not adhere to the agar. They are both non-haemolytic and CAMP (Christie, Atkins, and Munch-Petersen) negative. Non-symbiotic growth is observed.

They are positive in the catalase, oxidase, porphyrin, nitrate, alanine aminopeptidase and alkaline phosphatase tests as well as ornithine decarboxylase and indole formation tests whereas negative reactions are obtained in the Simmons citrate test, acid is not formed from mucate and an alkaline reaction is not obtained from malonate. Negative reactions are also observed for the H_2S/Triple Sugar Iron (TSI) test, urease test, methyl red, Voges–Proskauer, gas from nitrate, arginine dehydrolase, lysine decarboxylase, phenylalanine deaminase, gelatinase, growth on Tween 20 and 80, gas from D-glucose within 3-14 days and growth on McConkey medium.

Acid is formed from xylitol, D-ribose, D-xylose, dulcitol, D-fructose, D-galactose, D-glucose, D-mannose, sucrose, trehalose, maltose and dextrin within 3-14 days. Acid is not formed from glycerol, meso-erythritol, D-adonitol, D-arabitol, L-arabinose, L-xylose, D-arabinose, myo-inositol, D-mannitol, D-sorbitol, L-rhamnose, D-fucose, L-fucose, L-sorbose, cellobiose, lactose, melibiose, melezitose, raffinose, D-glycogen and inulin after 14 days.

This has been isolated from a cat bite of a human.

Other morphological similar organisms

Actinobacillus species

The genus *Actinobacillus* currently has 19 species and 2 subspecies that are animal pathogens, the only exceptions being *Actinobacillus ureae* and *Actinobacillus hominis*, which appear to be highly adapted to humans[11]. *Actinobacillus actinomycetemcomitans* has been re-classified to genus *Aggregatibacter* as *Aggregatibacter actinomycetemcomitans*[12].

Members of the genus *Actinobacillus* are small, Gram negative, pleomorphic, coccobacillary rods that are facultatively anaerobic. They are arranged singly and in pairs; rarely in chains. They are positive for urease, β-galactosidase and nitrate tests and negative for indole production, gelatinase, lysine, ornithine and arginine production. Variable reactions occur for the catalase and oxidase tests. The optimum growth temperature is 37°C and they all have complex nutritional requirements; growth requires enriched media and is improved by a 5 % - 10 % CO_2 atmosphere. Most strains will grow on MacConkey agar apart from *A. pleuropneumoniae* and some strains of *A. suis*. The colonies are non-haemolytic, translucent and 1-2mm in diameter on blood agar.

The medically important *Actinobacillus* species are;

Actinobacillus ureae (Formerly *Pasteurella ureae*)

They have the same phenotypic characteristics as *Pasteurella* species. As the name suggests, *A. ureae* is urease positive and 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*.

The other biochemically similar *Actinobacillus* species may be differentiated from *A. ureae* by acid production from carbohydrates (lactose and xylose or trehalose which *A.ureae* does not utilize) and by their ability to grow on MacConkey agar.

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[6].

They have been isolated from sputum and tracheal secretions in humans.

Actinobacillus hominis

They have the same phenotypic characteristics as *Pasteurella* species. *A. hominis* is phenotypically relatively homogeneous but can be difficult to differentiate from other *Actinobacillus* species unless extensive biochemical testing is performed. Mannose-positive strains of *A. hominis* are especially difficult to differentiate from *A. equuli*[13].

A. hominis has primarily been found in the sputum and tracheal secretions in patients with chronic respiratory tract diseases or pneumonia, although systemic infections have been reported. It is assumed that they also colonize the respiratory tract of healthy individuals.

Aggregatibacter species

For information on *Aggregatibacter* species please see UK SMI ID 12 - Identification of Haemophilus species and the HACEK Group of organisms.

Bibersteinia species[14]

This genus is a member of the family *Pasteurellaceae* as defined by Olsen et al. and it has one species only, *Bibersteinia trehalosi* that was transferred from the genus *Pasteurella*[15,16]. They are Gram negative, non-motile, rod-shaped or pleomorphic with cells occurring singly and in pairs or short chains depending upon the growth stage. Colonies on blood agar are round, regular, greyish or yellowish, semi-transparent at the periphery and are about 2mm in diameter after 24hr at 37°C. Some strains are haemolytic and are CAMP positive. Endospores are not formed. Growth is mesophilic and facultatively anaerobic or microaerophilic. They are positive for nitrate reduction, Porphyrin, phosphatase and alanine aminopeptidase test.

