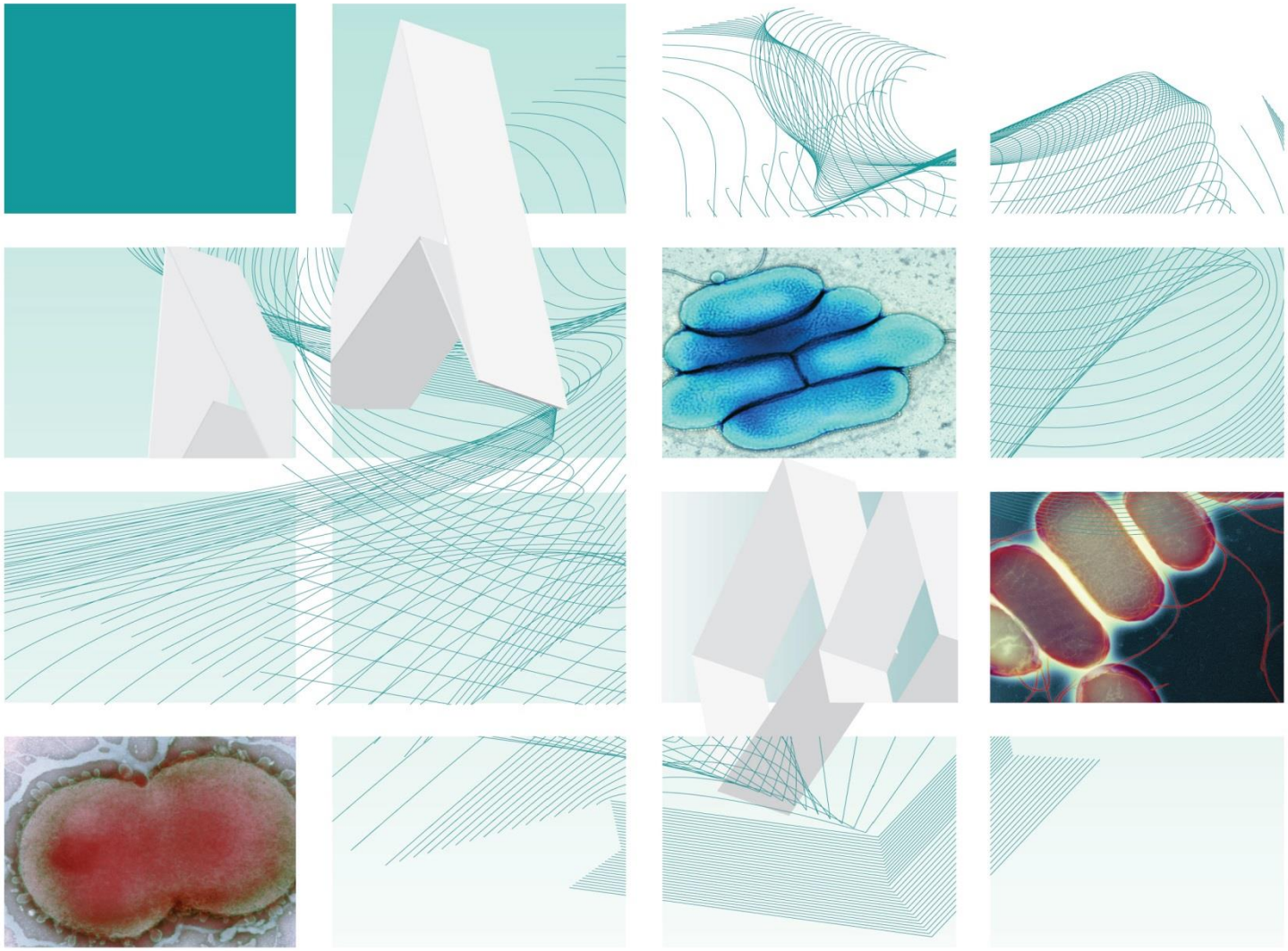
UK Standards for Microbiology Investigations

Identification of *Haemophilus* species and the HACEK group of organisms



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Contents

Amendment table

Each UK 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.

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Amendment number/date	7/08.01.2020
Issue number discarded	3
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Anticipated next review date*	08.01.2023
Section(s) involved	**Amendment**
Whole document	Document presented in a new format. Reorganisation of some text. Document and references updated.
Section 4.1	The taxonomy of *Haemophilus* species and other HACEK Group of organisms have been updated
Section 8.4	Table 2 amended to indicate positive and negative growth.
Appendix 1 and 2	Flowcharts updated
Appendix 3	Table: Aerobic growth Characteristics of HACEK group organisms moved from section 8:3 to appendix 3
References	References updated

*Reviews can be extended up to five years subject to resources available.

1 General information

View general information related to UK SMIs.

2 Scientific information

View scientific information related to UK SMIs.

3 Scope of document

This UK 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 UK SMI includes both biochemical tests and automated methods for the identification of microorganisms. Some biochemical tests may not be done routinely in the laboratory except in cases where confirmation by an alternative technique is required or automated methods are not available.

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

4 Introduction

4.1 Taxonomy/characteristics

There are currently 25 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*, *H. sputorum and H. seminalis*[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 reclassified 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 reclassified as *Aggregatibacter segnis*[4].

H. influenzae is the type species.

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 to 37°C. They are facultatively anaerobic, non-acid-fast, non-spore forming and non-motile.

