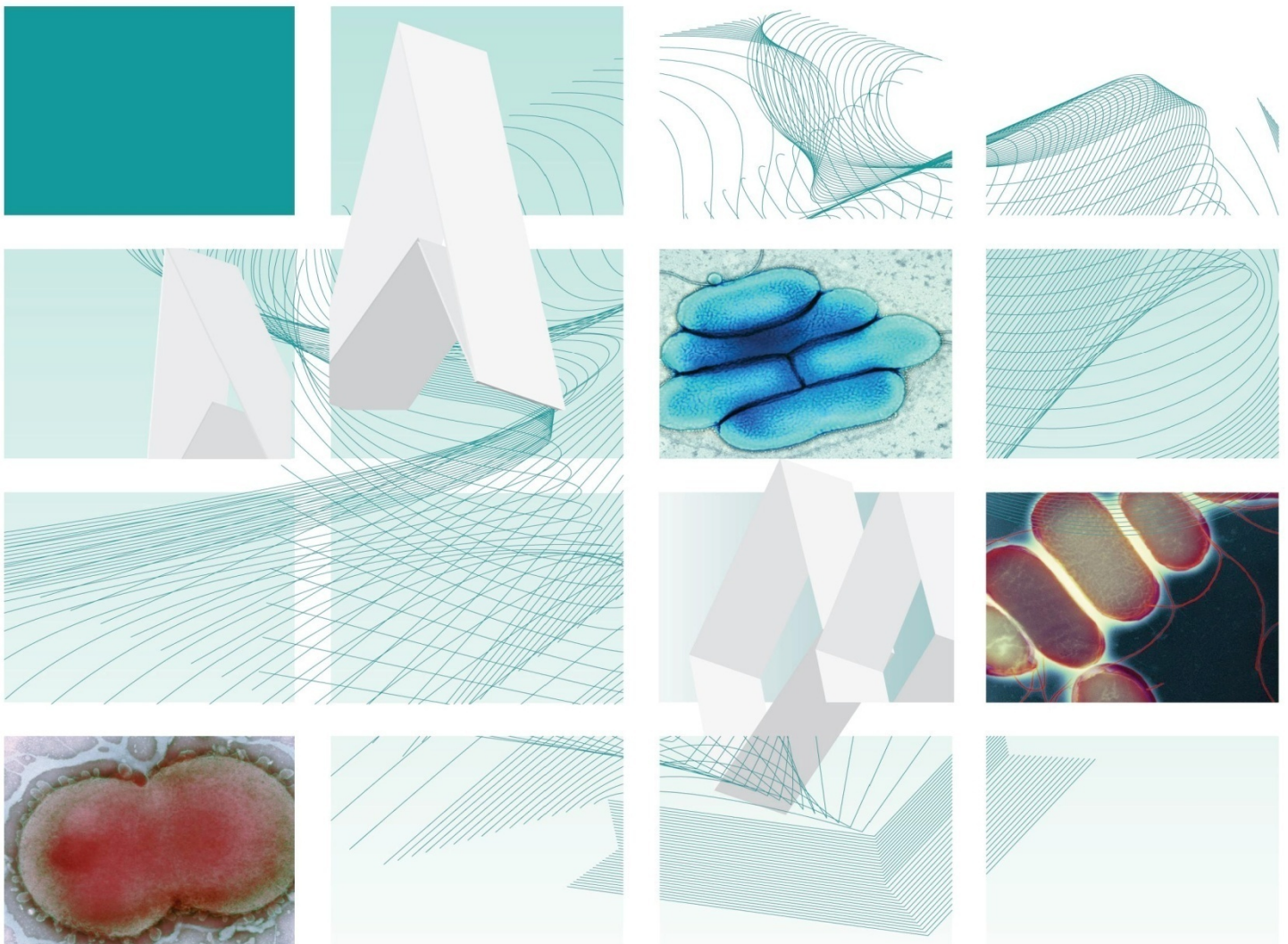




# UK Standards for Microbiology Investigations

## Identification of *Haemophilus* species and the HACEK Group of Organisms



## Acknowledgments

---

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see <https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee>).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the Medical Editors for editing the medical content.

For further information please contact us at:

Standards Unit  
Microbiology Services  
Public Health England  
61 Colindale Avenue  
London NW9 5EQ

E-mail: [standards@phe.gov.uk](mailto:standards@phe.gov.uk)

Website: <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>

UK Standards for Microbiology Investigations are produced in association with:



Logos correct at time of publishing.

## Contents

---

ACKNOWLEDGMENTS .....	2
AMENDMENT TABLE .....	4
UK STANDARDS FOR MICROBIOLOGY INVESTIGATIONS: SCOPE AND PURPOSE.....	6
SCOPE OF DOCUMENT .....	9
INTRODUCTION .....	9
TECHNICAL INFORMATION/LIMITATIONS.....	19
1 SAFETY CONSIDERATIONS .....	20
2 TARGET ORGANISMS.....	20
3 IDENTIFICATION.....	20
4A IDENTIFICATION OF <i>HAEMOPHILUS</i> SPECIES.....	26
4B IDENTIFICATION OF HACEK GROUP.....	27
5 REPORTING .....	28
6 REFERRALS.....	28
7 NOTIFICATION TO PHE OR EQUIVALENT IN THE DEVOLVED ADMINISTRATIONS .....	30
REFERENCES .....	31



NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at [www.nice.org.uk/accreditation](http://www.nice.org.uk/accreditation).

For full details on our accreditation visit: [www.nice.org.uk/accreditation](http://www.nice.org.uk/accreditation).

## Amendment Table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from [standards@phe.gov.uk](mailto:standards@phe.gov.uk).

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment No/Date.	6/03.02.15
Issue no. discarded.	2.3
Insert Issue no.	3
<b>Section(s) involved</b>	<b>Amendment</b>
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Introduction.	The taxonomy of <i>Haemophilus</i> species and other HACEK Group of organisms have been updated.  More information has been added to the Characteristics section. The medically important species are mentioned. Other HACEK organisms that are medically important are also mentioned and their characteristics described.  Section on Principles of Identification has been updated to include the MALDI-TOF.
Technical Information/Limitations.	Addition of information regarding Agar Media and X & V factor Testing and Incubation.
Safety considerations.	This section has been updated to include handling of <i>Haemophilus</i> species and laboratory acquired infections.
Target Organisms.	The section on the Target organisms has been updated and clearly presented.
Identification.	Updates have been done on 3.2, 3.3 and 3.4 to reflect standards in practice.  Section 3.4.1 has 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 <i>Haemophilus</i> species and other HACEK Group of organisms has been done for easy guidance.

Identification of *Haemophilus* species and the HACEK Group of Organisms

Reporting.	Subsections 5.3 have been updated to reflect the information required on reporting practice.
Referral.	The addresses of the reference laboratories have been updated.
Whole document.	Document presented in a new format.
References.	Some references updated.

# UK Standards for Microbiology Investigations<sup>#</sup>: Scope and Purpose

---

## Users of SMIs

- SMIs are primarily intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK.
- SMIs provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests.
- SMIs provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

## Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages.

Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

## Equal Partnership Working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies.

The list of participating societies may be found at <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>.

Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process.

SMIs are developed, reviewed and updated through a wide consultation process.

---

<sup>#</sup>Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

## Quality Assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008.

SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development.

The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

## Patient and Public Involvement

The SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

## Information Governance and Equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions.

The development of SMIs are subject to PHE Equality objectives <https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity>. The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

## Legal Statement

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

SMIs are Crown copyright which should be acknowledged where appropriate.

### **Suggested Citation for this Document**

Public Health England. (2015). Identification of *Haemophilus* species and the HACEK Group of Organisms. UK Standards for Microbiology Investigations. ID 12 Issue 3. <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>



## Scope of Document

---

This SMI describes the identification of *Haemophilus* species and other members of the HACEK group (*Haemophilus* species, *Aggregatibacter actinomycetemcomitans* (formerly *Actinobacillus actinomycetemcomitans*), *Aggregatibacter aphrophilus* (formerly *Haemophilus aphrophilus* and *Haemophilus paraphrophilus*), *Cardiobacterium hominis*, *Eikenella corrodens* and *Kingella* species).

This SMI should be used in conjunction with other SMIs.

## Introduction

---

### Taxonomy

There are currently fourteen species of the genus *Haemophilus*<sup>1</sup>. The *Haemophilus* species associated with humans are *H. influenzae*, *H. aegyptius*, *H. haemolyticus*, *H. parainfluenzae*, *H. pittmaniae*, *H. parahaemolyticus*, *H. paraphrohaemolyticus*, *H. ducreyi* and *H. sputorum*<sup>2,3</sup>. Nucleic acid hybridisation studies and 16S rRNA sequence homologies suggest *H. ducreyi* does not belong in the genus *Haemophilus*, though it does seem to be a valid member of the family Pasteurellaceae. *Haemophilus aphrophilus* and *H. paraphrophilus* have been re-classified as a single species on the basis of multilocus sequence analysis, *Aggregatibacter aphrophilus*, which includes V-factor dependent and V-factor independent isolates. *H. segnis* has been re-classified as *Aggregatibacter segnis*<sup>4</sup>.

*H. influenzae* is the type species.

### Characteristics

*Haemophilus* species are Gram negative spherical, oval or rod-shaped cells less than 1µm in width, variable in length, with marked pleomorphism, and sometimes forming filaments. The optimum growth temperature is 35–37°C. They are facultatively anaerobic and non-motile.

Members of the *Haemophilus* genus are typically cultured on blood agar plates as all species require at least one of the following blood factors for growth: haemin (factor X) and/or nicotinamide adenine dinucleotide (factor V). Chocolate agar is an excellent *Haemophilus* growth medium as it allows for increased accessibility to these factors. Alternatively, *Haemophilus* is sometimes cultured using the "Staph streak" technique: both *Staphylococcus* and *Haemophilus* organisms are cultured together on a single blood agar plate. In this case, *Haemophilus* colonies will frequently grow in small "satellite" colonies around the larger *Staphylococcus* colonies because the metabolism of *Staphylococcus* produces the necessary blood factor by-products required for *Haemophilus* growth. All *Haemophilus* species grow more readily in an atmosphere enriched with CO<sub>2</sub>; *H. ducreyi* and some nontypable *H. influenzae* strains will not form visible colonies on culture plates unless grown in CO<sub>2</sub>-enriched atmosphere. *Aggregatibacter aphrophilus* and *Haemophilus paraphrohaemolyticus* require CO<sub>2</sub> for primary isolation.