Negative reactions occur for Simmons' citrate, malonate-base, growth in the presence of KCN, Voges–Proskauer, methyl red and urease tests. Negative tests are further observed with ONPG, arginine dehydrolase, lysine decarboxylase, ornithine decarboxylase, phenylalanine deaminase, indole, gelatinase and hydrolysis of Tweens 20 and 80. Acid is formed from D-ribose, D-mannitol, D-sorbitol, D-fructose, D-glucose, D-mannose, maltose, sucrose, D-trehalose and dextrin while acid is not produced from adonitol, D-arabitol, D-arabinose, L-arabinose, myoerythritol, dulcitol, D-fucose, L-fucose, D-galactose, D-glycogen, inulin, lactose, D-melibiose, D-melezitose, L-rhamnose, L-sorbose, D-turanose, xylitol, D-xylose or L-xylose.

Variable reactions occur for the catalase and oxidase tests and the production of acid from glycerol, myoinositol, cellobiose, raffinose, aesculin, amygdalin, arbutin, gentiobiose and salicin. Variable reactions are also obtained in the β-glucosidase and β-glucosidase tests.

The medically important *Bibersteinia* species is;

Bibersteinia trehalosi (*Previously known as P. haemolytica biotype T and then, P. trehalosi)*[5,14]

Cells are small rods and coccobacilli. Endospores are not formed. Growth is aerobic and facultatively anaerobic, mesophilic. Surface colonies grown aerobically on sheep blood agar are round, greyish, semi-transparent, and about 2.5mm in diameter after 48hr at 37°C; they usually have a pronounced zone of haemolysis (often double) and occasionally produce some greening of the erythrocytes. They are notable for giving a weak or negative reaction for catalase and urease, in producing acid from sorbitol and trehalose but not from galactose, and for often showing delayed fermentation in Hugh-Leifson medium. This organism is distinguished from *P. haemolytica* sensu stricto (now known as *Mannheimia haemolytica* (formerly *P. haemolytica* biotype A) by fermenting trehalose but not L-arabinose and usually not galactose or D-xylose.

Avibacterium species[17]

This genus is a member of the family *Pasteurellaceae* and it currently has 5 species[18]. They are Gram negative, non-motile, rod-shaped or pleomorphic with cells occurring singly and in pairs or short chains depending upon the growth stage. Colonies on sheep-blood agar are non-haemolytic, greyish, opaque, but eventually translucent at the periphery, with a butyrous consistency, smooth and shiny, circular and raised with an entire margin. Some isolates show symbiotic growth. Major differences are consequently observed in the size of colonies after 24hr incubation (pinpoint up to almost 2mm in diameter). No growth occurs on MacConkey agar. Pigment production is variable. Endospores are not formed. Growth is mesophilic and facultatively anaerobic or microaerophilic. They are positive for oxidase reaction,

nitrate reduction, porphyrin and alanine aminopeptidase tests. The reaction in Hugh–Leifson medium with D-glucose is fermentative without gas production and acid is formed from D-fructose, D-mannose and sucrose. Negative reactions occur for Simmons' citrate, mucate-acid, malonate-base, H_2S/tri-sugar iron (TSI), growth in the presence of KCN, Voges– Proskauer, methyl red and urease tests. Negative tests are further observed with arginine dehydrolase, lysine decarboxylase, phenylalanine deaminase, indole, gelatinase and Tween 20 and 80 hydrolysis. Acid is not produced from meso-erythritol, adonitol, L-xylose, dulcitol, D-fucose, L-rhamnose, L-sorbose, cellobiose, D-melibiose, D-melezitose, D-glycogen, inulin and aesculin.

Variable reactions occur for catalase, phosphatase, ornithine decarboxylase and *o*-nitrophenyl-β-D- galactopyranoside (ONPG) and *p*-nitrophenyl-β- D- galactoside (PNPG) tests and the production of acid from glycerol, D-arabitol, xylitol, L-arabinose, D-arabinose, D-ribose, D-xylose, meso-inositol, D-mannitol, D-sorbitol, L-fucose, D-galactose, lactose, maltose, trehalose, raffinose and dextrin.