Members of the *Haemophilus* genus are typically cultured on blood or chocolate agar plates as all species require either or both of two blood factors for growth: haemin (factor X) and/or nicotinamide adenine dinucleotide (factor V). Chocolate agar is an excellent growth medium for *Haemophilus sp* as it allows for increased accessibility to these factors. Blood agar contains free V, but not X factor. *Haemophilus* species requiring X factor can be cultured on blood agar 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 grow in small "satellite" colonies around the larger *Staphylococcus* colonies because the metabolism of *Staphylococcus* produces the necessary X blood factor required for *Haemophilus* growth.

All *Haemophilus* species grow more readily in an atmosphere enriched with CO_2, *H. ducreyi* and some non-typeable *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.

The nature of specimen for the diagnosis of *Haemophilus* depends on the infection being evaluated. For example, blood and cerebrospinal fluid (CSF) cultures to be performed for patients with meningitis. Middle ear fluid for patients with otitis media. Lower respiratory secretion for patients suspected to have bronchopulmonary infections due to *Haemophilus* species. Obtain blood culture for bacterial pneumonia[5].

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

Haemophilus influenzae[6]

H. influenzae is facultatively anaerobic, small, non-motile Gram-negative bacterium in the family *Pasteurellaceae*. 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 colonies show no β-haemolysis on blood agar (with additional X factor). They require both the X and V factors for growth.

H. influenzae is positive for oxidase, catalase, nitrate reduction and phosphatase. Eleven to eighty nine percent of strains are positive for indole production and 80 to 89% of strains are positive for urease and ornithine decarboxylase tests. It is also negative for ONPG, H_2S production and aesculin hydrolysis[6].

Pittman described six antigenically distinct capsular types of *H. influenzae*, designated ‘a’ to ‘f’ based on the polysaccharide composition of the capsular structure. Isolates that do not express a polysaccharide capsule are referred to as non-capsulated or non-typeable. There is also a biotyping scheme for *H. influenzae* based on a series of biochemical reactions (indole, ornithine decarboxylase and urease production). There are eight biotypes of *H. influenzae* (I-VIII)[4].

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

Before the introduction of a vaccine against serotype b (Hib), the majority of infections were caused by serotype b strains which caused meningitis, epiglottitis, orbital cellulitis and bacteraemia (generally biotypes I and II of this species). However, all types of *H. influenzae* (including non-typeable strains) can cause systemic infections such as meningitis, bacteraemia, septic arthritis and cellulitis. Most non-typeable *H. influenzae* strains fall into biotypes II to VI and can cause acute conjunctivitis, otitis media, sinusitis, tracheobronchitis and pneumonia as well as invasive diseases[7].

Haemophilus parainfluenzae[6]

H. parainfluenzae is facultatively anaerobic, small, non-motile Gram-negative bacterium in the family *Pasteurellaceae*. There is no growth on MacConkey or CLED agar and no β-haemolysis on sheep blood cells. V factor but not X factor is required for growth.

The organism is positive for oxidase, nitrate reduction and H_2S production. Eleven to eighty nine percent of strains are positive for catalase, ONPG, ornithine decarboxylase and urease. It is negative for indole production and aesculin hydrolysis.

H. parainfluenzae has been associated with some cases of acute otitis media, sinusitis and chronic bronchitis[5]. The organism has been isolated from clinical specimens – respiratory secretions (from the lower airways, oropharynx, and nasopharynx), abscesses and sputum. Although it has been isolated from sputum, it is considered a part of the normal oral flora and not reported as significant[8].

Haemophilus haemolyticus[6]

H. haemolyticus is Gram negative, non-motile and non-spore-forming short to medium length rods. There is no growth on MacConkey or CLED agar. They classically show β-haemolysis on blood agar (with additional X factor), although non-haemolytic isolates have been reported[9]. They also require X and V factors for growth.

The organism is positive for oxidase, catalase, nitrate reduction, phosphatase, urease and H_2S production. Some strains of *H. haemolyticus* (11 to 89%) are positive for indole production. It is negative for ONPG, ornithine decarboxylase and aesculin hydrolysis.

H. haemolyticus is a commensal of the respiratory tract but does occasionally cause invasive disease[10].

Haemophilus parahaemolyticus[11]

H. parahaemolyticus 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.

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.

The organism is positive for oxidase, nitrate reduction, H_2S production and urease tests. Some strains of *H. parahaemolyticus* (11 to 89%) are positive for catalase, ONPG, ornithine decarboxylase and produce acid from $_D$-galactose. It is negative for indole production and aesculin hydrolysis[6].

H. parahaemolyticus is associated frequently with acute pharyngitis and occasionally cause sub-acute endocarditis.

Haemophilus paraphrohaemolyticus[12]

Cells are Gram negative, non-motile and non-spore-forming short to medium length rods measuring 0.75 to 2.5µm and 0.4 to 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.

The organism is positive for catalase, oxidase, nitrate reduction, H_2S production and urease tests. Eleven to eighty nine percent of strains are positive for ONPG and produces acid from D-galactose. It is negative for ornithine decarboxylase, indole production and aesculin hydrolysis[6].

