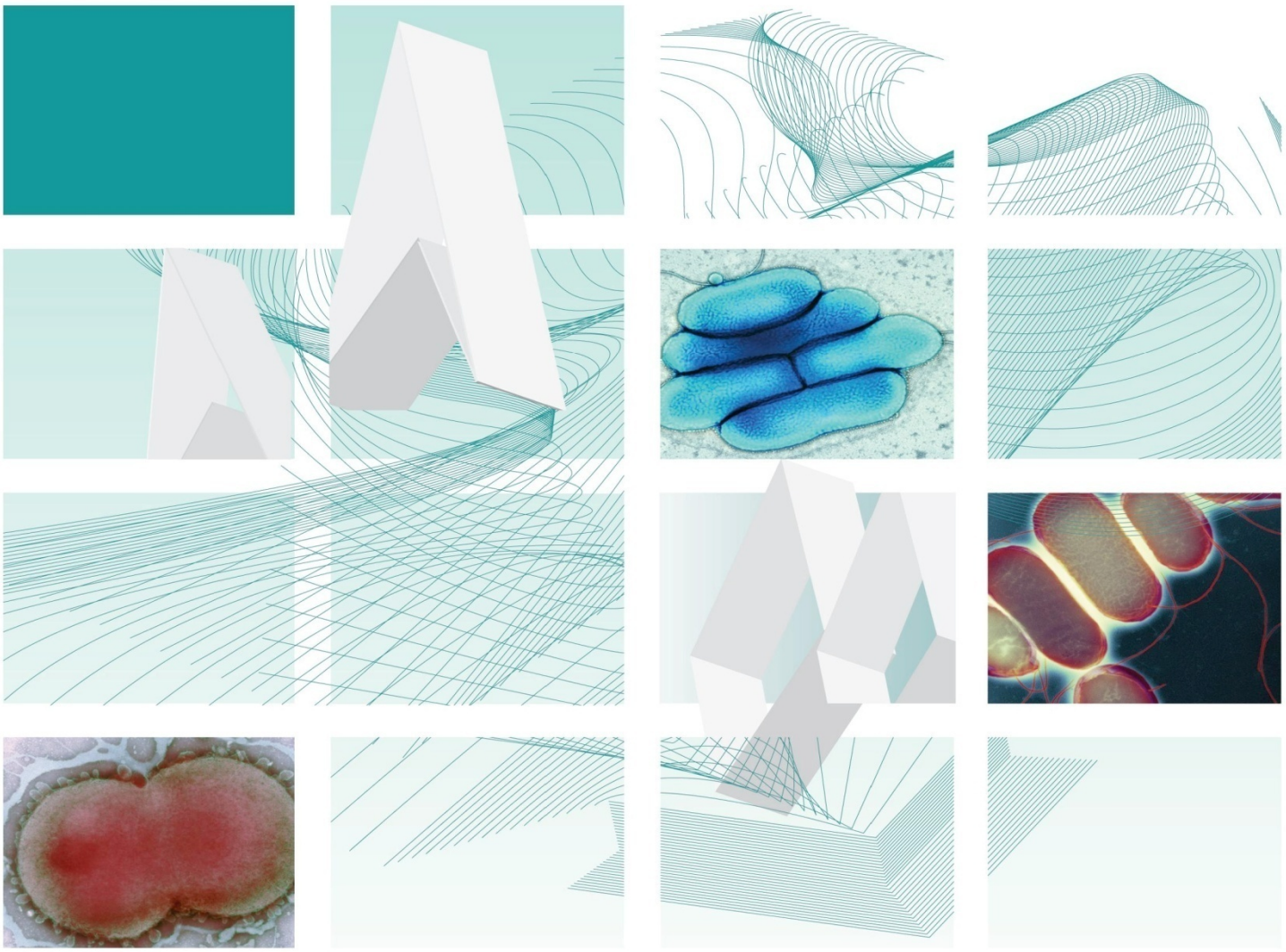
Public Health England

NHS

UK Standards for Microbiology Investigations

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



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Acknowledgments

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UK Standards for Microbiology Investigations are produced in association with:

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Contents

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.

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
Section(s) involved	**Amendment**
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Introduction.	The taxonomy of *Haemophilus* 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 *Haemophilus* 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 *Haemophilus* species and other HACEK Group of organisms has been done for easy guidance.

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[#]: 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.

[#]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.

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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*[1]. 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*[2,3]. 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*[4].

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_2; *H. ducreyi* and some nontypable *H. influenzae* strains will not form visible colonies on culture plates unless grown in CO_2-enriched atmosphere. *Aggregatibacter aphrophilus* and *Haemophilus paraphrohaemolyticus* require CO_2 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[5].