They are commonly isolated from birds – both wild and domestic.

The medically *important Avibacterium* species are;

Avibacterium gallinarum (formerly *P. gallinarum*)

Growth on blood agar is non-symbiotic, with most strains producing a greyish-yellow pigment and showing a more even colony development if incubated under 5–10% CO_2. Catalase and phosphatase reactions are positive. ONPG test is variable. Acid is produced from D-ribose, D-galactose, maltose, trehalose and dextrin. Acid is not produced from L-arabinose, D-mannitol or D-sorbitol. Acid production from glycerol, D-arabitol, D-arabinose, D-xylose, meso-inositol, L-fucose, lactose and raffinose is variable.

This was originally from the sinus of a *chicken but has been isolated from humans.*

Avibacterium volantium (formerly P. volantium)

Growth on blood agar is symbiotic. On blood agar, colonies are smooth, convex, and non-haemolytic. A yellowish pigment is produced by some isolates. Growth on MacConkey agar is negative. Catalase, phosphatase and ONPG reactions are positive. Acid is produced from D-ribose, D-mannitol, D-galactose, maltose, trehalose and dextrin. Acid is not produced from glycerol, D-arabitol, L-arabinose, meso-inositol or raffinose. Acid production from D-arabinose, D-xylose, D-sorbitol, L-fucose and lactose is variable.

They have been isolated from the wattles of domestic fowl and from the human tongue.

Mannheimia species[19]

This is a new genus within the family *Pasteurellaceae*. They consist of Gram negative, non-motile rods or coccobacilli. Endospores are not formed. Growth is mesophilic and facultatively anaerobic or microaerophilic. Glucose is fermented without gas production. Oxidase reaction is normally positive but might be variable. They are positive for catalase, alkaline phosphatase and nitrate reduction tests. Simmon's citrate, urease and arginine dihydrolase tests are negative and there is no fermentation of adonitol or L-sorbose, trehalose and D-mannose. All strains ferment mannitol.

There are currently 6 species and have been isolated from ruminants[20].

The medically important *Mannheimia* species is;

Mannheimia haemolytica (formerly P. *haemolytica biotype A*)

Cells are small rods and coccobacilli. Colonies are smooth and greyish on blood agar and are 1-2mm in diameter after 24hr incubation. Most strains show a characteristic β-haemolysis on bovine blood agar. D-sorbitol, D-xylose, maltose and dextrin are fermented. No strains ferment L-arabinose or glucosides. Strains are negative for ornithine decarboxylase and NPG (β-glucosidase) and positive for ONPF (α-fucosidase).

They have been isolated from pneumonia in cattle and sheep and from septicaemia in lambs and mastitis in ewes. Some of the serotypes are probably part of the resident microflora of the upper respiratory tract of ruminants[19]. In humans, they have been isolated from wound infections[21].

4.4 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.

5 Technical Information/Limitations

Commercial Identification Systems

Attempts to identify *A. hominis* by automatic identification systems may lead to misidentifications eg, *A. hominis* strains has resulted in doubtful profiles (ID 32E) or no identifications (Vitek), because *A. hominis* is not included in the databases of the systems[13].

6 Safety Considerations[22-38]

Pasteurella species are Hazard Group 2 organisms.

Two cases of laboratory acquired infections have been reported, both of which were associated with working with laboratory animals[39].

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

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[30].

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

Compliance with postal and transport regulations is essential.

7 Target organisms

Pasteurella species reported to have caused human infections[2,3,10]

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. caballi, P. oralis*

Other species reported to have caused human infections[6,14,17]

Actinobacillus ureae (formerly *P. ureae*), *Actinobacillus hominis, Bibersteinia trehalosi* (Previously known as *P. haemolytica* biotype T and then, *P. trehalosi*), *Avibacterium volantium* (formerly *P. volantium*), *Avibacterium gallinarum* (formerly *P. gallinarum*), *Mannheimia haemolytica* (formerly *P. haemolytica* biotype A)

8 Identification

8.1 Microscopic appearance

Gram stain (UK SMI TP 39 - Staining Procedures)

Pasteurella species are spherical, ovoid or rod-shaped Gram negative rods or coccobacilli which occur singly or in pairs or short chains. Bipolar staining is common, capsules may be present.