H. paraphrohaemolyticus has been isolated from sputum, throat, pharynx and urethral discharge in humans[12].

Haemophilus aegyptius[13]

Cells are Gram negative, non-motile, non-spore-forming, non-encapsulated bacillus, 0.25 to 0.5µm by 1.0 to 2.5µm, with rounded ends and sometimes with a bipolar body. The organism is a facultative aerobe. It requires both haemin and V factors for growth. The optimum temperature is 35 to 37°C with a range of 25 to 40°C. 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.

It has been reported that *H. aegyptius* can be differentiated from *Haemophilus influenzae* by serological means and to a certain extent, by growth characteristics and biochemical reactions. However, it has been proposed that this strain should be reclassified as a biogroup within the *H. influenzae* species[14].

Haemophilus pittmaniae[15]

H. pittmaniae is 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 to 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.

H. pittmaniae is positive for porphyrin test, negative or weakly positive for catalase and oxidase tests. A small amount of gas is produced from glucose. Indole, urease, in lysine and ornithine decarboxylase and arginine dihydrolase tests are negative.

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

Haemophilus ducreyi[16]

Cells are Gram negative coccobacilli in “railroad track” arrangement. They grow best in microaerophilic conditions at 33 to 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 phenotypic testing of *Haemophilus* species’. *H. ducreyi* grows on Mueller-Hinton agar with 5% sheep blood in a CO_2 enriched atmosphere. It produces characteristic tan-yellow colonies that are highly self-adherent and can be ‘nudged’ intact over the surface of the agar.

The organism require X factor for growth and this can most easily be evaluated using the porphyrin test. It is 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[17].

Haemophilus sputorum[3]

Cells are non-motile, small regular rods, 0.3 to 0.5µm × 2.0 to 3.0µm, with occasional coccoid forms. Colonies on chocolate agar are convex, whitish, opaque, and reach a diameter of 0.5 to 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 dependent on V factor for growth.

It is positive for oxidase and give variable results on catalase tests. Cells produce β-galactosidase, urease, and leucinearylamidase. Species are negative for indole test, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, and phenylalanine arylamidase. H_2S is not or only weakly emitted (lead acetate test), IgA1 protease is not produced.

H. sputorum was originally isolated from a case of human tooth alveolitis and is occasionally involved in human infections. It has been isolated from blood and from the sputum of patients with cystic fibrosis[3].

Haemophilus massiliensis[18]

Cells are Gram negative, non-endospore forming, facultative anaerobes and non-motile bacilli. Colonies are non-haemolytic, round and light with a size of 0.5 to 1mm on blood enriched Colombia agar. Growth occurs between 25 and 45°C and optimal growth temperature is at 37°C. They are positive for catalase and oxidase.

It has been isolated from human peritoneal fluid[18].

Haemophilus seminalis[19]

Cells are Gram negative, non-motile, non-acid-fast and coccobacilli or rods with the size of 0.4 to 0.8µm by 0.6 to 1.8µm. Cells are facultatively anaerobic, growth occurs at 28 to 40°C and is enhanced in the presence of CO_2. They require V factor for growth, but not X factor. Nitrates are reduced to nitrites. They ferment D-glucose and sucrose. They exhibit good growth on Haemophilus chocolate 2 agar and chocolate agar with PolyVitex, but not on Columbia blood agar, BHI agar, CHAB agar, MH agar or lysogeny broth agar. Colonies are smooth, low, convex, greyish and translucent on Haemophilus chocolate 2 agar.

It was recently isolated from human semen[19].

Other HACEK group of organisms

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[20]. The identification is considered together with the clinical details and the isolates may be identified further if clinically indicated.

Aggregatibacter species[4]

They are members of the family *Pasteurellaceae*. The genus *Aggregatibacter* contains 4 species, *Aggregatibacter actinomycetemcomitans, Aggregatibacter aphrophilus, Aggregatibacter segnis* and *Aggregatibacter kilianii* [21].

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 to 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 humans; they are part of the human oral flora and are occasionally recovered from other body sites, including blood and brain, and as causes of infective endocarditis and abscesses.

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

Aggregatibacter actinomycetemcomitans[4]

They are small rods, 0.3 to 0.5µm by 0.5 to 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.

The strain 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 to 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 to 7 days.

A. actinomycetemcomitans is 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. It is 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.

It is mostly found on dental surfaces. *A. actinomycetemcomitans* has regularly been isolated together with *Actinomyces* species from human actinomycosis. It has sometimes been found in 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*.

Cells are Gram negative, short regular rods, 0.5 × 1.5 to 1.7µm with occasional filamentous forms. They require 5 to 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 whilst others are V factor independent. Colonies on chocolate agar are high convex, opaque, granular and yellowish and reach a diameter of 1.0 to 1.5mm within 24hr.

Variable fermentation is observed with galactose and raffinose. H_2O_2 is not decomposed; ONPG is hydrolysed. The organism is catalase and urease negative, and oxidase variable.

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

A.aphrophilus is a member of the normal flora of the human oral cavity and pharynx. It 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]

Cells are small, pleomorphic rods, often showing a predominance of irregular, filamentous forms. Growth on chocolate agar is slow and 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 to 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. Catalase and β-galactosidase (hydrolysis of ONPG) are variably present. They are negative for oxidase, indole, urease and ornithine decarboxylase tests.