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

Haemophilus influenzae[6]

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 β-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_2S 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[6].

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[4].

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[7].

H. influenzae has been isolated from respiratory secretions, CSF, sputum and blood culture[7].

Haemophilus parainfluenzae[6]

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 β-haemolysis on sheep cells. They require V factor but not the X factor for growth.

They are positive for oxidase, nitrate reduction and H_2S 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)[4].

They have been associated with some cases of acute otitis media, sinusitis and chronic bronchitis[5].

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[8].

Haemophilus haemolyticus[6]

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 β-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_2S 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[9]

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_2S 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[6].

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

Haemophilus paraphrohaemolyticus[10]

They are Gram negative, non-motile and non-spore-forming short to medium length rods measuring 0.75- 2.5µm and 0.4-.0.5µm. They grow well at 37°C both in air and in air with added CO_2.

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_2. 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_2S 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[6].

It has been isolated from sputum, throat, pharynx, thumb print and urethral discharge in humans[10].

Haemophilus aegyptius[11]

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[12]

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 leucinearylamidase, 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[13]

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_2. 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_2 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)[14].

Haemophilus sputorum[3]

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 leucinearylamidase; the species are negative for indole test, arginine di-hydrolase, lysine decarboxylase, ornithine decarboxylase, and phenylalanine arylamidase. H_2S 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 orginially 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[3].

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[15]. 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[4]

This is a member of the family Pasteurellaceae. The genus *Aggregatibacter* contains 3 species, *Aggregatibacter actinomycetemcomitans, Aggregatibacter aphrophilus* and *Aggregatibacter segnis*[16].

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_2. 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[4]

(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_2 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[4]

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_2 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_2O_2 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*[4]**

(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_2. 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[17]**

The genus *Cardiobacterium* contains 2 species, *Cardiobacterium hominis* and *Cardiobacterium valvarum*[18,19]. 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_2. 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_2 may be required by some strains on primary isolation. The optimum growth temperature is 30-37°C.

They are positive for oxidase, H_2S 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[17]

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_2. 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_2 may be required by some strains on primary isolation. The optimum growth temperature is 30-37°C.

They are positive for oxidase, H_2S 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[20].

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

Cardiobacterium valvarum[21]

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_2S 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[18].

Eikenella corrodens[22]

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 dacrboxylase 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[23]

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

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[25].

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[26]

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 β-hydroxybutyrate in mineral medium, acid production from maltose unless serum was present, starch hydrolysis and urease production. Isolated in the respiratory tract of man[27].

K. kingae[28]

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 β-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[29]

They are Gram negative rods or coccobacilli approximately 0.6-0.7µm in diameter by 1-3µm long with rounded ends. Cells can form pairs or chains. Cells have monopolar fimbriae up to 10µm 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 0.5µg of menadione per mL in both anaerobic and aerobic environments with CO_2. 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*[30]**

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, α-glycosidase, β-galactosidase, or β-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_2. 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_2[31].

For more information on technical limitation for the X and V Factor Test, see TP 38 – X and V Factor Test.

1 Safety Considerations[32-48]

Haemophilus influenzae is a Hazard Group 2 organism, and, 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[49]. The organism infects primarily by the respiratory route (inhalation), autoinoculation or ingestion in laboratory workers[50].

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_2 at 35-37°C for 24-48hr.

Blood agar incubated in 5-10% CO_2 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**
A. actinomycetemcomitans	Will not grow in air but grows in air + CO_2. 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.
A. aphrophilus	Requires added CO_2 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 *H. paraphrophilus)* whereas others are V-factor-independent (formerly *H. aphrophilus)*. Non-haemolytic.
A. segnis	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.
C. hominis	Some strains will not grow without added CO_2. May require X-factor on primary isolation. Colonies smooth, convex and opaque. 1-2mm at 48hr. Slight α-haemolysis.
C. valvarum	Grows best in air +5% CO_2. Slow growing, colonies smooth, round, opaque and glistening, 0.6-0.8mm after 48hr. Some strains show slight α-haemolysis, others are non-haemolytic.
E. corrodens	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_2.
K. kingae	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_2.
K. denitrificans	Non-haemolytic. 2 types of colony: a spreading, corroding type and a smooth, convex type.
K. oralis	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.
K. potus	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
H. influenzae	+	+	(+)
H. aegyptius	+	+	+
H. ducreyi	-	+	Unknown
H. haemolyticus	+	+	+
H. parainfluenzae	d	+	d
H. pittmaniae	d	d	-
H. parahaemolyticus	d	+	+
H. paraphrohaemolyticus	+	+	+
H. sputorum	V	+	+
A. actinomycetemcomitans	+	+	-
A. aphrophilus	-	-	-
C. hominis	-	+	-
C. valvarum	-	+	-
E. corrodens	-	+	-
K. kingae	-	+	-
K. denitrificans	-	+	-
K. oralis	-	+	-
K. potus	-	+	-