On chocolate blood agar, colonies are small and grey, round, convex, which may be iridescent, and these develop in 24 hours. Iridescence is seen with capsulated strains.

Carbohydrates are catabolised with the production of acid. A few species produce gas. Nitrates are reduced to nitrites.

These have been isolated from abscess, respiratory secretions, middle ear fluid, CSF, purulent sputum and blood culture<sup>5</sup>.

The medically important *Haemophilus* species are described as follows;

### ***Haemophilus influenzae***<sup>6</sup>

They are small, non-motile Gram negative bacterium in the family *Pasteurellaceae*. They are facultatively anaerobic. On chocolate blood agar, colonies are small and grey, round, convex, which may be iridescent, and these develop in 24 hours. Iridescence is seen with capsulated strains. There is no growth on MacConkey or CLED agar and show no  $\beta$ -haemolysis on sheep red blood cells. They also require the X and V factors for growth.

They are positive for oxidase, catalase, nitrate reduction and phosphatase. Eleven to eighty nine percent of strains are positive for indole production and 80-89% of strains are positive for urease and ornithine decarboxylase tests. They are also negative for ONPG, H<sub>2</sub>S production and aesculin hydrolysis. Acid is produced from D-Glucose, D-Galactose, Maltose, D-Ribose and D-xylose and not from lactose, D-mannitol, D-Mannose, sucrose, Inulin, Trehalose, Raffinose, L-Rhamnose, L-Sorbose, D-Sorbitol, Fructose and Melibiose<sup>6</sup>.

Pittman described six antigenically distinct capsular types of *H. influenzae*, designated a-f based on the polysaccharide composition of the capsular structure. There is also a biotyping scheme for *H. influenzae* based on a series of biochemical reactions (indole, ornithine decarboxylase and urease production). There are 8 biotypes of *H. influenzae* (I-VIII). They can either be typable or non-typable<sup>4</sup>.

Systemic infections such as meningitis, epiglottitis, orbital cellulitis and bacteraemia are caused by capsular type b strains which generally fall within the biotypes I and II of this species. Most non-typable *H. influenzae* strains fall into biotypes II to VI and can cause acute conjunctivitis, otitis media, sinusitis, tracheobronchitis and pneumonia<sup>7</sup>.

*H. influenzae* has been isolated from respiratory secretions, CSF, sputum and blood culture<sup>7</sup>.

### ***Haemophilus parainfluenzae***<sup>6</sup>

They are small, non-motile Gram negative bacterium in the family *Pasteurellaceae*. They are facultatively anaerobic. There is no growth on MacConkey or CLED agar and show no  $\beta$ -haemolysis on sheep cells. They require V factor but not the X factor for growth.

They are positive for oxidase, nitrate reduction and H<sub>2</sub>S production. Acid is produced from Fructose, D-Galactose, D-Glucose, Maltose, Sucrose and D-Mannose. Eleven to eighty nine percent of strains are positive for catalase, ONPG, Ornithine decarboxylase and Urease. They are negative for indole production and aesculin hydrolysis. Acid is not produced from D-Adonitol, L-Arabinose, Cellobiose, Dulcitol, D-Sorbitol, L-Sorbose, Trehalose, D-Xylose, Glycerol, Inulin, Lactose, D-Mannitol, Melibiose, Raffinose, L-Rhamnose, D-Ribose and Salicin.

There are eight biotypes of *Haemophilus parainfluenzae* (I-VIII) based on a series of biochemical reactions (indole, ornithine decarboxylase and urease production)<sup>4</sup>.

They have been associated with some cases of acute otitis media, sinusitis and chronic bronchitis<sup>5</sup>.

*H. parainfluenzae* has been isolated from clinical specimens – respiratory secretions (from the lower airways, oropharynx, and nasopharynx), abscess and sputum. Although it has been isolated from sputum, it is considered a part of the normal oral flora and so not reported as significant<sup>8</sup>.

### ***Haemophilus haemolyticus*<sup>6</sup>**

They are Gram negative, non-motile and non-spore-forming short to medium length rods. There is no growth on MacConkey or CLED agar and show  $\beta$ -haemolysis on sheep cells. They also require the X and V factors for growth.

They are positive for oxidase, catalase, nitrate reduction, phosphatase, urease and H<sub>2</sub>S production. Some strains of *H. haemolyticus* (11-89%) are positive for Indole production. Acid is produced from D-Galactose, D-Glucose, Maltose and D-Ribose and about 11-89% of strains produce acid from D- Xylose. They are negative for ONPG, ornithine decarboxylase and aesculin hydrolysis. Acid is not produced from D-Adonitol, L-Arabinose, Cellobiose, Dulcitol, Glycerol, Inulin, Lactose, D-Mannitol, D-Mannose, Melibiose, Raffinose, L-Rhamnose, Salicin, D-Sorbitol, L-Sorbose, Sucrose and Trehalose.

### ***Haemophilus parahaemolyticus*<sup>9</sup>**

These usually differ morphologically from other haemophilic bacteria in that they are larger, stain more heavily and unevenly, and occur in long tangled thread forms with much pleomorphism.

The colonies tend to be larger, less translucent, and on blood agar, they are surrounded by a large colourless zone of haemolysis. In broth, there is stringy floccular sediment with clear supernatant. The V factor but not X factor is required for growth.

They are positive for oxidase, nitrate reduction, H<sub>2</sub>S production and urease tests. Some strains of *H. parahaemolyticus* (11-89%) are positive for catalase, ONPG, Ornithine decarboxylase and produce acid from D-Galactose. Acid is also produced from fructose, D-Glucose, maltose and sucrose. They are negative for indole production and aesculin hydrolysis. Acid is not produced from D-Adonitol, L-Arabinose, Cellobiose, Dulcitol, Glycerol, Inulin, Lactose, D- Mannitol, D-Mannose, Melibiose, Raffinose, L-Rhamnose, Salicin, D-Sorbitol, L-Sorbose, D-Xylose and Trehalose<sup>6</sup>.

The bacteria are associated frequently with acute pharyngitis and occasionally cause sub-acute endocarditis.

### ***Haemophilus paraphrohaemolyticus*<sup>10</sup>**

They are Gram negative, non-motile and non-spore-forming short to medium length rods measuring 0.75- 2.5 $\mu$ m and 0.4-.0.5 $\mu$ m. They grow well at 37°C both in air and in air with added CO<sub>2</sub>.

On blood agar plate, the colonies are smooth, round and dome-shaped and they also produce large zones of clear haemolysis. Chocolate agar promotes larger colonies than blood agar, irrespective of the presence or absence of CO<sub>2</sub>. The V factor but not X factor is required for growth. No growth is observed on inspissated serum or on MacConkey or CLED agar.

They are positive for catalase, oxidase, nitrate reduction, H<sub>2</sub>S production and urease tests. Acid is produced from fructose, D-Glucose, Maltose and sucrose. Eleven to

eighty nine percent of strains are positive for ONPG and produces acid from D-Galactose. They are negative for ornithine decarboxylase, Indole production and aesculin hydrolysis. Acid is not produced from D-Adonitol, L-Arabinose, Cellobiose, Dulcitol, Glycerol, Inositol, inulin, lactose, D-Mannitol, D-Mannose, Melibiose, Raffinose, L-Rhamnose, D-Ribose, Salicin, D-Sorbitol, L-Sorbose, Trehalose and D-Xylose<sup>6</sup>.

It has been isolated from sputum, throat, pharynx, thumb print and urethral discharge in humans<sup>10</sup>.

### ***Haemophilus aegyptius*<sup>11</sup>**

They are Gram negative, non-motile, non-spore-forming, non-encapsulated bacillus, 0.25-0.5µm by 1.0-2.5µm, with rounded ends and sometimes with a bipolar body. It is a facultative aerobe. It requires both haemin and V factors for growth. The optimum temperature is 35-37°C with a range of 25-40°C. The colonies on blood agar are small and dew-drop-like without haemolysis; on transparent agar, they have a bluish tinge in transmitted light; and in semifluid medium they are granular to fluffy. They are soluble in sodium desoxycholate, reduce nitrates to nitrites, and do not produce indole. Slight acidity is formed from glucose and galactose; reaction on levulose is variable and on xylose negative. It agglutinates human red cells.

*Haemophilus aegyptius* can be differentiated from *Haemophilus influenzae* by serological means and to a certain extent, by growth characteristics and biochemical reactions.

### ***Haemophilus pittmaniae*<sup>12</sup>**

They are non-motile, facultatively anaerobic, Gram negative, small, pleomorphic rods, with occasional long, filamentous forms. Colonies on chocolate agar are greyish white and reach a diameter of 1-2mm after 24hr at 35°C. A distinct β-haemolytic zone is produced around the colonies on horse or sheep blood agar. They depend on V-factor for growth on brain heart infusion agar plates, but are capable of growth on blood plates due to release of V factor from lysed blood cells. They are positive for porphyrin test, negative or weakly positive in catalase and oxidase tests. Acid is produced from D-glucose, D-fructose, sucrose, D-mannose, D-galactose and maltose. A small amount of gas is produced from glucose. They also produce β-galactosidase (ONPG), alkaline phosphatase, acid phosphatase and leucine arylamidase, but not β-glucosidase (NPG), α-glucosidase (PNPG), β-glucosaminidase (GNAC), β-glucuronidase (PGUA) or α-fucosidase (ONPF).

They are negative for the indole, urease, in lysine and ornithine decarboxylase and arginine dihydrolase tests. Acid is not produced from lactose, D-xylose, D-mannitol, D-sorbitol, sorbose, melibiose, inulin, aesculin or amygdalin.

*Haemophilus pittmaniae* was originally isolated from human saliva and is part of the normal flora of the oral mucous membranes of man. It is an opportunistic pathogen and has been isolated from various sites of infection, including blood and bile.

### ***Haemophilus ducreyi*<sup>13</sup>**

Cells are Gram negative coccobacilli in "railroad track" arrangement. They grow best in microaerophilic conditions at 33-35°C in a humid atmosphere containing 5% CO<sub>2</sub>. The identification of *H. ducreyi* growing from cultured specimens is not easy because the organism often cannot grow in the media used for routine biochemical testing; *H. ducreyi* grows on Mueller-Hinton agar with 5% sheep blood in a CO<sub>2</sub> enriched

atmosphere. They produce characteristic tan-yellow colonies that are highly self-adherent and can be 'nudged' intact over the surface of the agar. Furthermore, identification is not easy because *H. ducreyi* is asaccharolytic.