A. segnis is found in 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.

Aggregatibacter kilianii[22]

Cells are short, regular rods (0.5 by 1.5 to 1.7 mm), with occasional filamentous forms. They are Gram-negative, nonmotile, facultatively anaerobic. Colonies on chocolate agar incubated in air supplemented with 5 to 10% extra CO_2 are highly convex, granular, yellowish, and opaque and reach a diameter of 1.0 to 1.5mm within 24 hr. When plates are incubated without extra CO_2, the growth characteristically shows very small colonies interspersed with a few larger colonies. Both V-factor and X-factor are not required.

A decisive phenotypic test for identification of *A. kilianii* is alanine-phenylalanine-proline arylamidase, which is positive for *A. kilianii* and negative for the 3 other aggregatibacter species. *A. kilianii* can be distinguish from *A. aphrophilus* and *A.segnis* by testing for N-acetylglucosamine and from *A. actinomycetemcomitans* by testing for catalase and β-galactosidase (ONPG). *A. kilianii* is negative for indole, urease and ornithine decarboxylase tests.

A. kilianii is a commensal of the upper respiratory tract of humans. It is occasionally involved in human infections and has been isolated from conjunctivitis, wounds, abdominal abscesses, and blood.

Cardiobacterium species[23]

The genus *Cardiobacterium* contains 2 species, *Cardiobacterium hominis* and *Cardiobacterium valvarum*[24,25]. Cells are pleomorphic or straight rods, 0.5 to 0.75µm in diameter and 1 to 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 to 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 utilise galactose, lactose, raffinose and xylose.

Cardiobacterium hominis is the type species.

Cardiobacterium hominis[23]

Cells are Gram negative pleomorphic to short, non-motile rods. Growth on blood agar is poor. *C. hominis* does not require X or V factor, 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 to 37°C.

The organism is positive for oxidase, H_2S production, indole (weakly), and negative for nitrate reduction, catalase, urease and aesculin hydrolysis. Dextrose, fructose, maltose, mannitol, sucrose, sorbitol, and mannose is utilized but do not utilize galactose, lactose, raffinose and xylose.

C. hominis can be distinguished from other members of the HACEK group and from *Pasteurella, Brucella*, *Streptobacillus moniliformis* and *Bordetella parapertussis* by the following characteristics: absence of catalase activity, positive oxidase reaction, production of indole and absence of nitrate production[26].

Cardiobacterium valvarum[27]

Cells are fastidious Gram negative regular, pleomorphic to short rods. All strains are facultatively anaerobic and non-motile. Some strains have an acidulous smell. Its preferred culture medium is sheep blood agar, and visible colonies appear after an incubation period of 3 days. 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 2-day incubation and reach a diameter of 2.2mm after 4 days.

Microscopically, *C. valvarum* appears readily decolourised 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 × 2 to 4µm. On chocolate agar, it is smaller and pleomorphic.

The organism is positive for the production of indole, cytochrome oxidase, and H_2S but negative for catalase production, urea hydrolysis, aesculin hydrolysis, and nitrate reduction. Dextrose, fructose, sorbitol, and mannose is utilized, like *C. hominis*, but unlike *C. hominis,* does not utilize maltose, sucrose, or mannitol.

C. valvarum was first isolated in 2001 from the blood of a 37 year old man with endocarditis. *C. valvarum* is present in subgingival pockets and dental plaques, and all the reported cases of endocarditis have been in people who had recently undergone a dental procedure or had oral infection[24].

Eikenella species[28]

The genus *Eikenella* contained a single species *Eikenella corrodens* for many years. In November 2019 three more species*, Eikenella exigua, Eikenella halliae* and *Eikenella longinqua* were isolated.

Eikenella corrodens[29,30]

Cells are straight, non-branching, non-sporing, slender Gram-negative rods, 0.3 to 0.4 × 1.5 to 4µm in length. Colonies may be very small on blood agar after overnight incubation or may not be visible for several days. 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 to 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.

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

E. corrodens was originally isolated from human sputum, it is now recognised as a coloniser of the oral mucous membranes, the upper respiratory tract and possibly the gastrointestinal tract.

Eikenella exigua[28,31]

Eikenella exigua is slow growing facultatively anaerobic, short and slender Gram-negative rod, with occasional strains having scant growth when grown in 5% CO_2. It grows poorly (after 5 days) or not at all under aerobic conditions. On blood agar colonies are visible after 1 to 3 days of incubation in a microaerophilic or anaerobic atmosphere. Colonies are small (≤0.5 mm) and translucent with a caramel odour. Pitting of agar may or may not be observed on agar plates. It is catalase and oxidase negative and nitrate not reduced to nitrite. It is non-motile and indole negative. Aesculin, urea and gelatin hydrolysis is not detected.

It has been detected in samples from brain abscess, bone and pleural empyema. It has also been isolated from a submandibular abscess and parotid gland and is probably a commensal of the human oropharyngeal microbiota.

Eikenella halliae[28]

Eikenella halliae is facultative anaerobes and grow best in 5% CO_2, under microaerophilic or strict anaerobic conditions with scanty growth when grown aerobically. Colonies are adherent to agar and pitting or haemolysis on blood agar are not observed. Colonies are approximately 1mm in diameter, flat, opaque or translucent with regular flat edges after 24 h on SBA. Optimal growth is at 35 to 37 °C with no or scant growth at 25°C or 42°C. Cells are slender, medium length, Gram-stain-negative non-motile rods. It is catalase-negative and oxidase-positive and do not ferment, oxidize nor assimilate sugars. Nitrate is reduced to nitrite, indole negative, urease not detected and neither gelatin or aesculin is hydrolysed.