Actinobacillus species are Gram negative, pleomorphic coccobacillary rods that are arranged singly or in pairs, and rarely in chains. They give the characteristic “Morse-code” appearance.

Bibersteinia and *Avibacterium* species are Gram negative, rod-shaped or pleomorphic with cells occurring singly and in chains or short chains depending on the growth stage.

Mannheimia species are Gram negative small rods and coccobacilli.

8.2 Primary isolation media

Blood agar incubated in 5-10% CO_2 at 35-37°C for 16–48hr.

8.3 Colonial appearance

Organism	**Characteristics of growth on blood agar after incubation in 5-10% CO_2 at 35-37°C for 16–48hr.**
Pasteurella species	Colonies are grey and viscous, but rough irregular colonies occur frequently.
Bibersteinia species	Colonies on blood agar are round, regular, greyish or yellowish, semi-transparent at the periphery and are about 2mm in diameter. Some strains are haemolytic.

Avibacterium species	Colonies on sheep-blood agar are non-haemolytic, greyish, opaque, but eventually translucent at the periphery, with a butyrous consistency, smooth and shiny, circular and raised with an entire margin.
Actinobacillus species	Colonies are non-haemolytic, translucent and 1-2mm in diameter on blood agar.
Mannheimia species*	Colonies are smooth and greyish on blood agar and are 1-2mm in diameter after 24hr incubation. Most strains show a characteristic β-haemolysis on bovine blood agar.
*Freshly isolated strains of *M. haemolytica* produce clear zones of ß-haemolysis on blood agar.	

8.4 Test procedures

8.4.1 Biochemical tests

Oxidase test (UK SMI TP 26 - Oxidase Test)

Pasteurella species are oxidase positive except *P. bettyae.*

Catalase test (UK SMI TP 8 - Catalase Test)

Pasteurella species are catalase positive except *P. bettyae*, *P. caballi* and some strains of *A. hominis* which are catalase negative.

Growth on CLED or MacConkey agar

Pasteurella species, *Avibacterium* species, *Actinobacillus pleuropneumoniae* and some strains of *Actinobacillus suis* do not show growth on MacConkey agar but can grow poorly on some CLED agars.

However*, Actinobacillus* species and *Pasteurella aerogenes* grow well on MacConkey agar.

Sensitivity to penicillin

Pasteurella species are typically penicillin susceptible and so a zone of inhibition around a 10-U penicillin disc may aid differentiation from other Gram negative bacilli[2].

8.4.2 Commercial identification systems

Laboratories should follow manufacturer's instructions and rapid tests and kits must be validated and be shown to be fit for purpose prior to use.

8.4.3 Matrix-Assisted Laser Desorption/Ionisation - Time of Flight Mass Spectrometry (MALDI-TOF MS)

This has been shown to be a rapid and powerful tool because of its reproducibility, speed and sensitivity of analysis. The advantage of MALDI-TOF MS as compared with other identification methods is that the results of the analysis are available within a few hours rather than several days. The speed and the simplicity of sample preparation and result acquisition associated with minimal consumable costs make this method well suited for routine and high-throughput use[40].

This method has been used to identify *P. multocida* and can also be used as an alternative to 16S rRNA sequencing for identification of difficult- to - identify bacterial strains[41].

8.4.4 Nucleic Acid Amplification Tests (NAATs)

PCR is usually considered to be a good method as it is simple, sensitive and specific. The basis for PCR diagnostic applications in microbiology is the detection of infectious agents and the discrimination of non-pathogenic from pathogenic strains by virtue of specific genes.

This technique has been used to identify and confirm *P. multocida* and *M. haemolytica* in infections[42].

PCR has also been used in direct detection of the *toxA* gene in toxigenic *P. multocida* specimens[43]. This has not only facilitated rapid clinical diagnoses and prompt therapy but has also facilitated epidemiology studies and screening to prevent transmission to clean herds by animal movement.

A PCR method based on 16S rDNA and specific for *P. pneumotropica* has also been described[44].