They require X factor for growth and this can most easily be evaluated using the porphyrin test. They are positive for oxidase and negative for catalase test.

*H. ducreyi* has been isolated from a number of ulcer specimens including leg, foot, perianal and genital (penis)<sup>14</sup>.

### ***Haemophilus sputorum*<sup>3</sup>**

Cells are non-motile, small regular rods, 0.3-0.5µm × 2.0-3.0µm, with occasional coccoid forms. Colonies on chocolate agar are convex, whitish, opaque, and reach a diameter of 0.5–1.5mm within 24hr. Zones of β-haemolysis are produced around colonies on horse or sheep blood agar; occasional strains are non-haemolytic and consequently fail to grow on blood agar. Cells are unable to synthesize nicotinamideadenine dinucleotide de novo, ie growth is dependent on V factor. Porphyrins are synthesized from δ -aminolevulinic acid that is X factor is not required.

They are positive for oxidase and give variable results on catalase tests. Cells produce β-galactosidase, urease, and leucine arylamidase; the species are negative for indole test, arginine di-hydrolase, lysine decarboxylase, ornithine decarboxylase, and phenylalanine arylamidase. H<sub>2</sub>S is not or only weakly emitted (lead acetate test), IgA1 protease is not produced.

Acid is produced from fermentation of d-glucose, fructose, D-maltose and maltotriose; acid is not produced from N-acetyl-β-D-glucosamine, D-xylose, D-ribose, D-mannose, lactose, and D-malate. Variable fermentation is observed with D-galactose.

*H. sputorum* was originally isolated from a case of human tooth alveolitis and is occasionally involved in human infections and has been isolated from blood, sputum of patients with cystic fibrosis, and tooth alveolitis<sup>3</sup>.

### **Other HACEK group of organisms**

For the identification of *Haemophilus* species in the HACEK group see above.

A systematic approach is used to differentiate the HACEK group of clinically encountered, morphologically similar, aerobic and facultatively anaerobic Gram negative rods mainly associated with endocarditis and infections from normally sterile sites. These organisms are oropharyngeal/respiratory tract commensals<sup>15</sup>. The identification is considered together with the clinical details and the isolates may be identified further if clinically indicated. Isolates of clinically significant HACEK organisms from cases of endocarditis and normally sterile sites may be referred to the Antimicrobial Resistance and Healthcare Associated Infections Reference Unit, PHE Microbiology Services, Colindale for confirmation of identification and MIC testing.

### ***Aggregatibacter* species<sup>4</sup>**

This is a member of the family Pasteurellaceae. The genus *Aggregatibacter* contains 3 species, *Aggregatibacter actinomycetemcomitans*, *Aggregatibacter aphrophilus* and *Aggregatibacter segnis*<sup>16</sup>.

They are Gram negative, non-motile, facultatively anaerobic rods or coccobacilli. Growth is mesophilic. Several species of the genus are capnophilic and primary isolation may require the presence of 5-10% CO<sub>2</sub>. There is no dependence on X factor and the requirement for V factor is variable. Granular growth in broth is

common. Colonies on sheep and horse blood agar are greyish white and non-haemolytic. Acid is produced from glucose, fructose and maltose, whereas arabinose, cellobiose, melibiose, melezitose, salicin and sorbitol are not fermented. The fermentation of galactose, lactose, mannitol, mannose, raffinose, sorbose, sucrose, trehalose and xylose is variable and may aid in identification to the species level. They are also positive for nitrate reduction and alkaline phosphatase production, but strains are negative in tests for indole, urease, ornithine and lysine decarboxylases and arginine dihydrolase. Oxidase reaction is negative or weak; catalase is variably present.

The species of the genus are intimately associated with man; they are part of the human oral flora and are occasionally recovered from other body sites, including blood and brain, as causes of endocarditis and abscesses.

The type species is *Aggregatibacter actinomycetemcomitans*, originally described as '*Bacterium actinomycetemcomitans*'.

### ***Aggregatibacter actinomycetemcomitans*<sup>4</sup>**

**(Previously known as *Actinobacillus actinomycetemcomitans* and then as *Haemophilus actinomycetemcomitans*).**

They are small rods, 0.3-0.5 x 0.5-1.5µm, which may exhibit irregular staining and may appear as cocci in broth or actinomycotic lesions. They may occur singly, in pairs or in small clumps. Small amounts of extracellular slime may be produced. Cells are non-motile.

It grows best under microaerophilic conditions with added CO<sub>2</sub> and is facultatively anaerobic. The optimal growth temperature is 37°C after 24hr incubation. Colonies on chocolate agar are small, with a diameter of ≤0.5mm after 24hr, but may exceed 1-2mm after 48hr. On primary isolation, the colonies are rough, textured and adherent and have an internal, opaque pattern described as star-like or like 'crossed cigars'. The rough phenotype is related to fimbriation and to the production of hexoseamine-containing exopolysaccharide. Cells from rough colonies grow in broth as granular, autoaggregated cells that adhere to the glass and leave a clear broth. X and V factors are not required. If extracellular slime is produced, cultures may be sticky on primary isolation. Surface cultures have low viability and may die within 5-7 days.

They are positive for catalase, oxidase and acid is produced from glucose, fructose, maltose and mannose, whereas arabinose, cellobiose, galactose, lactose, melibiose, melezitose, trehalose, raffinose, salicin, sorbitol and sucrose are not fermented. Variable fermentation is observed with mannitol and xylose. They are negative for urease and ONPG hydrolysis.

The key tests for discrimination between *Aggregatibacter actinomycetemcomitans* and V factor-independent strains of *Aggregatibacter aphrophilus* are catalase and ONPG, plus fermentation of lactose, sucrose and trehalose.

They are indigenous to man, with primary habitat on dental surfaces. *Aggregatibacter actinomycetemcomitans* has regularly been isolated together with *Actinomyces* species from human actinomycosis. It has been sometimes found in other pathological processes such as endocarditis, brain abscess and urinary tract infections.

### ***Aggregatibacter aphrophilus*<sup>4</sup>**

The species *Haemophilus aphrophilus* and *Haemophilus paraphrophilus* have been reclassified as a single species *Aggregatibacter aphrophilus*.

These are Gram negative, short regular rods, 0.5 x 1.5-1.7µm with occasional filamentous forms. They require 5-10% CO<sub>2</sub> for primary isolation. Growth may be enhanced by haemin, but porphyrins are synthesized from δ-aminolaevulinic acid and X factor is not required. Some isolates require V factor (formerly *H. paraphrophilus*) whilst others are V factor independent (formerly *H. aphrophilus*). The colonies on chocolate agar are high convex, opaque, granular and yellowish and reach a diameter of 1.0-1.5mm within 24hr.

Acid is produced from glucose, fructose, lactose, maltose, mannose, sucrose and trehalose, whereas arabinose, cellobiose, mannitol, melibiose, melezitose, salicin, sorbose, sorbitol and xylose are not fermented. Variable fermentation is observed with galactose and raffinose. H<sub>2</sub>O<sub>2</sub> is not decomposed; ONPG is hydrolysed. They are also catalase and urease negative, and oxidase variable.

Key tests for discrimination between V factor-dependent isolates of *Aggregatibacter aphrophilus* and strains of *H. parainfluenzae* biotype V (negative for indole, urease and ornithine decarboxylase) are fermentation of lactose and trehalose.

*Aggregatibacter aphrophilus* is a member of the normal flora of the human oral cavity and pharynx. May cause brain abscess and infective endocarditis and has been isolated from various other body sites including peritoneum, pleura, wound and bone.

#### ***Aggregatibacter segnis*<sup>4</sup>**

**(Formerly called *Haemophilus segnis*)**

Cells are small, pleomorphic rods, often showing a predominance of irregular, filamentous forms. Growth on chocolate agar is slow and the colonies are smooth or granular, convex, greyish-white or opaque and 0.5mm in diameter after 48hr incubation. Growth in broth and fermentation media is slow, and reactions are negative or weakly positive. The growth of some strains is enhanced by 5-10% CO<sub>2</sub>. V-factor but not X-factor is required.

Small amounts of acid result from the fermentation of glucose, fructose, galactose, sucrose and maltose. Fermentation of sucrose is usually stronger than fermentation of glucose. Arabinose, cellobiose, lactose, mannitol, mannose, melibiose, melezitose, raffinose, salicin, sorbose, sorbitol, trehalose and xylose are not fermented. Catalase and β-galactosidase (hydrolysis of ONPG) are variably present. They are negative for oxidase, indole, urease and ornithine decarboxylase tests.

*Aggregatibacter segnis* is a regular member of the human oral flora, particularly in dental plaque, and can be isolated from the pharynx. It has occasionally been isolated from human infections including infective endocarditis.

#### ***Cardiobacterium* species<sup>17</sup>**

The genus *Cardiobacterium* contains 2 species, *Cardiobacterium hominis* and *Cardiobacterium valvarum*<sup>18,19</sup>. Cells are pleomorphic or straight rods, 0.5–0.75µm in diameter and 1–3µm in length with rounded ends, and long filaments may occur. Cells are arranged singly, in pairs, in short chains and in rosette clusters. They are Gram negative, but parts of the cell may stain Gram positive.

Growth on blood agar is poor. They do not require X or V factors, but may show an apparent requirement for X factor on first isolation. Very small colonies are produced unless incubated in a humid aerobic or anaerobic atmosphere with 5% CO<sub>2</sub>. After incubation for 2 days, colonies are 1mm in diameter, smooth, opaque and butyrous and show slight α- haemolysis. Some strains may pit the agar. They are facultatively

anaerobic, but CO<sub>2</sub> may be required by some strains on primary isolation. The optimum growth temperature is 30-37°C.

They are positive for oxidase, H<sub>2</sub>S production, indole (weakly), and are negative for nitrate reduction, catalase, urea and aesculin hydrolysis. They utilize dextrose, fructose, maltose, mannitol, sucrose, sorbitol, and mannose but do not utilize galactose, lactose, raffinose and xylose.

*Cardiobacterium hominis* is the type species.