The type strain was recovered from an eye swab and one strain was isolated from a maxillary sinus.

Eikenella longinqua[28]

It is a slow growing, strict anaerobe with no growth aerobically or in 5 %CO_2, and scant growth microaerophilically. There is no pitting of agar or haemolysis on blood agar observed. Colonies are 0.5mm in diameter, rounded, transparent after 5 to 7 days growth on brucella agar. It grows at 35 to 37 °C. Cells are small to medium length, thin Gram-stain-negative rods. Growth in PY broth is not enhanced by the presence of glucose, bile, serum, tween or FF. Catalase and oxidase are negative. It does not ferment nor assimilate sugars. Nitrate is weakly reduced or not observed (method dependent). It is non-motile but ‘twitching motility’ observed. It is indole-negative. It does not hydrolyse urea, aesculin and gelatin. Arginine dihydrolase and proline arylamidase are detected in biochemical tests.

The type strain was recovered from a blood culture isolate of a patient.

Kingella species[32]

The genus *Kingella* comprises five species, *Kingella kingae, Kingella denitrificans*, *Kingella potus* and *Kingella oralis, Kingella negevensis*[33]. *Kingella indologenes* has been transferred to a new genus and classified as *Suttonella indologenes*[32].

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 decolourisation. Two types of colonies occur on blood agar; a spreading, corroding type and a smooth, convex type. They do not require X or

V factors. Growth is aerobic or facultatively anaerobic. The optimum growth temperature is 33 to 37°C[34].

It is 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. The strain 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.

Kingella denitrificans[35,36]

Previously designated CDC group TM-1. Cells 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.

The following test are positive: oxidase, growth at 30 and 37°C, fermentative in the O/F test, acid production from glucose, nitrate reduction, nitrite reduction, and production of gas from nitrite.

The following tests are negative: catalase, growth at 5 and 45°C, growth in the presence of 6% NaCl, growth on β-hydroxybutyrate in mineral medium, acid production from maltose unless serum was present, starch hydrolysis and urease production.

It is isolated in the respiratory tract.

Kingella kingae[37]

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, Gram negative, with some tendency to resist decolourisation. It is 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 to 0.5mm in diameter, with a small central papilla initially but after two 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 colonial type, which often arises in subcultures of the first type, is small, delicate, translucent or slightly opaque, 0.1 to 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.

Kingella kingae is aerobic and grow at room temperature but their optimal growth is at 33 to 37°C. The strain is relatively fastidious and growth on high quality nutrient agar is as good as that on blood agar.

It is 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 is not reduced or slightly reduced.

The organism is parasitic on human mucous membranes. Strains have been isolated from throat, nose, blood, bone lesions and joints.

Kingella oralis[38]

Cells are Gram negative rods or coccobacilli approximately 0.6 to 0.7µm in diameter by 1 to 3µm long with rounded ends. Cells can form pairs or chains and 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 5mg 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. *K.oralis* is 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.

Kingella potus[39]

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 to 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. It is negative for alkaline phosphatase, α-glycosidase, β-galactosidase, or β-glucuronidase activity. *K. potus* has been isolated from wounds caused by animal bites.

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

Kingella negevensis [40]

Cells are coccobacillary, non-spore-forming and non-motile, Gram negative, oxidase positive and exhibit aerobic and facultatively anaerobic capnophilic growth. *K.negevensis* is catalase, indole, lipase, alkaline phosphatase negative. After incubation at 37°C for 1 day on 5% sheep blood-enriched Columbia agar, colonies are β-haemolytic, round, pale yellow, smooth and 0.5 to 1 mm in diameter. It was first isolated from the oropharynx of healthy children.

5 Technical information/limitations

Agar media and X & V factor testing

The use of chocolate agar is preferable for species that require X and V factor for growth rather than blood agar or blood containing medium because of risk of carryover of X factor. The X and V factor testing 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

Please note that sometimes the X and V factor tests can give false V dependent results if incubated in CO_2[41].

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

Principles of isolation

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 with 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 Vaccine Preventable Bacteria Section, Respiratory and Vaccine Preventable Bacteria Reference Unit (RVPBRU), Public Health England, Colindale, for confirmation and typing.

6 Safety considerations[42-59]

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[60]. The organism infects primarily by the respiratory route (inhalation), autoinoculation or ingestion in laboratory workers[61].

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 UK SMI.

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

Compliance with postal and transport regulations is essential.

7 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, Haemophilus seminalis, Aggregatibacter aphrophilus (includes *Haemophilus aphrophilus* and *Haemophilus paraphrophilus*), *Aggregatibacter segnis* (formerly *Haemophilus segnis*), *Aggregatibacter actinomycetemcomitans* (formerly *Actinobacillus actinomycetemcomitans*), *Aggregatibacter kilianii, Cardiobacterium hominis, Cardiobacterium valvarum, Eikenella corrodens, Eikenella exigua, Eikenella halliae, Eikenella longinqua Kingella kingae, Kingella denitrificans, Kingella oralis, Kingella potus.*

8 Identification

8.1 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.

8.2 Primary isolation media

Chocolate agar incubated in 5 to 10% CO_2 at 35 to 37°C for 24 to 48hr.

Blood agar incubated in 5 to 10% CO_2 at 35 to 37°C for 24 to 48hr.

