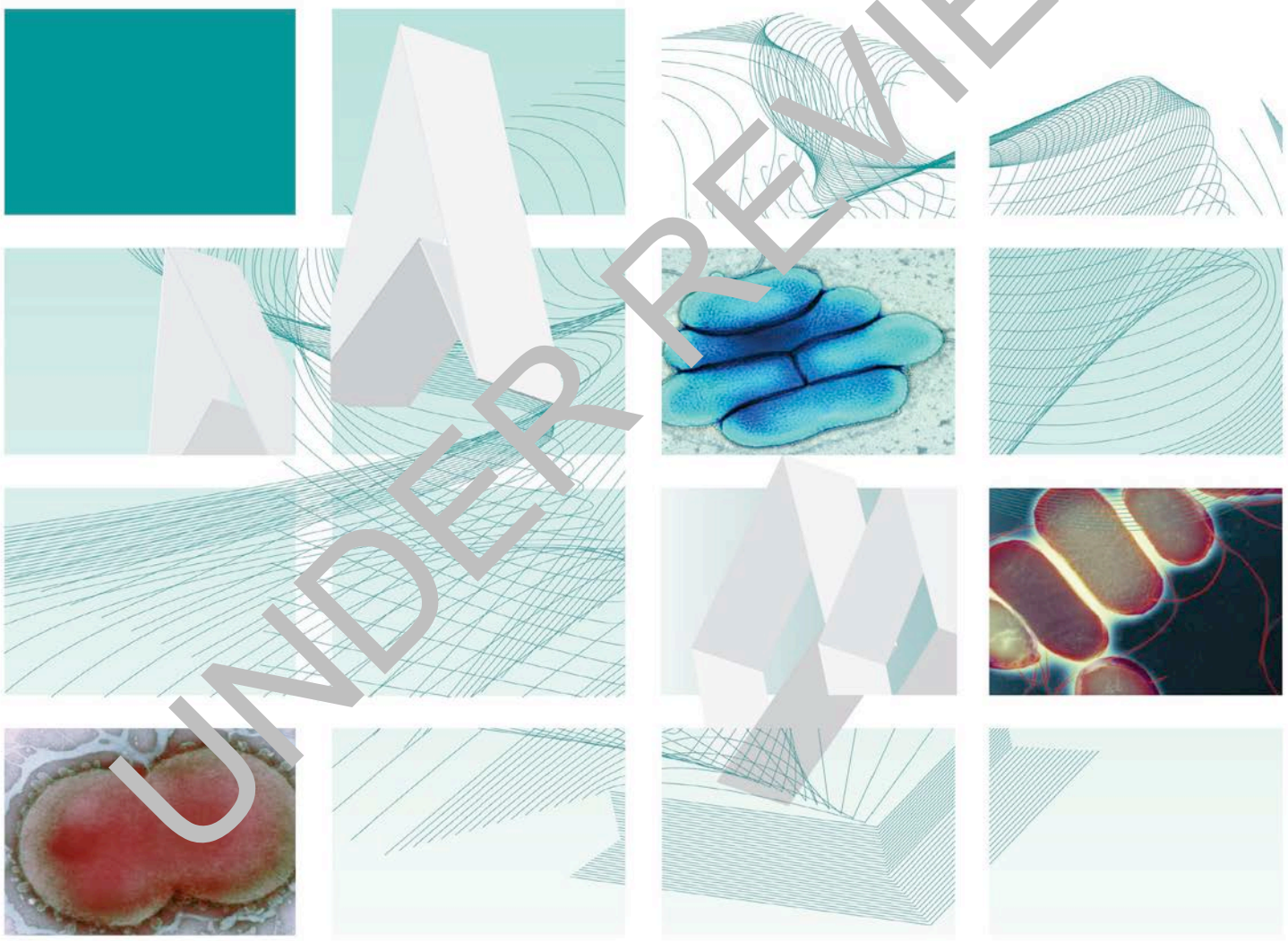
Public Health England

NHS

UK Standards for Microbiology Investigations

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



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Bacteriology – Identification | ID 12 | Issue no: 2.3 | Issue date: 11.03.14 | Page: 1 of 22

Acknowledgments

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website http://www.hpa.org.uk/SMI/Partnerships. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see http://www.hpa.org.uk/SMI/WorkingGroups).