### ***Cardiobacterium hominis*<sup>17</sup>**

They are Gram negative pleomorphic to short, non-motile rods. Growth on blood agar is poor. *C. hominis* does not require X or V factors, but may show an apparent requirement for X factor on first isolation. Very small colonies are produced unless incubated in a humid aerobic or anaerobic atmosphere with 5% CO<sub>2</sub>. After incubation for 2 days, colonies are 1mm in diameter, circular, smooth, entire, moist, glistening, opaque and butyrous and show slight α-haemolysis. Some strains may pit the agar. *C. hominis* is facultatively anaerobic, but CO<sub>2</sub> may be required by some strains on primary isolation. The optimum growth temperature is 30-37°C.

They are positive for oxidase, H<sub>2</sub>S production, indole (weakly), and are negative for nitrate reduction, catalase, urease and aesculin hydrolysis. They utilize dextrose, fructose, maltose, mannitol, sucrose, sorbitol, and mannose but do not utilize galactose, lactose, raffinose and xylose.

*C. hominis* could be distinguished from other members of the HACEK group and from *Pasteurella*, *Brucella*, *Streptobacillus moniliformis* and *Bordetella parapertussis*. The main characteristics of *C. hominis*, distinguishing it from other closely related organisms are absence of catalase activity, positive oxidase reaction, production of indole and absence of nitrate production<sup>20</sup>.

They have been isolated from cerebrospinal fluid, blood as well as from nose and throat in healthy individuals.

### ***Cardiobacterium valvarum*<sup>21</sup>**

They are fastidious Gram negative regular, pleomorphic to short rods. All strains are facultatively anaerobic and non-motile. Some strains have an acidulous. Its preferred culture medium is sheep blood agar, and visible colonies appear after an incubation period of 3 days. The colonies are round, elevated, opaque, smooth, and glistening. However, the colonies hardly reach 1mm after extended incubation. Therefore, *C. valvarum* is more fastidious than *C. hominis*, whose colonies appear after a two-day incubation and reach a diameter of 2.2mm after 4 days. Microscopically, *C. valvarum* appears readily decolorized by acetone alcohol, and the cellular morphology varies depending on culture medium. When grown on blood agar, it is a fairly large regular rod, measuring 1 by 2 to 4µm. On chocolate agar, it is smaller and pleomorphic.

They are positive for the production of indole, cytochrome oxidase, and H<sub>2</sub>S but negative for catalase production, urea hydrolysis, aesculin hydrolysis, and nitrate reduction. It utilizes dextrose, fructose, sorbitol, and mannose, like *C. hominis*, but unlike *C. hominis*, does not utilize maltose, sucrose, or mannitol.

It was first isolated in 2001 from the blood of a 37 year old man with endocarditis.

*Cardiobacterium valvarum* is present in subgingival pockets and dental plaques, and



all the reported cases of endocarditis have been in persons who had recently undergone a dental procedure or had oral infection<sup>18</sup>.

### ***Eikenella corrodens***<sup>22</sup>

The genus *Eikenella* contains only one species, *Eikenella corrodens*. Cells are straight, un-branched, non-sporing, slender Gram negative rods, 0.3-0.4 x 1.5-4µm in length.

Colonies may be very small on blood agar after overnight incubation or may not be visible for several days. The colonies have moist, clear centres surrounded by flat, and sometimes spreading, growth. Pitting of the medium may occur and yellow colouration may be seen in older cultures due to cell density. There may be colonial variation and spreading growth may vary between colonies of the same isolate. *E. corrodens* is non-haemolytic but a slight greening may occur around the colonies. Haemin is usually required for aerobic growth and rare strains remain X-dependent after further subculture. The optimum growth temperature is 35-37°C. *E. corrodens* is non-motile, but 'twitching' motility may be produced on some media. Strains are facultatively anaerobic and capnophilic. It may be confused with *Bacteroides ureolyticus*, which also exhibits pitting or corroding, but unlike *E. corrodens* is an obligate anaerobe and urease positive.

They are positive for oxidase, ornithine decarboxylase and nitrate reduction and are negative for acidification of carbohydrates, production of indole, aesculin hydrolysis, catalase and urease tests.

*Eikenella corrodens* exists in dental plaque of both healthy people and periodontitis patients and can cause infections. Other clinical sources include head and neck infections and respiratory tract infections.

### ***Kingella* species**<sup>23</sup>

The genus *Kingella* comprises four species, *Kingella kingae*, *Kingella denitrificans*, *Kingella potus* and *Kingella oralis*<sup>24</sup>. *Kingella indologenes* has been transferred to a new genus and classified as *Suttonella indologenes*<sup>23</sup>.

*Kingella* species are straight rods, 1.0µm in length with rounded or square ends. They occur in pairs and sometimes short chains. Endospores are not formed. Cells are Gram negative, but tend to resist decolourization. Two types of colonies occur on blood agar; a spreading, corroding type and a smooth, convex type. It does not require X or V factors. Growth is aerobic or facultatively anaerobic. The optimum growth temperature is 33-37°C<sup>25</sup>.

They are non-motile, oxidase positive, catalase negative and urease negative. Glucose and other carbohydrates are fermented with the production of acid but not gas.

*Kingella* species may grow on *Neisseria* selective agar and therefore may be misidentified as pathogenic *Neisseria* species. They can be differentiated from *Moraxella* and *Neisseria* species by a catalase test. Most *Kingella* species are catalase negative; *Moraxella* and most *Neisseria* species (except *Neisseria elongata*) are catalase positive.

### ***K. denitrificans***<sup>26</sup>

Previously designated CDC group TM-1. They are Gram negative, non-motile, plump rods 1.0µm in width. Small, translucent non-haemolytic colonies are produced on

blood agar after 48hr of incubation at 37°C. Colonies may show pitting of the medium. Growth occurs anaerobically on blood agar. They are positive for oxidase, growth at 30 and 37°C, fermentative result in the O/F test, acid production from glucose, nitrate reduction, nitrite reduction, and production of gas from nitrite.

They are also negative for catalase, growth at 5 and 45°C, growth in the presence of 4 and 6% NaCl, growth on  $\beta$ -hydroxybutyrate in mineral medium, acid production from maltose unless serum was present, starch hydrolysis and urease production. Isolated in the respiratory tract of man<sup>27</sup>.

### ***K. kingae***<sup>28</sup>

The cells are coccoid to medium-sized rods, very much like those of *Moraxella* but slightly smaller, have square ends, and occur in pairs and short chains. They are Gram negative, with some tendency to resist decolourisation. They are also non-motile, non-encapsulated and no endospores are produced. On blood agar, two types of colonies occur; colonies of freshly isolated strains appear as small depressions, 0.1-0.5mm in diameter, with a small central papilla initially but after 2 or more days incubation, there is considerable spreading growth and thin granular zones of growth often surround the colonies. Colonies when scrapped shows corrosion marks on the agar surface. The second colony, which often arises in subcultures of the first type, is small, delicate, translucent or slightly opaque, 0.1-0.6mm in diameter after 20hr on blood agar, low hemispherical, and smooth. On further incubation, the colonies increase in size but there is no evidence of corrosion or spreading. Both types of colonies are surrounded by distinct zones of  $\beta$ -haemolysis; their consistencies are soft or coherent and are not pigmented.

They are aerobic and grow at room temperature but their optimal growth is at 33-37°C. They are relatively fastidious and growth on high quality nutrient agar is as good as that on blood agar.

They are negative for catalase and urease tests. No acid is produced from fructose, lactose, saccharose, arabinose, xylose, rhamnose, mannitol, dulcitol, sorbitol, or glycerol. Gelatin and serum are not liquefied. Nitrate are not reduced or slightly reduced.

They are parasitic on human mucous membranes. Strains have been isolated from throat, nose, blood, bone lesions and joints.

### ***K. oralis***<sup>29</sup>

They are Gram negative rods or coccobacilli approximately 0.6-0.7 $\mu$ m in diameter by 1-3 $\mu$ m long with rounded ends. Cells can form pairs or chains. Cells have monopolar fimbriae up to 10 $\mu$ m long. There is a tendency to resist Gram decolourisation. Not motile by means of flagella, but cells form spreading colonies. They are aerobic or facultatively anaerobic. Growth is supported by 5% sheep blood agar supplemented with 5 mg of haemin per litre and 0.5 $\mu$ g of menadione per mL in both anaerobic and aerobic environments with CO<sub>2</sub>. They do not grow on MacConkey agar. Colonies are round with slightly irregular borders and flat to umbonate, and each colony has a granular periphery. Colonies appear to corrode the agar surface. They are positive for oxidase test and negative for nitrate, nitrite, indole, urease and aesculin hydrolysis tests. Acid is not produced from lactose, maltose, mannitol, sucrose, and xylose.

The habitat of *K. oralis* appears to be human dental plaque and has been isolated from a supragingival plaque sample from a patient with adult periodontitis.

### ***K. potus***<sup>30</sup>

Cells are gram negative, non-spore-forming, non-motile rods. They are aerobic, DNase positive, oxidase positive, and catalase negative. Colonies are circular, low convex, yellow-pigmented, smooth, entire, approximately 1.5-2mm in diameter, and friable on Columbia blood agar after 48hr of incubation at 37°C. Colonies are non-haemolytic. Non-diffusible yellow pigments are produced. Nitrate and nitrite are not reduced. Aesculin and urea are not hydrolysed. Indole is not produced. Acid is not produced from fructose, glucose, mannose, mannitol, maltose, lactose, or sucrose. No alkaline phosphatase,  $\alpha$ -glycosidase,  $\beta$ -galactosidase, or  $\beta$ -glucuronidase activity is detected. This has been isolated from the human wound caused by a bite from a kinkajou.

Tests that are useful in distinguishing *Kingella potus* from other *Kingella* species and members of the genus *Neisseria* are DNase test and its ability to pigment.

### **Principles of Identification**

Colonies on blood or chocolate agar may be presumptively identified by colonial morphology, Gram stain, haemolysis and requirement for X and V factors and CO<sub>2</sub>. The porphyrin synthesis test (see [TP 29 – Porphyrin Synthesis \(ALA\) Test](#)) may be used to differentiate haemin producing *Haemophilus* species. Identification is confirmed by commercial biochemical tests, serotyping with type-specific antisera and/or referral to a Reference Laboratory.

Full identification using for example, MALDI-TOF MS can be used to identify *Haemophilus* isolates to species level.