8.3 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 section 4.1 and appendix 3).

8.4 Test procedures

Tests listed below are no longer carried out routinely in laboratories. They may be useful in some cases where further identification is required.

Catalase Test (TP 8 - Catalase test)

Oxidase Test (TP 26 - Oxidase test)

Urease Test (TP 36 – Urease test)

Table 1: Summary of the biochemical tests:

Organism	Catalase	Oxidase	Urease
H. influenzae	+	+	(+)
H. parainfluenzae	d	+	d
H. haemolyticus	+	+	+
H. parahaemolyticus	d	+	+
H. paraphrohaemolyticus	+	+	+
H. aegyptius	+	+	+
H. pittmaniae	d	d	-
H. ducreyi	-	+	Unknown
H. sputorum	V	+	+
H. massiliensis	+	+	+
H. seminalis	+	+	+
A. actinomycetemcomitans	+	+	-
A. aphrophilus	-	-	-
A. segnis	V	-	-
A. kilianii	-	Unknown	-
C. hominis	-	+	-
C. valvarum	-	+	-
E. corrodens	-	+	-
E. exigua	-	-	-
E. halliae	-	+	-
E. longinqua	-	-	-
K. denitrificans	-	+	-
K. kingae	-	+	-
K. oralis	-	+	-
K. potus	-	+	-
K. negevensis	-	+	-

+ = 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).

Table 2: Summary of X and V test results

Organism	X factor	V factor	X + V factor	Porphyrin
H. influenzae[a]	-	-	+	-
H. parainfluenzae	-	+	+	+
H. haemolyticus[b]	-	-	+	-
H. parahaemolyticus	-	+	+	+
H. paraphrohaemolyticus	-	+	+	+
H. aegyptius	-	-	+	-
H. pittmaniae	-	+	+	+
H. ducreyi	+	-	+	+
H. sputorum	-	+	+	+

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

[b]Traditionally described as β-haemolytic on horse blood agar, but non-haemolytic strains exist[14]

Serotyping *H. influenzae* with commercial type-specific antisera and PCR

The presence of capsule polysaccharide can be detected by slide agglutination using commercial antisera. If positive, the individual serotype (a to f) can also be determined using antisera. Slide agglutination can sometimes generate ambiguous results and so the capsule type can be confirmed using multiple PCRs directed at targets within the capsule gene operon[62,63].

Some multi-species meningitis latex agglutination detection kits include antiserum against *H. influenzae* serotype b alone because of its historical dominance in causing meningitis and its relevance in detecting vaccine failures. However, it should be noted that not all latex agglutination detection kits are suitable for use on bacterial suspensions of *H. influenzae* (according to the manufacturer's instructions).

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.

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

This technique has 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[64]. MALDI-TOF MS can be used for identification and characterisation of different

members of the genus *Haemophilus*, as well as it can accurately identify the HACEK organisms despite their fastidious nature[14,65].

This technique can be used to rapidly distinguish between *C. hominis* and *C. valvarum*[66] and can provide rapid differentiation of *H. influenzae* and *H. aemolyticus*[67].

Nucleic Acid Amplification Tests (NAATs)

PCRs have been developed to detect *H.* influenzae and *H. parainfluenzae* in clinical specimens and some have been incorporated into commercial multi-pathogen detection systems. PCR methods are also used to confirm the species of *H.influenzae* isolates and serotype them[62,63]. They have also been developed to help discriminate non-typeable *Haemophilus influenzae* from non-haemolytic *H. haemolyticus*[68,10].

PCR has been used to identify *H. ducreyi* in clinical specimens. Commercial multiplex PCR assay 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[69].

16s rRNA PCR assay followed by sequencing and analysis has been used for the identification of difficult and serious infections due to fastidious microorganisms – *Cardiobacterium hominis*. 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[70].

8.5 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 are difficult to implement for routine bacterial identification in a clinical laboratory and may be better sourced from a reference 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*[27,71]. It has equally been used for identifying *Aggregatibacter* species[14].

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*[72,73]. It may be used to study the epidemiology *of H. ducreyi* and chancroid.

Ribotyping 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[74].

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 characterising epidemiologically related isolates. 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[75,76].

This has been used successfully to identify and discriminate between strains of non-typeable *Haemophilus influenzae*[77].

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. A scheme was developed for *H. influenzae*[78] and has been extensively used, not only within this species, but also more widely across the *Haemophilus* genus[14]. For example, a version of MLST was used to describe the 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[9,14,15].

8.6 Storage and referral

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

9 Reporting

9.1 Infection Specialist

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

Certain clinical conditions must be notified to the laboratory associated infection specialist. Typically, these will include:

- Facial cellulitis
- Septic arthritis
- Osteomyelitis
- Epiglottitis, pneumonia, mastoiditis or empyema thoracis

Follow local protocols for reporting to clinician.

9.2 Preliminary identification

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

9.3 Confirmation of identification

Following serotyping of *H. influenzae*, appropriate X and V and/or commercial identification kit or platform (e.g. MALDI-TOF MS) results and/or the Reference Laboratory report.

9.4 Health Protection Team (HPT)

Refer to local agreements in devolved administrations.

9.5 Public Health England[79]

Refer to current guidelines on SGSS reporting.