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

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UK Standards for Microbiology Investigations are produced in association with:

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.	5/11.03.14
Issue no. discarded.	2.2
Insert Issue no.	2.3
Section(s) involved	**Amendment**
Whole document.	Document has been transferred to a new template to reflect the Health Protection Agency's transition to Public Health England. Front page has been redesigned. Status page has been renamed as Scope and Purpose and updated as appropriate. Professional body logos have been reviewed and updated. Standard safety and notification references have been reviewed and updated. Scientific content remains unchanged.

Amendment No/Date.	4/27.06.12
Issue no. discarded.	2.1
Insert Issue no.	2.2
Section(s) involved	**Amendment**
Whole document.	Minor formatting amendments.
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 http://www.hpa.org.uk/SMI/Partnerships. 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.

Information Governance and Equality

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

The development of SMIs are subject to PHE Equality objectives http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317133470313. The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal Statement

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

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Suggested Citation for this Document

Public Health England. (2014). Identification of *Haemophilus* species and the HACEK Group of Organisms. UK Standards for Microbiology Investigations. ID 12 Issue 2.3. http://www.hpa.org.uk/SMI/pdf.

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 thirteen species of *Haemophilus*. The *Haemophilus* species associated with humans are *H. influenzae, H. aegyptius, H. haemolyticus*, *H. parainfluenzae, H. pittmaniae, H. parahaemolyticus, H. paraphrohaemolyticus* and *H. ducreyi*[1]. 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[2]. *H. segnis* has been re-classified as *Aggregatibacter segnis*[2]. There are 8 biotypes of *Haemophilus influenzae* (I-VIII) and eight biovars of *Haemophilus parainfluenzae* (I-VIII)[2]. Pittman described six antigenically distinct capsular types of *H. influenzae*, designated a-f.

Characteristics

Haemophilus are Gram negative spherical, oval or rod-shaped cells less than 1µm in width, variable in length, with marked pleomorphism, and sometimes forming filaments. Small, round convex, colonies, which may be iridescent, develop in 24hr on chocolate blood agar. Iridescence is seen with capsulated strains.

All species require preformed growth factors present in blood, particularly X factor (protoporphyrin IX or protoheme) and/or V factor (nicotinamide adenine dinucleotide (NAD) or NAD phosphate (NADP)). On blood agar, *H. influenzae* exhibits satellitism around colonies of *Staphylococcus aureus* (a source of V factor). *Aggregatibacter aphrophilus* and *Haemophilus paraphrohaemolyticus* require CO_2 for primary isolation. Carbohydrates are catabolised with the production of acid. A few species produce gas. The optimum growth temperature is 35–37°C. They are facultatively anaerobic and non-motile. Nitrates are reduced to nitrites.

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 haem producing *Haemophilus* species. Identification is confirmed by commercial biochemical tests, serotyping with type-specific antisera and/or referral to a Reference Laboratory. Isolates of *H. influenzae* from normally sterile sites should be sent to the *Haemophilus* Reference Unit, Respiratory and Systemic Infection

Laboratory, Public Health England, Microbiology Services Division, Colindale, for confirmation and typing.

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[3]. 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 should be referred to the Antimicrobial Resistance and Healthcare Associated Infections Reference Unit, PHE Microbiology Services Division, Colindale for confirmation of identification and MIC testing.

***Aggregatibacter actinomycetemcomitans*[4,5]**

Aggregatibacter actinomycetemcomitans (formerly *Actinobacillus actinomycetemcomitans*) is a Gram negative coccobacillus or short rod, 0.3-0.5 x 0.5-1.5μm, which may exhibit irregular staining. *A. actinomycetemcomitans* is mostly bacillary, but cocci are interspersed. Occasional longer forms up to 6μm may occur. Cells are arranged singly, in pairs, or (more rarely) in chains. Small amounts of extracellular slime may be produced.

A. actinomycetemcomitans does not require X or V factors. 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 blood or chocolate agar may be less than 0.5mm and enlarge to 1mm after several days incubation. The colonies on blood or chocolate agar may be firm, adherent, star-shaped, sometimes with rough surfaces and pitting, and may be difficult to remove from the agar surface. If extracellular slime is produced, cultures may be sticky on primary isolation. Surface cultures have low viability and may die within five to seven days. Cells are non-motile. It is catalase and oxidase positive, and urease negative.