8.5 Further Identification

Conventional identification of *Pasteurella* and related bacteria remains a challenge to many laboratories. Most commercial identification systems are likely to overlook species other than *P. multocida*, and further phenotypic characterization is long, fastidious, and sometimes inconclusive or misleading.

Rapid Molecular Methods

Molecular methods have had an enormous impact on the taxonomy of *Pasteurella*. Analysis of gene sequences has increased understanding of the phylogenetic relationships of *Pasteurella* species and related organisms and has resulted in the recognition of numerous new species. Molecular techniques have made identification of many species more rapid and precise than is possible with phenotypic techniques.

A variety of rapid typing methods have been developed for isolates from clinical samples; these include molecular techniques such as *sodA* Sequencing, 16S rDNA gene sequencing, Multilocus Sequence Analysis (MLSA), and Ribotyping. All of these approaches enable subtyping of unrelated strains, but do so with different accuracy, discriminatory power, and reproducibility.

However, some of these methods remain accessible to reference laboratories only and are difficult to implement for routine bacterial identification in a clinical laboratory.

16S rDNA (rRNA) gene sequencing

A genotypic identification method, 16S rDNA gene sequencing is used for phylogenetic studies and has subsequently been found to be capable of re-classifying bacteria into completely new species, or even genera. It has also been used to describe new species that have never been successfully cultured and to show distinct differences between subspecies as well.

This has been used to differentiate between the *P. multocida* subspecies. The 16S rDNA sequence is identical for *P. multocida* subsp. *multocida* and *Pasteurella multocida* subsp. *gallicida* but differs from that of *P. multocida* subsp. *septica*[45].

This has also been used to describe new species; *Pasteurella* species B was classified as *Pasteurella oralis* using the sequence of the 16S rRNA gene of these strains based on *rpoB* sequence as well as to delineate six new species in the genus *Mannheimia* based on their sequences and to reclassify some *Pasteurella* species to the genus *Avibacterium*[10,17,19].

It has been recommended that 16S rRNA gene sequencing be used as supplementary genotyping method for identification of *P. mairii, P. aerogenes* and *Actinobacillus rossi*[4].

sodA Sequencing

Sequence based identification is a convenient alternative which should be recommended when dealing with unusual or atypical isolates and when performing epidemiological studies, despite its cost.

The *sodA* sequence method provides a rapid and accurate tool for species identification and the high discriminative power related to the *sodA* gene variability has been particularly helpful in recognizing closely related species or subspecies of *Pasteurella*, which cannot be achieved with the same confidence through 16S rRNA gene analysis[21].

The sequencing of *sodA* in *Haemophilus* and *Actinobacillus* species has been in progress and should provide new insights on the phylogeny of the family *Pasteurellaceae*. This convenient genetic approach might help to investigate the distribution of *Pasteurella* species in human infections.

Ribotyping

This technique involves the fingerprinting of genomic DNA restriction fragments that contain all or part of the genes coding for the 16S and 23S rRNA.

The use of ribotyping with EcoR1 is a reliable tool for separation of isolates related to either *P. aerogenes* or *Actinobacillus rossi*[4].

Multilocus Sequence Analysis (MLSA)

Multilocus sequence analysis (MLSA) has been suggested as an alternative to 16S rRNA gene analysis and recommended for improving identification at the species level of members of the *Pasteurellaceae*.