Isolates of *H. influenzae* from normally sterile sites should be sent to the *Haemophilus* Reference Unit, Respiratory and Systemic Infection Laboratory, Public Health England, Colindale, for confirmation and typing.

## **Technical Information/Limitations**

---

### **Agar Media and X & V factor Testing**

The use of chocolate agar is more preferable for X and V factor testing rather than blood agar or blood containing medium because of risk of carryover of X factor. This test could also be done using a basic nutrient agar but for which the X and V discs have been validated in case it had trace factors that could influence the results, usually identifying *H. influenzae* as *H. parainfluenzae*. Manufacturers' instructions should be followed when performing this test.

### **Incubation**

The X and V factor tests could sometimes give false V dependent results if incubated in CO<sub>2</sub><sup>31</sup>.

For more information on technical limitation for the X and V Factor Test, see [TP 38 – X and V Factor Test](#).

## 1 Safety Considerations<sup>32-48</sup>

---

*Haemophilus influenzae* is a Hazard Group 2 organism, and, in some cases the nature of the work may dictate full Containment Level 3 conditions. All laboratories should handle specimens as if potentially high risk.

*H. influenzae* can cause serious invasive disease, especially in young children. Invasive disease is usually caused by encapsulated strains of the organism.

Laboratory acquired infections have been reported<sup>49</sup>. The organism infects primarily by the respiratory route (inhalation), autoinoculation or ingestion in laboratory workers<sup>50</sup>.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet. For the urease test, a urea slope is considered safer than a liquid medium. The use of needles, syringes, or other sharp objects should be strictly limited and eye protection must be used where there is a known or potential risk of exposure to splashes.

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

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

Compliance with postal and transport regulations is essential.

## 2 Target Organisms

---

### HACEK group reported to have caused human infection

*Haemophilus influenzae*, *Haemophilus parainfluenzae*, *Haemophilus haemolyticus*, *Haemophilus parahaemolyticus*, *Haemophilus paraphrohaemolyticus*, *Haemophilus aegyptius*, *Haemophilus pittmaniae*, *Haemophilus ducreyi*, *Haemophilus sputorum*, *Aggregatibacter aphrophilus* (includes *Haemophilus aphrophilus* and *Haemophilus paraphrophilus*), *Aggregatibacter segnis* (formerly *Haemophilus segnis*), *Aggregatibacter actinomycetemcomitans* (formerly *Actinobacillus actinomycetemcomitans*), *Cardiobacterium hominis*, *Cardiobacterium valvarum*, *Eikenella corrodens*, *Kingella kingae*, *Kingella denitrificans*, *Kingella oralis*, *Kingella potus*

## 3 Identification

---

### 3.1 Microscopic Appearance

#### Gram stain ([TP 39 - Staining Procedures](#))

*Haemophilus* species are small coccobacilli or longer rod-shaped Gram negative cells, variable in length with marked pleomorphism and sometimes forming filaments.

Other HACEK organisms produce spherical, oval or rod-shaped Gram negative cells which may be variable in length with marked pleomorphism or filament formation.

### 3.2 Primary Isolation Media

Chocolate agar incubated in 5-10% CO<sub>2</sub> at 35-37°C for 24-48hr.

Blood agar incubated in 5-10% CO<sub>2</sub> at 35-37°C for 24-48hr.

### 3.3 Colonial Appearance

*Haemophilus* species are small, round, convex colonies, which may be iridescent and develop after 24hr incubation on chocolate agar. Satellitism of *H. influenzae* may be seen around colonies of *S. aureus* on blood agar.

Colonial morphology of other HACEK organisms varies with species and isolation medium (see subheading “Characteristics” and table below).

#### Aerobic growth Characteristics of HACEK group organisms

HACEK group organisms	Characteristics of growth on blood agar after aerobic incubation at 35-37°C for 16-48hr
<i>A. actinomycetemcomitans</i>	Will not grow in air but grows in air + CO <sub>2</sub> . Minute colonies at 24hr, 1mm at 48hr. Firm, adherent, star-shaped colonies with rough surface and which may produce pitting of the agar. Some strains may be sticky. Non-haemolytic.
<i>A. aphrophilus</i>	Requires added CO <sub>2</sub> for primary isolation. Opaque, yellowish colonies 1.0-1.5mm at 24hr. X-factor enhances growth but there is not an absolute requirement for it. Some isolates require V factor (formerly <i>H. paraphrophilus</i> ) whereas others are V-factor-independent (formerly <i>H. aphrophilus</i> ). Non-haemolytic.
<i>A. segnis</i>	Growth on chocolate agar is slow and the colonies are smooth or granular, convex, greyish-white or opaque and 0.5mm in diameter after 48hr incubation.
<i>C. hominis</i>	Some strains will not grow without added CO <sub>2</sub> . May require X-factor on primary isolation. Colonies smooth, convex and opaque. 1-2mm at 48hr. Slight α-haemolysis.
<i>C. valvarum</i>	Grows best in air +5% CO <sub>2</sub> . Slow growing, colonies smooth, round, opaque and glistening, 0.6-0.8mm after 48hr. Some strains show slight α-haemolysis, others are non-haemolytic.
<i>E. corrodens</i>	Colonies very small, moist, clear centres surrounded by flat growth. Pitting may occur. Spreading is rare and usually confined to a very small area around the colony. Non-haemolytic. Colonies 0.5-1mm after 48hr. Requires 5-10% CO <sub>2</sub> .
<i>K. kingae</i>	2 types of colony: a spreading, corroding type and a smooth, convex type. Small zone of β-haemolysis. Cells are often capsulate, producing mucoid colonies. Does not require 5-10% CO <sub>2</sub> .
<i>K. denitrificans</i>	Non-haemolytic. 2 types of colony: a spreading, corroding type and a smooth, convex type.
<i>K. oralis</i>	Colonies are round with slightly irregular borders and flat to umbonate, and each colony has a granular periphery. Colonies appear to corrode the agar surface.
<i>K. potus</i>	Colonies are circular, low convex, yellow- pigmented, smooth, entire, approximately 1.5-2mm in diameter, and friable on Columbia blood agar after 48hr of incubation at 37°C. Colonies are non-haemolytic.

**Note 1:** For descriptions of *Haemophilus* species, see subheading “Characteristics”

### 3.4 Test Procedures

#### 3.4.1 Biochemical tests

These tests are no longer done routinely in laboratories except in cases where there are doubts, and may be useful.

**Catalase Test** ([TP 8 - Catalase Test](#))

**Oxidase Test** ([TP 26 - Oxidase Test](#))

**Urease Test** ([TP 36 – Urease Test](#))

Summary of the biochemical tests:

Organism	Catalase	Oxidase	Urease
<i>H. influenzae</i>	+	+	(+)
<i>H. aegyptius</i>	+	+	+
<i>H. ducreyi</i>	-	+	Unknown
<i>H. haemolyticus</i>	+	+	+
<i>H. parainfluenzae</i>	d	+	d
<i>H. pittmaniae</i>	d	d	-
<i>H. parahaemolyticus</i>	d	+	+
<i>H. paraphrohaemolyticus</i>	+	+	+
<i>H. sputorum</i>	V	+	+
<i>A. actinomycetemcomitans</i>	+	+	-
<i>A. aphrophilus</i>	-	-	-
<i>C. hominis</i>	-	+	-
<i>C. valvarum</i>	-	+	-
<i>E. corrodens</i>	-	+	-
<i>K. kingae</i>	-	+	-
<i>K. denitrificans</i>	-	+	-
<i>K. oralis</i>	-	+	-
<i>K. potus</i>	-	+	-

+ = positive, - = Negative, (+) = 80-89% positive, d= 11-89% positive, V= variable result

#### Growth requirement for X and V factors

This is used to distinguish *Haemophilus* species ([TP 38 - X and V Factor Test](#) or [TP 29 – Porphyrin Synthesis \(ALA\) Test](#)).

### Summary of X and V test results

Organism	X factor	V factor	X + V factor	Porphyrin
<i>H. influenzae</i> <sup>a</sup>	No growth	No growth	Growth	Negative
<i>H. haemolyticus</i> <sup>b</sup>	No growth	No growth	Growth	Negative
<i>H. parainfluenzae</i>	No growth	Growth	Growth	Positive
<i>H. pittmaniae</i>	No growth	Growth	Growth	Positive
<i>H. parahaemolyticus</i>	No growth	Growth	Growth	Positive
<i>H. paraphrohaemolyticus</i>	No growth	Growth	Growth	Positive
<i>Haemophilus ducreyi</i>	Growth	No growth	Growth	Positive
<i>Haemophilus sputorum</i>	No growth	Growth	Growth	Positive

<sup>a</sup>*H. aegyptius* is indistinguishable from *H. influenzae* biotype III in normal laboratory tests.

<sup>b</sup>β-haemolytic on horse blood agar.

#### 3.4.2 Serotyping *H. influenzae* with commercial type-specific antisera

#### 3.4.3 Commercial identification Systems

Several commercial identification systems that use biochemical or enzymatic substrates are available for identification of *Haemophilus* species. The manufacturer's instructions should be followed precisely when using these kits. In many cases, the commercial identification system may not reflect recent changes in taxonomy.

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

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 as compared with other identification methods is that the results of the analysis are available within a few minutes to 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<sup>51</sup>.

MALDI- TOF MS has been used to describe and characterise new specie, *Haemophilus sputorum*<sup>3</sup>. Members of the species yield a unique MALDI-TOF mass spectrum distinct from other related *Haemophilus* species.

MALDI- TOF MS can be used to accurately identify the HACEK organisms despite their fastidious nature<sup>52,53</sup>. This technique can also be used for rapid discrimination of *Haemophilus influenzae*, *H. parainfluenzae* and *H. haemolyticus*, although, there are suggestions of misidentifications of commensal *H. haemolyticus* as *H. influenzae*<sup>54</sup>. This could be resolved by the addition of a suitable *H. haemolyticus* reference spectrum to the system's database as well as alternative tests being applied in case of ambiguous test results on isolates from seriously ill patients.

This has also been used to rapidly distinguish between *C. hominis* and *C. valvarum*<sup>55</sup>.

#### 3.4.5 Nucleic Acid Amplification Tests (NAATs)

PCR has been used to identify *H. ducreyi* in clinical specimens. Orle et al. reported on the development of a commercial multiplex PCR assay that permits the simultaneous

amplification of DNA targets from *H. ducreyi*, *T. pallidum*, and Herpes Simplex Virus types 1 and 2 directly from genital ulcer specimens<sup>56</sup>.