9.6 Infection prevention and control team

N/A

10 Referral to reference laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory see the Specialist and reference microbiology: laboratory tests and services page on GOV.UK for user manuals and request forms

Organisms with unusual or unexpected resistance, or associated with a laboratory or clinical problem, or an anomaly that requires investigation should be sent to the appropriate 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:

England and Wales

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

Scotland

https://www.hps.scot.nhs.uk/a-to-z-of-topics/reference-laboratories/

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

Haemophilus influenzae

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

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

Telephone: +44 (0) 20 8327 7887

HACEK group and *Haemophilus* species for identification

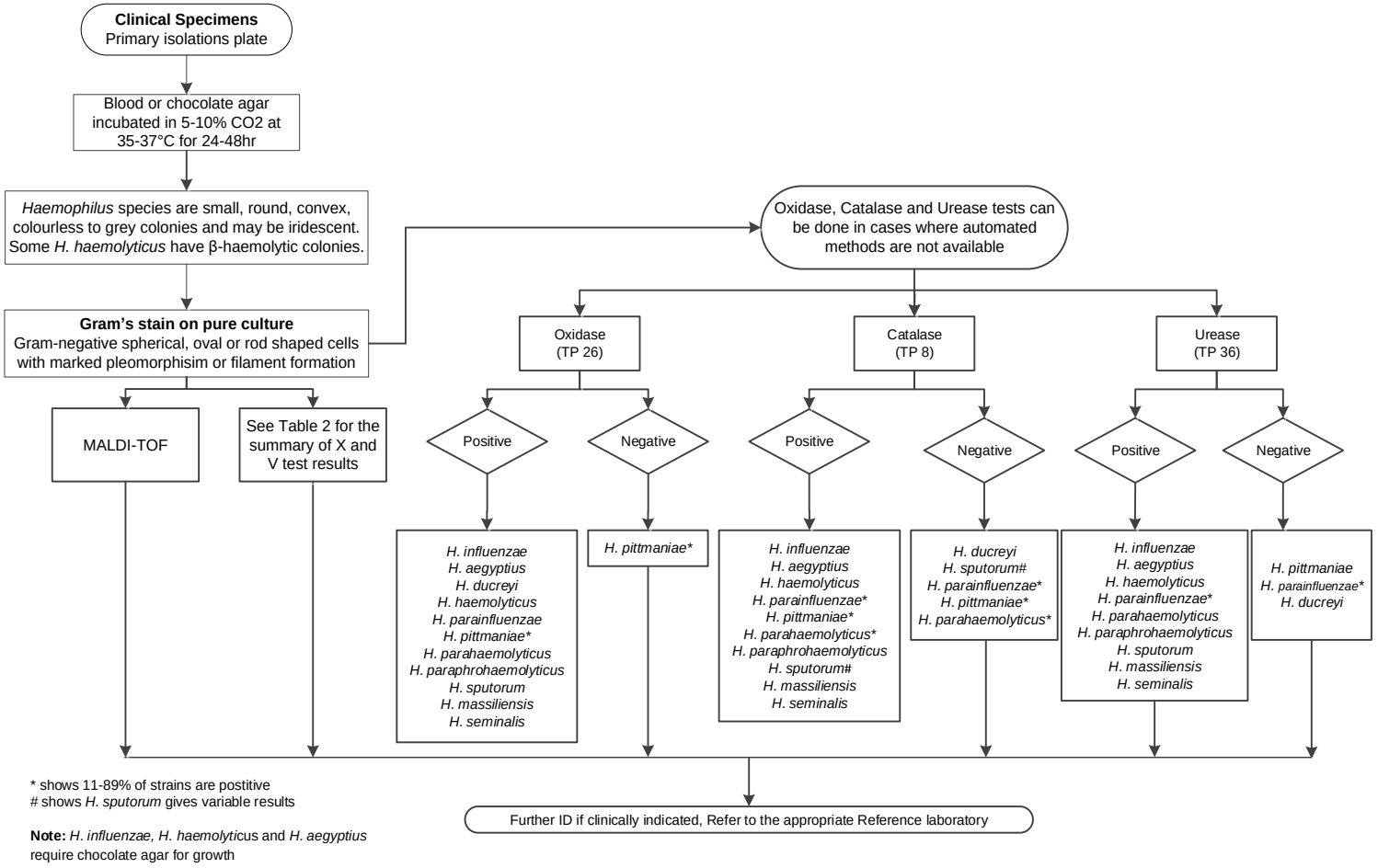
Bacterial Identification Section (BIDS)
Antimicrobial Resistance and Healthcare Associated Infections Reference Unit (AMRHAI)
Public Health England
61 Colindale Avenue
London
NW9 5EQ