+ = 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
H. influenzae[a]	No growth	No growth	Growth	Negative
H. haemolyticus[b]	No growth	No growth	Growth	Negative
H. parainfluenzae	No growth	Growth	Growth	Positive
H. pittmaniae	No growth	Growth	Growth	Positive
H. parahaemolyticus	No growth	Growth	Growth	Positive
H. paraphrohaemolyticus	No growth	Growth	Growth	Positive
Haemophilus ducreyi	Growth	No growth	Growth	Positive
Haemophilus sputorum	No growth	Growth	Growth	Positive

[a]*H. aegyptius* is indistinguishable from *H. influenzae* biotype III in normal laboratory tests.

[b]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[51].

MALDI- TOF MS has been used to describe and characterise new specie, *Haemophilus sputorum*[3]. 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[52,53]. 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*[54]. 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*[55].

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[56].

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*[57]. 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 [58]. 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*[59].

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*[21,60]. It has equally been used for identifying *Aggregatibacter* species[53].

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*[61,62]. 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[63].

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[64,65].

This has been used successfully to identify and discriminate between strains of non-typeable *Haemophilus influenzae*[66].

Multilocus Sequence Typing (MLST)

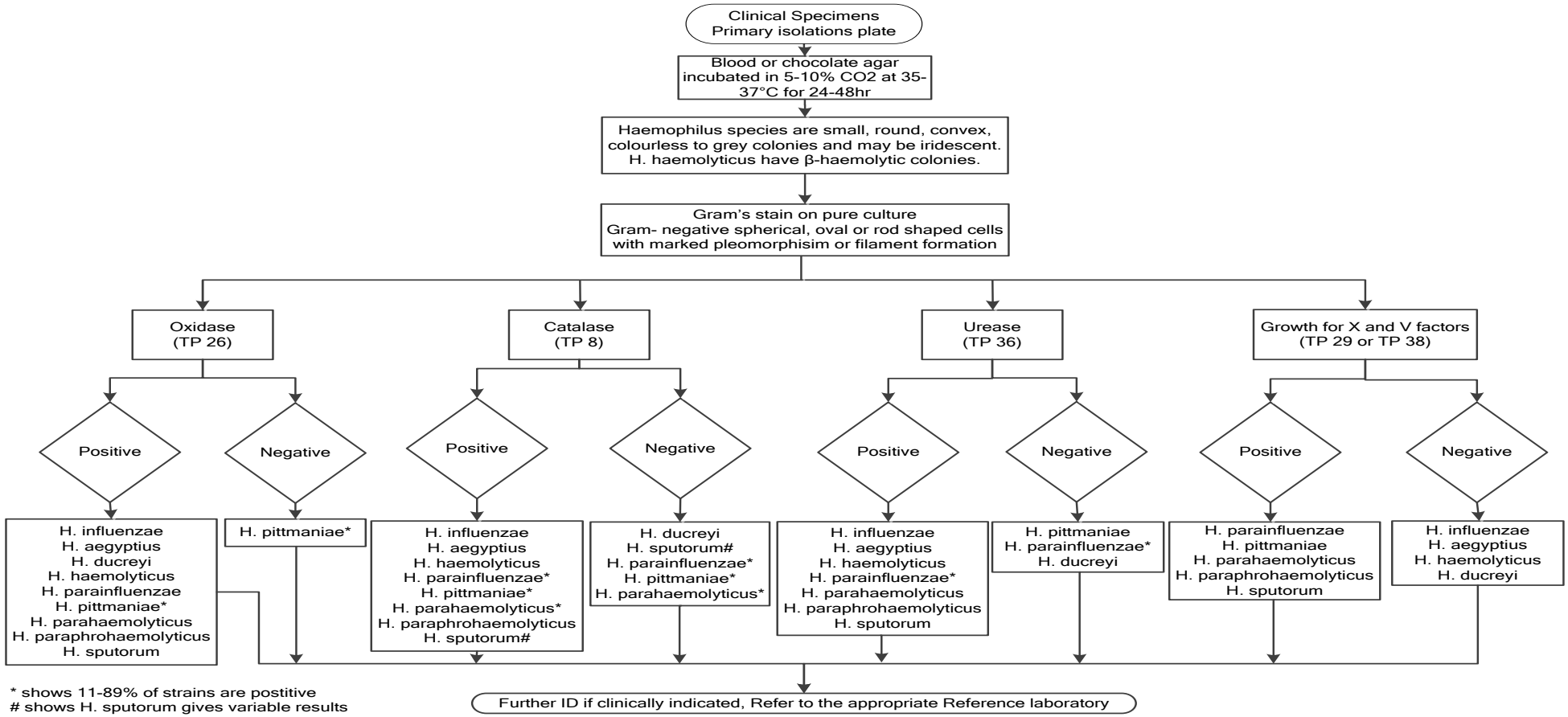
Multilocus sequence typing (MLST) is a tool that is widely used for phylogenetic typing of bacteria. MLST is based on PCR amplification and sequencing of internal fragments of a number (usually 6 or 7) of essential or housekeeping genes spread around the bacterial chromosome. MLST has been extensively 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 specie*, 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)[12,67].