16s rRNA PCR assay followed by sequencing and analysis has been used for rapid identification of difficult and serious infections due to fastidious microorganisms – *Cardiobacterium hominis*<sup>57</sup>. In addition, this method can also be used to discriminate *C. hominis* from *C. valvarum*, which has recently been found to be responsible for endocarditis.

*H. haemolyticus* and *H. influenzae* differ from other *Haemophilus* species because they require haemin (X factor) and NAD (V factor) for growth. *H. haemolyticus* can easily be distinguished from encapsulated *H. influenzae* because *H. influenzae* isolates produce one of the six structurally distinct capsules that can be easily determined by slide agglutination assay, whereas *H. haemolyticus* has never been shown to produce a capsule. However, due to the high similarity in morphology, biochemistry, and genetics between *H. haemolyticus* and non-encapsulated or nontypeable *H. influenzae*, distinguishing the two by standard microbiology methods has been challenging. A new PCR assay has been developed and this has proved to be a superior method for discrimination of non-typeable *Haemophilus influenzae* from closely related *Haemophilus* species with the added potential for quantification of *H. influenzae* directly from specimens. It has also been suggested it would be suitable for routine non-typeable *Haemophilus influenzae* surveillance and to assess the impact of antibiotics and vaccines, on *H. influenzae* carriage rates, carriage density, and disease<sup>58</sup>. The *hpd*- and *iga*- based PCR assays can be used in combination with standard microbiological methods to improve the identification of *H. haemolyticus* from non-typeable *Haemophilus influenzae*<sup>59</sup>.

### 3.5 Further Identification

#### Rapid Molecular Methods

Molecular methods have had an enormous impact on the taxonomy of *Haemophilus*. Analysis of gene sequences has increased understanding of the phylogenetic relationships of *Haemophilus* 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 Pulsed Field Gel Electrophoresis (PFGE), Multilocus Sequence Typing (MLST), Ribotyping, and 16S rRNA gene sequencing. 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 rRNA gene sequencing

A genotypic identification method, 16S rRNA 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.

This has been used for better discrimination of closely related species such as *C. hominis* and *C. valvarum*<sup>21,60</sup>. It has equally been used for identifying *Aggregatibacter* species<sup>53</sup>.



## Ribotyping

Ribotyping is based on restriction fragment length polymorphisms of rRNA genes, which are highly conserved and are usually present in multiple copies on the genome. Ribotyping does however present some disadvantages; it is labour intensive and requires costly enzymes and materials. Nevertheless, ribotyping provides a highly reproducible and reliable reference typing system.

This has been used to identify and characterise *H. ducreyi* and it was found to be highly reproducible and that it discriminated among strains of *H. ducreyi*<sup>61,62</sup>. It may be used to study the epidemiology of *H. ducreyi* and chancroid.

This has also been used successfully in the identification of *H. influenzae* and may help to understand the molecular characteristics of outbreaks, endemicity and value of vaccination<sup>63</sup>.

## Pulsed Field Gel Electrophoresis (PFGE)

PFGE detects genetic variation between strains using rare-cutting restriction endonucleases, followed by separation of the resulting large genomic fragments on an agarose gel. PFGE is known to be highly discriminatory and a frequently used technique for outbreak investigations and has gained broad application in characterizing epidemiologically related isolates. However, the stability of PFGE may be insufficient for reliable application in long-term epidemiological studies. However, due to its time-consuming nature (30hr or longer to perform) and its requirement for special equipment, PFGE is not used widely outside the reference laboratories<sup>64,65</sup>.

This has been used successfully to identify and discriminate between strains of non-typeable *Haemophilus influenzae*<sup>66</sup>.

## Multilocus Sequence Typing (MLST)

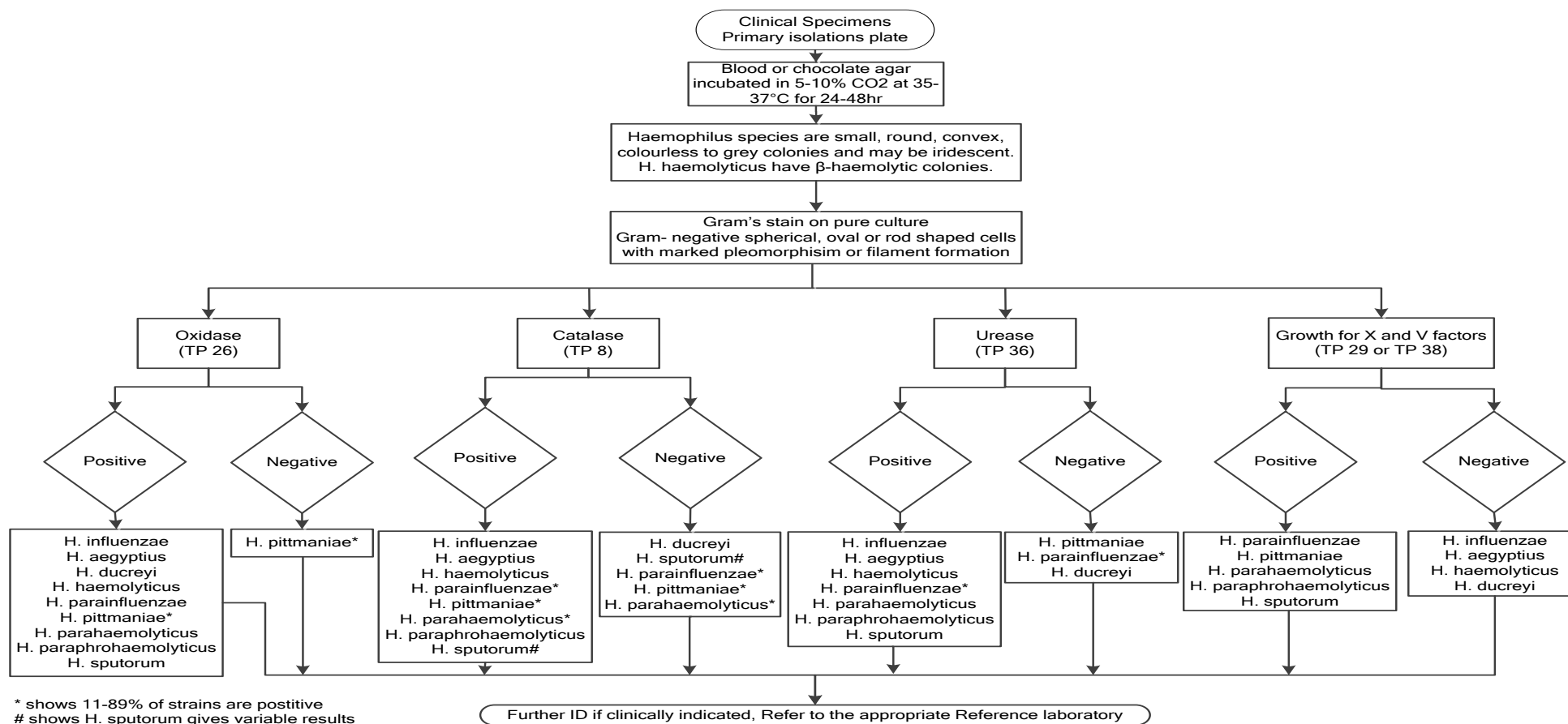
Multilocus sequence typing (MLST) is a tool that is widely used for phylogenetic typing of bacteria. MLST is based on PCR amplification and sequencing of internal fragments of a number (usually 6 or 7) of essential or housekeeping genes spread around the bacterial chromosome. MLST has been extensively used as the one of the main typing methods for analysing the genetic relationships within the genus *Haemophilus* population.

This has been used to describe new species, *H. pittmaniae* and to also separate *H. haemolyticus* and *H. influenzae* into distinct clusters using concatenated sequences of multiple genes, including the 16S rRNA gene, *adk* (adenylate kinase gene), *pgi* (glucose-6-phosphate isomerase gene), *recA* (recombination protein gene), and *infB* (translation initiation factor 2 gene)<sup>12,67</sup>.

## 3.6 Storage and Referral

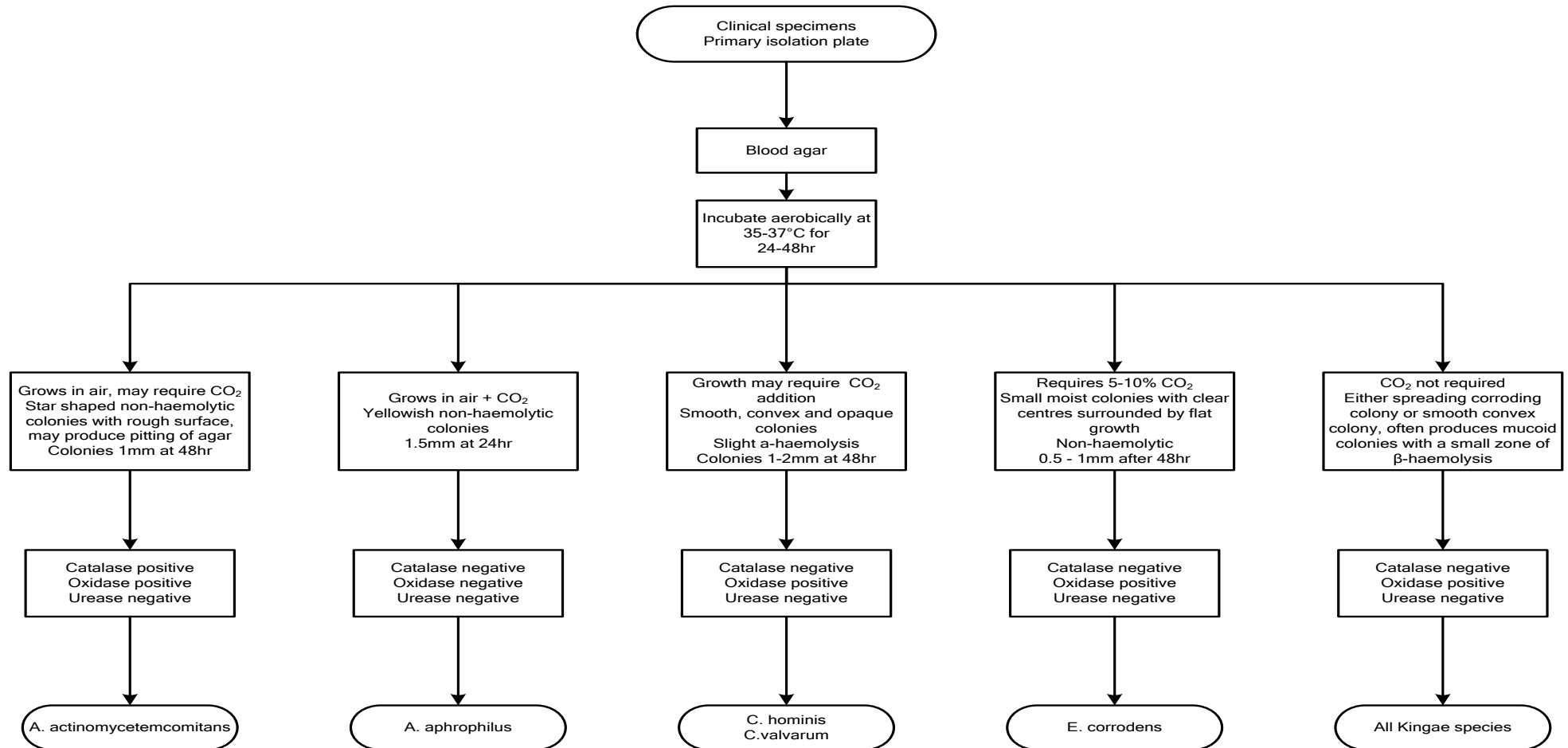
If required, save pure isolate on a chocolate agar slope for referral to the Reference Laboratory.