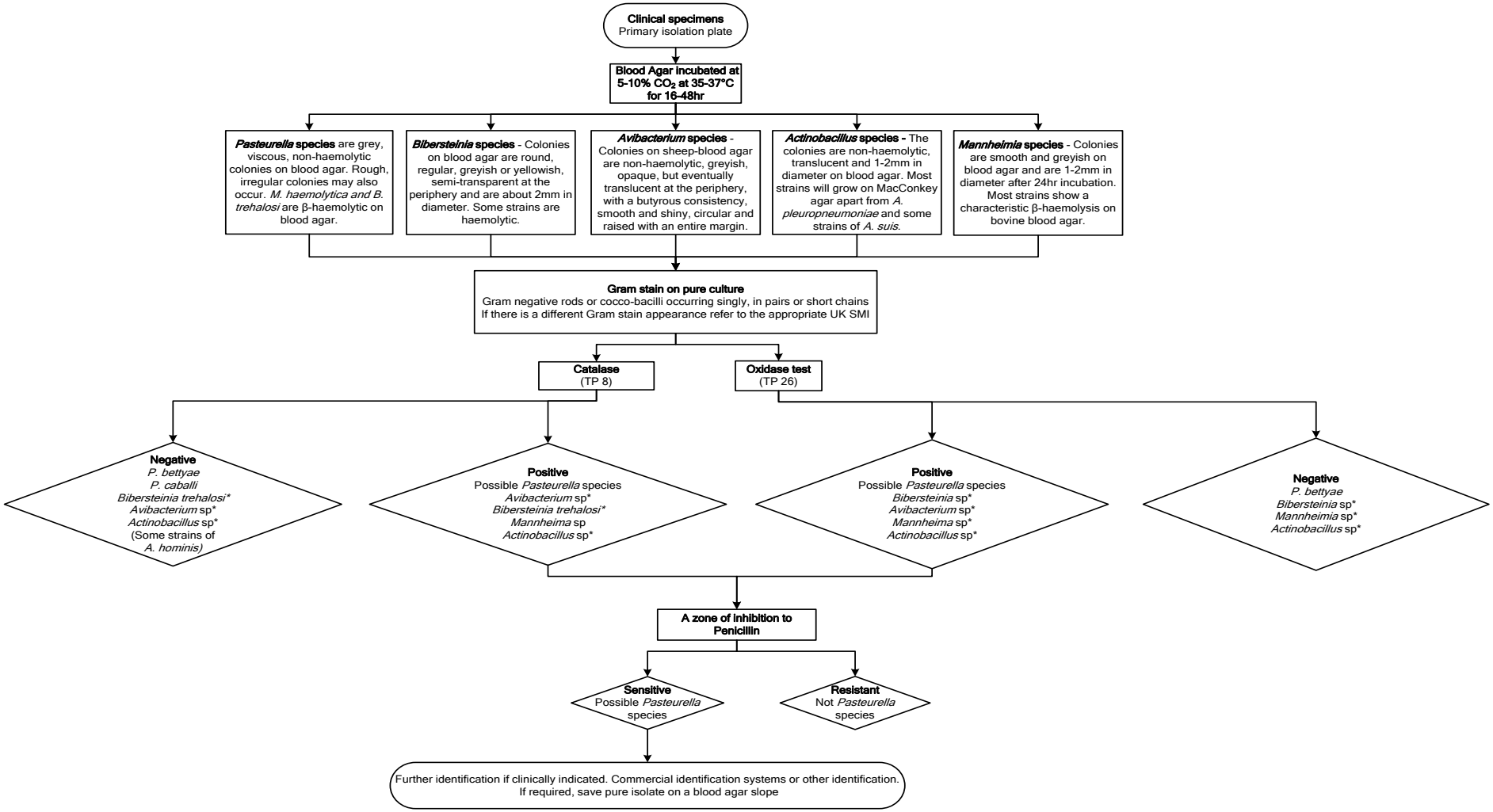
This has shown that *Avibacterium* may need to be reclassified leaving only two or three species in the genus. Major difficulties have been experienced in resolving the phylogeny of members of this genus[46].

This method is being used to demonstrate grouping *P. multocida* for further characterisation as to common potential virulence properties and for the design of specific PCR tests to detect this population[47].

8.6 Storage and Referral

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

9 Identification of Pasteurella species and Morphologically Similar Organisms



* These species give variable results in these tests

10 Reporting

10.1 Presumptive Identification

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

10.2 Confirmation of Identification

N/A

10.3 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.

10.4 CCDC

Refer to local Memorandum of Understanding.

10.5 UK Health Security Agency[48]

Refer to current guidelines on CIDSC and COSURV reporting.

10.6 Infection Prevention and Control Team

N/A

11 Referral to reference laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory see user manuals and request forms

Contact appropriate reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

England

Wales

Scotland

Northern Ireland

Note: In case of sending away to laboratories for processing, ensure that specimen is placed in appropriate package and transported accordingly.

12 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.

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An explanation of the reference assessment used is available in the scientific information section.

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