3.6 Storage and Referral

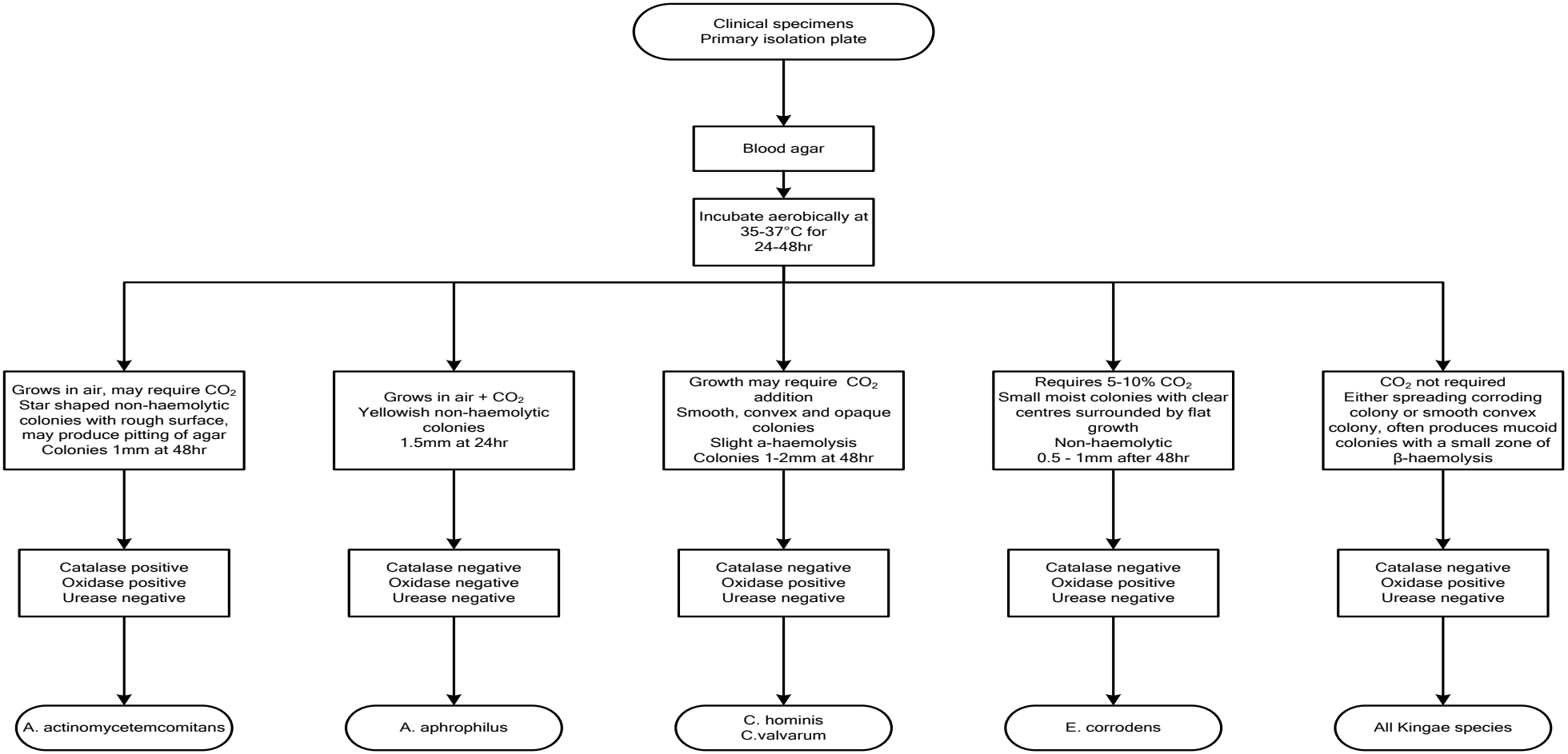
If required, save pure isolate on a chocolate agar slope for referral to the Reference Laboratory.

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[68]

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[68,69] or Equivalent in the Devolved Administrations[70-73]

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 (HCAIs) 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[70,71], Wales[72] and Northern Ireland[73].

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