## 4a Identification of *Haemophilus* species



This flowchart is for guidance only.

## 4b Identification of HACEK group



This flowchart is for guidance only.

## 5 Reporting

---

### 5.1 Presumptive Identification

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

### 5.2 Confirmation of Identification

Following serotyping of *H. influenzae*, appropriate X and V and/or commercial identification kit results and/or the Reference Laboratory report.

### 5.3 Medical Microbiologist

Inform the medical microbiologist of all positive cultures from normally sterile sites.

According to local protocols, the medical microbiologist should also be informed of presumptive or confirmed *Haemophilus* species or other member of the HACEK group of organisms when the request bears relevant information eg:

- Meningitis or brain abscess
- Facial cellulitis
- Septic arthritis
- Osteomyelitis
- Epiglottitis, pneumonia, mastoiditis or empyema thoracis
- Septicaemia or endocarditis

Follow local protocols for reporting to clinician.

### 5.4 CCDC

Refer to local Memorandum of Understanding.

### 5.5 Public Health England<sup>68</sup>

Refer to current guidelines on CIDSC and COSURV reporting.

### 5.6 Infection Prevention and Control Team

N/A

## 6 Referrals

---

### 6.1 Reference Laboratory

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

***Haemophilus influenzae* from cases of invasive disease (isolates from normally sterile sites)**

*Haemophilus* Reference Unit  
Respiratory and Vaccine Preventable Bacteria Reference Unit (RVPBRU)  
Microbiology Services  
Public Health England  
61 Colindale Avenue  
London  
NW9 5EQ

<https://www.gov.uk/rvpbru-reference-and-diagnostic-services>

Telephone: +44 (0) 208 327 7331/ 6091/ 7330

**HACEK group and *Haemophilus* species for identification**

Antimicrobial Resistance and Healthcare Associated Infections Reference Unit (AMRHAI)  
Microbiology Services  
Public Health England  
61 Colindale Avenue  
London  
NW9 5EQ

<https://www.gov.uk/amrhai-reference-unit-reference-and-diagnostic-services>

Telephone: +44 (0) 208 3276511

***Haemophilus ducreyi***

The Sexually Transmitted Bacteria Reference Unit (STBRU) currently provides a full reference service for the molecular testing for *Haemophilus ducreyi*.

Sexually Transmitted Bacteria Reference Unit  
Bacteriology Reference Department  
Microbiology Services  
Public Health England  
61 Colindale Avenue  
London  
NW9 5EQ

<https://www.gov.uk/stbru-reference-and-diagnostic-services>

Tel: +44 (0) 20 8327 6464

Contact PHE's main switchboard: Tel. +44 (0) 20 8200 4400

England and Wales

<https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services>

Scotland

<http://www.hps.scot.nhs.uk/reflab/index.aspx>

Northern Ireland

<http://www.belfasttrust.hscni.net/Laboratory-MortuaryServices.htm>

## 7 Notification to PHE<sup>68,69</sup> or Equivalent in the Devolved Administrations<sup>70-73</sup>

---

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

**Note:** The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAs) and Creutzfeldt–Jakob disease (CJD) under ‘Notification Duties of Registered Medical Practitioners’: it is not noted under ‘Notification Duties of Diagnostic Laboratories’.

<https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010>

Other arrangements exist in [Scotland](#)<sup>70,71</sup>, [Wales](#)<sup>72</sup> and [Northern Ireland](#)<sup>73</sup>.

## References

---

1. Euzeby,JP. Genus *Haemophilus*. 2013.
2. Brook I. Bacteriology of chronic sinusitis and acute exacerbation of chronic sinusitis. Arch Otolaryngol Head Neck Surg 2006;132:1099-101.
3. Nørskov-Lauritsen N, Bruun B, Andersen C, Kilian M. Identification of haemolytic *Haemophilus* species isolated from human clinical specimens and description of *Haemophilus sputorum* sp. nov. Int J Med Microbiol 2012;302:78-83.
4. Nørskov-Lauritsen N, Kilian M. Reclassification of *Actinobacillus actinomycetemcomitans*, *Haemophilus aphrophilus*, *Haemophilus paraphrophilus* and *Haemophilus segnis* as *Aggregatibacter actinomycetemcomitans* gen. nov., comb. nov., *Aggregatibacter aphrophilus* comb. nov. and *Aggregatibacter segnis* comb. nov., and emended description of *Aggregatibacter aphrophilus* to include V factor-dependent and V factor-independent isolates. Int J Syst Evol Microbiol 2006;56:2135-46.
5. Ledebøer NA, Doern GV. *Haemophilus*. In: Versalovic J, Carroll KC, Funke G, Jorgensen JH, Landry ML, Warnock DW, editors. Manual of Clinical Microbiology. 10th ed. Washington DC: American Society for Microbiology (ASM Press); 2011. p. 588-602.
6. Holt JG, Krieg NR, Sneath PHA, et al. Facultatively Anaerobic Gram- Negative Rods SUBGROUP 3: Genus *Haemophilus*. Bergey's Manual of Determinative Bacteriology. 9th ed. Baltimore, Maryland: Williams and Wilkins; 1994. p. 195-280.
7. Musher DM. *Haemophilus* Species. In: Baron S, editor. Medical Microbiology. 4th ed. University of Texas Medical Branch at Galveston; 1996.
8. Foweraker JE, Cooke NJ, Hawkey PM. Ecology of *Haemophilus influenzae* and *Haemophilus parainfluenzae* in sputum and saliva and effects of antibiotics on their distribution in patients with lower respiratory tract infections. Antimicrob Agents Chemother 1993;37:804-9.
9. PITTMAN M. A classification of the hemolytic bacteria of the genus *Haemophilus*: *Haemophilus haemolyticus* Bergey et al. and *Haemophilus parahaemolyticus* nov spec. J Bacteriol 1953;65:750-1.
10. Zinnemann K, Rogers KB, Frazer J, Devaraj SK. A haemolytic V-dependent CO<sub>2</sub>-preferring *Haemophilus* species (*Haemophilus paraphrohaemolyticus* nov. spec.). J Med Microbiol 1971;4:139-43.
11. PITTMAN M, DAVIS DJ. Identification of the Koch-Weeks bacillus (*Hemophilus aegyptius*). J Bacteriol 1950;59:413-26.
12. Nørskov-Lauritsen N, Bruun B, Kilian M. Multilocus sequence phylogenetic study of the genus *Haemophilus* with description of *Haemophilus pittmaniae* sp. nov. Int J Syst Evol Microbiol 2005;55:449-56.
13. Alfa M. The laboratory diagnosis of *Haemophilus ducreyi*. Can J Infect Dis Med Microbiol 2005;16:31-4.
14. Trees DL, Morse SA. Chancroid and *Haemophilus ducreyi*: an update. Clin Microbiol Rev 1995;8:357-75.
15. Howard AJ, Ison CA. *Haemophilus*, *Gardnerella* and other bacilli. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie & McCartney Practical Medical Microbiology. 14th ed. Edinburgh: Churchill Livingstone; 1996. p. 459-60.