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

Telephone: +44 (0) 20 8327 6511 / 7887

Appendix 1: Identification of *Haemophilus* species

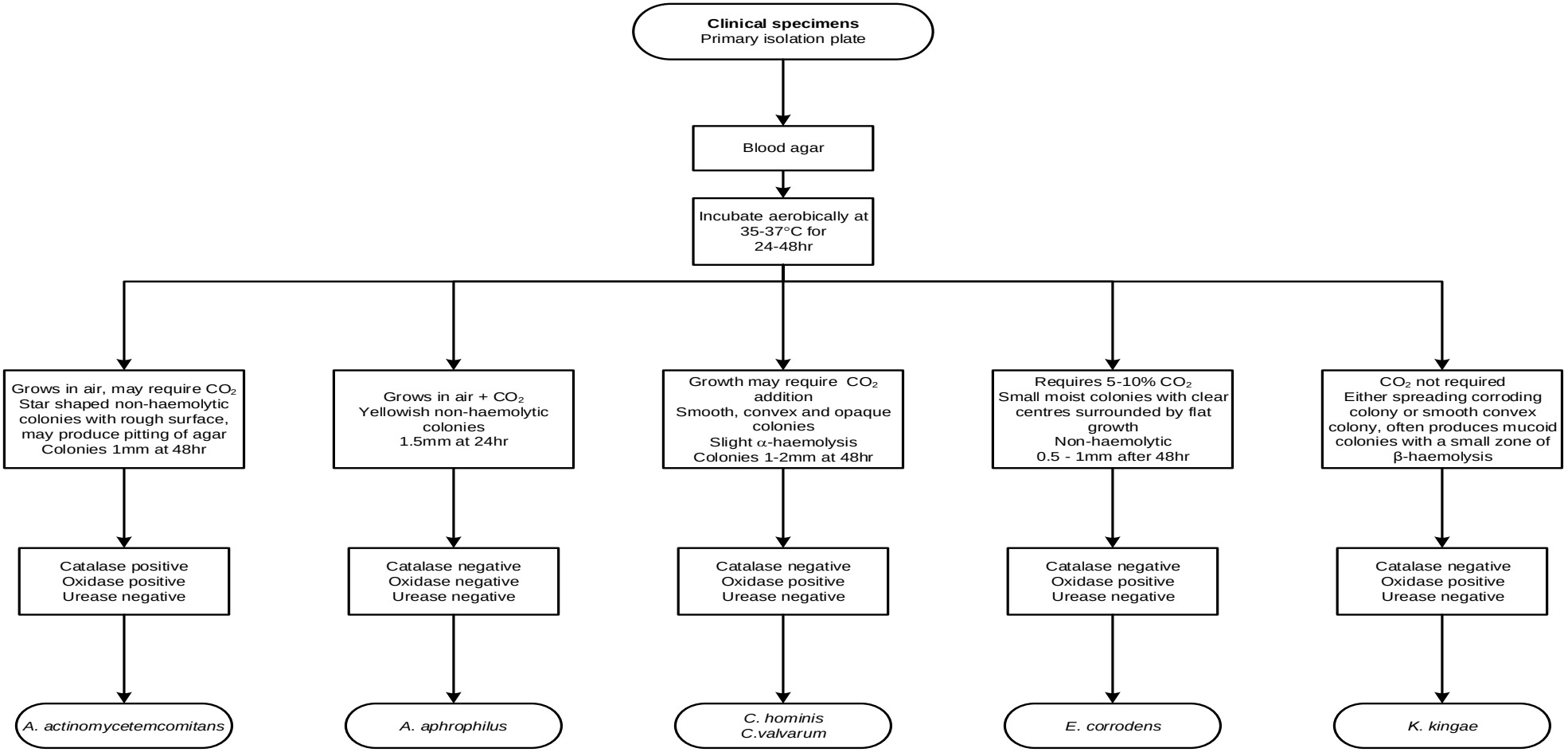
This flowchart provides a summary of information presented in section 8 (tables 1 and 2). An accessible text description of this flowchart is provided on the UK SMI ID 12 download page.



This flowchart is for guidance only.

Appendix 2: Identification of HACEK group

This flowchart provides a summary of information presented in section 4.1 and section 8 (table 1). An accessible text description of this flowchart is provided on the UK SMI ID 12 download page.



This flowchart is for guidance only.

Appendix 3: Colonial appearance of HACEK group organisms

HACEK group organisms	Characteristics of growth
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.
A. kilianii	Colonies on chocolate agar are highly convex, granular, yellowish, and opaque and reach a diameter of 1.0 to 1.5mm within 24hr. When plates are incubated without extra CO_2, the growth will show very small colonies interspersed with a few larger colonies.
C. hominis	Some strains will not grow without added CO_2. May require X-factor on primary isolation. Colonies smooth, convex and opaque. 1 to 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 to 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 to 1mm after 48hr. Requires 5 to 10% CO_2.
Eikenella exigua	Colonies on blood agar are small (≤0.5 mm) and translucent with a caramel odour. It is facultatively anaerobic and grows poorly (after 5 days) or not at all under aerobic conditions.

Eikenella halliae	Colonies are approximately 1mm in diameter, flat, opaque or translucent with regular flat edges after 24hr on SBA. Optimal growth at 35 to 37°C with no or scant growth at 25°C or 42°C. Cells are slender, medium length, Gram-stain-negative rods.
Eikenella longinqua	Colonies are 0.5mm in diameter, rounded, transparent after 5 to 7 days growth on brucella agar. Grows at 35 to 37 °C. it is slow growing and strict anaerobic.
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 to 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 to 2mm in diameter, and friable on Columbia blood agar after 48hr of incubation at 37°C. Colonies are non-haemolytic.
K. negevensis	After incubation at 37°C for 1 day on 5% sheep blood-enriched Columbia agar, colonies are β-haemolytic, round, pale yellow, smooth and 0.5 to 1mm in diameter
Note 1: For descriptions of *Haemophilus* species, see subheading “Taxonomy/characteristics”	

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For the information for the evidence grade ratings given, refer to the scientific information link above in section 2.

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