## Identification of *Haemophilus* species and the HACEK Group of Organisms

16. Euzeby,JP. List of Prokaryotic names with Standing in Nomenclature - Genus *Aggregatibacter*. 2014.
17. SLOTNICK IJ, DOUGHERTY M. FURTHER CHARACTERIZATION OF AN UNCLASSIFIED GROUP OF BACTERIA CAUSING ENDOCARDITIS IN MAN: *CARDIOBACTERIUM HOMINIS* GEN. ET SP. N. *Antonie Van Leeuwenhoek* 1964;30:261-72.
18. Han XY, Meltzer MC, Woods JT, Fainstein V. Endocarditis with ruptured cerebral aneurysm caused by *Cardiobacterium valvarum* sp. nov. *J Clin Microbiol* 2004;42:1590-5.
19. Euzeby,JP. Genus *Cardiobacterium*. 2013.
20. Shivaprakasha S, Radhakrishnan K, Kamath P, Karim P. Late prosthetic valve endocarditis due to *Cardiobacterium hominis*, an unusual complication. *Indian J Med Microbiol* 2007;25:64-6.
21. Chen M, Kemp M, Bruun NE, Bangsberg JM, Hojlyng N, Hesselbjerg A, et al. *Cardiobacterium valvarum* infective endocarditis and phenotypic/molecular characterization of 11 *Cardiobacterium* species strains. *J Med Microbiol* 2011;60:522-8.
22. Mutters R. Actinobacillus, Capnocytophaga, Eikenella, Kingella and other Fastidious or Rarely Encountered Gram Negative Rods. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Tenover FC, editors. *Manual of Clinical Microbiology*. 7th ed. Washington DC: American Society for Microbiology; 1999. p. 561-71.
23. Dewhirst FE, Paster BJ, La Fontaine S, Rood JI. Transfer of *Kingella indologenes* (Snell and Lapage 1976) to the genus *Suttonella* gen. nov. as *Suttonella indologenes* comb. nov.; transfer of *Bacteroides nodosus* (Beveridge 1941) to the genus *Dichelobacter* gen. nov. as *Dichelobacter nodosus* comb. nov.; and assignment of the genera *Cardiobacterium*, *Dichelobacter*, and *Suttonella* to *Cardiobacteriaceae* fam. nov. in the gamma division of Proteobacteria on the basis of 16S rRNA sequence comparisons. *Int J Syst Bacteriol* 1990;40:426-33.
24. Euzeby,JP. List of Prokaryotic names with Standing in Nomenclature - Genus *Kingella*. 2013.
25. Group 4 Gram-negative Aerobic/ Microaerophilic Rods and Cocci. In: Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST, editors. *Bergey's Manual of Determinative Bacteriology*. 9th ed. Baltimore: Williams and Wilkins; 1994. p. 85-94.
26. Snell JJS, LAPAGE SP. Transfer of some saccharolytic *Moraxella* species to *Kingella* Henriksen and Bovre 1976, with descriptions of *Kingella indologenes* sp.nov. and *Kingella denitrificans* sp. nov. *International Journal of Systematic Bacteriology* 1976;26:451-8.
27. Hollis DG, Weaver RE, Riley PS. Emended description of *Kingella denitrificans* (Snell and Lapage 1976): correction of the maltose reaction. *J Clin Microbiol* 1983;18:1174-6.
28. Henriksen SD, Bovre K. Transfer of *Moraxella kingae* Henriksen and Bovre to the Genus *Kingella* gen. nov. in the family *Neisseriaceae*. *International Journal of Systematic Bacteriology* 1976;26:447-50.
29. Dewhirst FE, Chen CK, Paster BJ, Zambon JJ. Phylogeny of species in the family *Neisseriaceae* isolated from human dental plaque and description of *Kingella oralis* sp. nov [corrected]. *Int J Syst Bacteriol* 1993;43:490-9.
30. Lawson PA, Malnick H, Collins MD, Shah JJ, Chattaway MA, Bendall R, et al. Description of *Kingella potus* sp. nov., an organism isolated from a wound caused by an animal bite. *J Clin Microbiol* 2005;43:3526-9.
31. Jones AM. *Haemophilus influenzae* and *H. parainfluenzae*: the influence of media and CO<sup>2</sup> on differentiation using X, V and XV discs. *Med Lab Sci* 1982;39:189-91.



## Identification of *Haemophilus* species and the HACEK Group of Organisms

32. European Parliament. UK Standards for Microbiology Investigations (SMIs) use the term "CE marked leak proof container" to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU *in vitro* Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: "The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes".
33. Official Journal of the European Communities. Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on *in vitro* diagnostic medical devices. 7-12-1998. p. 1-37.
34. Health and Safety Executive. Safe use of pneumatic air tube transport systems for pathology specimens. 9/99.
35. Department for transport. Transport of Infectious Substances, 2011 Revision 5. 2011.
36. World Health Organization. Guidance on regulations for the Transport of Infectious Substances 2013-2014. 2012.
37. Home Office. Anti-terrorism, Crime and Security Act. 2001 (as amended).
38. Advisory Committee on Dangerous Pathogens. The Approved List of Biological Agents. Health and Safety Executive. 2013. p. 1-32
39. Advisory Committee on Dangerous Pathogens. Infections at work: Controlling the risks. Her Majesty's Stationery Office. 2003.
40. Advisory Committee on Dangerous Pathogens. Biological agents: Managing the risks in laboratories and healthcare premises. Health and Safety Executive. 2005.
41. Advisory Committee on Dangerous Pathogens. Biological Agents: Managing the Risks in Laboratories and Healthcare Premises. Appendix 1.2 Transport of Infectious Substances - Revision. Health and Safety Executive. 2008.
42. Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. MMWR Surveill Summ 2012;61:1-102.
43. Health and Safety Executive. Control of Substances Hazardous to Health Regulations. The Control of Substances Hazardous to Health Regulations 2002. 5th ed. HSE Books; 2002.
44. Health and Safety Executive. Five Steps to Risk Assessment: A Step by Step Guide to a Safer and Healthier Workplace. HSE Books. 2002.
45. Health and Safety Executive. A Guide to Risk Assessment Requirements: Common Provisions in Health and Safety Law. HSE Books. 2002.
46. Health Services Advisory Committee. Safe Working and the Prevention of Infection in Clinical Laboratories and Similar Facilities. HSE Books. 2003.
47. British Standards Institution (BSI). BS EN12469 - Biotechnology - performance criteria for microbiological safety cabinets. 2000.
48. British Standards Institution (BSI). BS 5726:2005 - Microbiological safety cabinets. Information to be supplied by the purchaser and to the vendor and to the installer, and siting and use of cabinets. Recommendations and guidance. 24-3-2005. p. 1-14
49. Collins CH, Kennedy.D.A. Laboratory acquired infections. In: Woburn MA, editor. Laboratory acquired infection: History, incidence, causes and prevention. 4 ed. 1999. p. 1-37.

## Identification of *Haemophilus* species and the HACEK Group of Organisms

50. Jacobson JT, Orlob RB, Clayton JL. Infections acquired in clinical laboratories in Utah. *Journal of Clinical Microbiology* 1985;21:486-9.
51. Barbuddhe SB, Maier T, Schwarz G, Kostrzewa M, Hof H, Domann E, et al. Rapid identification and typing of listeria species by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *Appl Environ Microbiol* 2008;74:5402-7.
52. Couturier MR, Mehinovic E, Croft AC, Fisher MA. Identification of HACEK clinical isolates by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J Clin Microbiol* 2011;49:1104-6.
53. Norskov-Lauritsen N. Classification, identification, and clinical significance of haemophilus and aggregatibacter species with host specificity for humans. *Clin Microbiol Rev* 2014;27:214-40.
54. Frickmann H, Christner M, Donat M, Berger A, Essig A, Podbielski A, et al. Rapid discrimination of *Haemophilus influenzae*, *H. parainfluenzae*, and *H. haemolyticus* by fluorescence in situ hybridization (FISH) and two matrix-assisted laser-desorption-ionization time-of-flight mass spectrometry (MALDI-TOF-MS) platforms. *PLoS One* 2013;8:e63222.
55. Wallet F, Loiez C, Decoene C, Courcol R. Rapid identification of *Cardiobacterium hominis* by MALDI-TOF mass spectrometry during infective endocarditis. *Jpn J Infect Dis* 2011;64:327-9.
56. Orle KA, Gates CA, Martin DH, Body BA, Weiss JB. Simultaneous PCR detection of *Haemophilus ducreyi*, *Treponema pallidum*, and herpes simplex virus types 1 and 2 from genital ulcers. *J Clin Microbiol* 1996;34:49-54.
57. Gatselis N, Malli E, Papadamou G, Petinaki E, Dalekos GN. Direct detection of *Cardiobacterium hominis* in serum from a patient with infective endocarditis by broad-range bacterial PCR. *J Clin Microbiol* 2006;44:669-72.
58. Binks MJ, Temple B, Kirkham LA, Wiertsema SP, Dunne EM, Richmond PC, et al. Molecular surveillance of true nontypeable *Haemophilus influenzae*: an evaluation of PCR screening assays. *PLoS One* 2012;7:e34083.
59. Anderson R, Wang X, Briere EC, Katz LS, Cohn AC, Clark TA, et al. *Haemophilus haemolyticus* isolates causing clinical disease. *J Clin Microbiol* 2012;50:2462-5.
60. Hoffman MJ, Macrie BD, Taiwo BO, Qi C. Prosthetic valve/conduit infection caused by *Cardiobacterium valvarum*. *Infection* 2010;38:245-6.
61. Brown TJ, Ison CA. Non-radioactive ribotyping of *Haemophilus ducreyi* using a digoxigenin labelled cDNA probe. *Epidemiol Infect* 1993;110:289-95.
62. Sarafian SK, Woods TC, Knapp JS, Swaminathan B, Morse SA. Molecular characterization of *Haemophilus ducreyi* by ribosomal DNA fingerprinting. *J Clin Microbiol* 1991;29:1949-54.
63. Wang CC, Siu LK, Chen MK, Yu YL, Lin FM, Ho M, et al. Use of automated riboprinter and pulsed-field gel electrophoresis for epidemiological studies of invasive *Haemophilus influenzae* in Taiwan. *J Med Microbiol* 2001;50:277-83.
64. Liu D. Identification, subtyping and virulence determination of *Listeria monocytogenes*, an important foodborne pathogen. *J Med Microbiol* 2006;55:645-59.
65. Brosch R, Brett M, Catimel B, Luchansky JB, Ojienyi B, Rocourt J. Genomic fingerprinting of 80 strains from the WHO multicenter international typing study of *listeria monocytogenes* via pulsed-field gel electrophoresis (PFGE). *Int J Food Microbiol* 1996;32:343-55.
66. Pettigrew MM, Foxman B, Ecevit Z, Marrs CF, Gilsdorf J. Use of pulsed-field gel electrophoresis, enterobacterial repetitive intergenic consensus typing, and automated ribotyping to assess

## Identification of *Haemophilus* species and the HACEK Group of Organisms

genomic variability among strains of nontypeable *Haemophilus influenzae*. *J Clin Microbiol* 2002;40:660-2.

67. McCrea KW, Xie J, LaCross N, Patel M, Mukundan D, Murphy TF, et al. Relationships of nontypeable *Haemophilus influenzae* strains to hemolytic and nonhemolytic *Haemophilus haemolyticus* strains. *J Clin Microbiol* 2008;46:406-16.
68. Public Health England. Laboratory Reporting to Public Health England: A Guide for Diagnostic Laboratories. 2013. p. 1-37.
69. Department of Health. Health Protection Legislation (England) Guidance. 2010. p. 1-112.
70. Scottish Government. Public Health (Scotland) Act. 2008 (as amended).
71. Scottish Government. Public Health etc. (Scotland) Act 2008. Implementation of Part 2: Notifiable Diseases, Organisms and Health Risk States. 2009.
72. The Welsh Assembly Government. Health Protection Legislation (Wales) Guidance. 2010.
73. Home Office. Public Health Act (Northern Ireland) 1967 Chapter 36. 1967 (as amended).