Aggregatibacter aphrophilus

The species *Haemophilus aphrophilus* and *Haemophilus paraphrophilus* have been reclassified as a single species *Aggregatibacter aphrophilus*[1]. These are Gram negative, short regular bacilli, 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 X-factor is not an absolute requirement. Some isolates require V-factor (formerly *H. paraphrophilus*) whilst others are V-factor independent (formerly *H. aphrophilus*). The colonies on chocolate agar are opaque, granular and yellowish, catalase and urease negative, and oxidase variable.

Aggregatibacter segnis

Formerly called *Haemophilus segnis*. Cells are small and pleomorphic with a preponderance of filamentous forms. Growth on chocolate agar is slow and the colonies are smooth, greyish-white or opaque, and 0.5mm in diameter after 48hr incubation. The growth of some strains is enhanced by 5-10% CO_2. *A. segnis* requires V-factor but not X-factor.

Cardiobacterium hominis[6]

The genus *Cardiobacterium* contains two species, *Cardiobacterium hominis* and *Cardiobacterium valvarum*[7]. 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. *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 two days, colonies are 1mm in diameter, smooth, opaque and butyrous, and 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. It is non-motile, oxidase positive and catalase and urease negative.

Eikenella corrodens[4]

The genus *Eikenella* contains only one species, *Eikenella corrodens*. Cells are straight, unbranched, non-sporing, slender Gram negative rods, 0.2-0.4 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 many 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, oxidase positive, catalase negative, urease negative 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.

Kingella species[5]

The genus *Kingella* comprises three species, *Kingella kingae, Kingella denitrificans* and *Kingella oralis*. *Kingella indologenes* has been transferred to a new genus and classified as *Suttonella indologenes*[8].

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. It does not require X or V factors. Growth is aerobic or facultatively anaerobic. The optimum growth temperature is 33-37°C. *Kingella kingae* colonies are surrounded by a distinct zone of β-haemolysis on blood agar. *Kingella* species 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.

Technical Information/Limitations

N/A

1 Safety Considerations[9-25]

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

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

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

Compliance with postal and transport regulations is essential.

2 Target Organisms[6,26-47]

HACEK group reported to have caused human infection

Haemophilus influenzae

*Aggregatibacter aphrophilus (*includes *H. aphrophilus* and *H. paraphrohaemolyticus)*

Haemophilus parainfluenzae

*Aggregatibacter segnis (*formerly *H. segnis)*

Haemophilus parahaemolyticus

*Aggregatibacter actinomycetemcomitans (*formerly *Actinobacillus actinomycetemcomitans)*

Cardiobacterium hominis and *Cardiobacterium valvarum*

Eikenella corrodens

Kingella kingae

3 Identification

3.1 Microscopic Appearance

Gram stain (TP 27 - 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 16-48hr.

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

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 Introduction and 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. Haemin (X-factor) enhances growth but there is not an absolute requirement for X-factor. Some isolates require V factor (formerly H. paraphrophilus) whereas others are V factor-independent (formerly H. aphrophilus). Non-haemolytic.
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, 0.5-0.8mm 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	Two 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. Two types of colony: a spreading, corroding type and a smooth, convex type.
Note 1: For descriptions of *Haemophilus* species see *Haemophilus* species (Introduction) Note 2: In some cases it may be possible to use commercial biochemical identification kits	

3.4 Test Procedures

Biochemical tests[27]

Summary of biochemical tests:

Organism	Catalase	Oxidase	Urease
H. influenzae	+	+	(+)
H. aegyptius	+	+	+
H. haemolyticus	+	+	+
H. parainfluenzae	d	+	d

H. pittmaniae	d	d	-
H. parahaemolyticus	d	+	+
H. paraphrohaemolyticus	+	+	+
A. actinomycetemcomitans	+	+	-
A. aphrophilus	-	-	-
C. hominis	-	+	-
K. kingae	-	+	-
E. corrodens	-	+	-

Growth requirement for X and V factors

This is used to distinguish *Haemophilus* species (TP 38 - X and V Factor Test) or Porphyrin synthesis test TP 29 - Porphyrin Synthesis (ALA) Test

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

Commercial identification kit

Note: In many cases, the commercial identification kit may not reflect recent changes in taxonomy.

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

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

[b]β-haemolytic on horse blood agar.

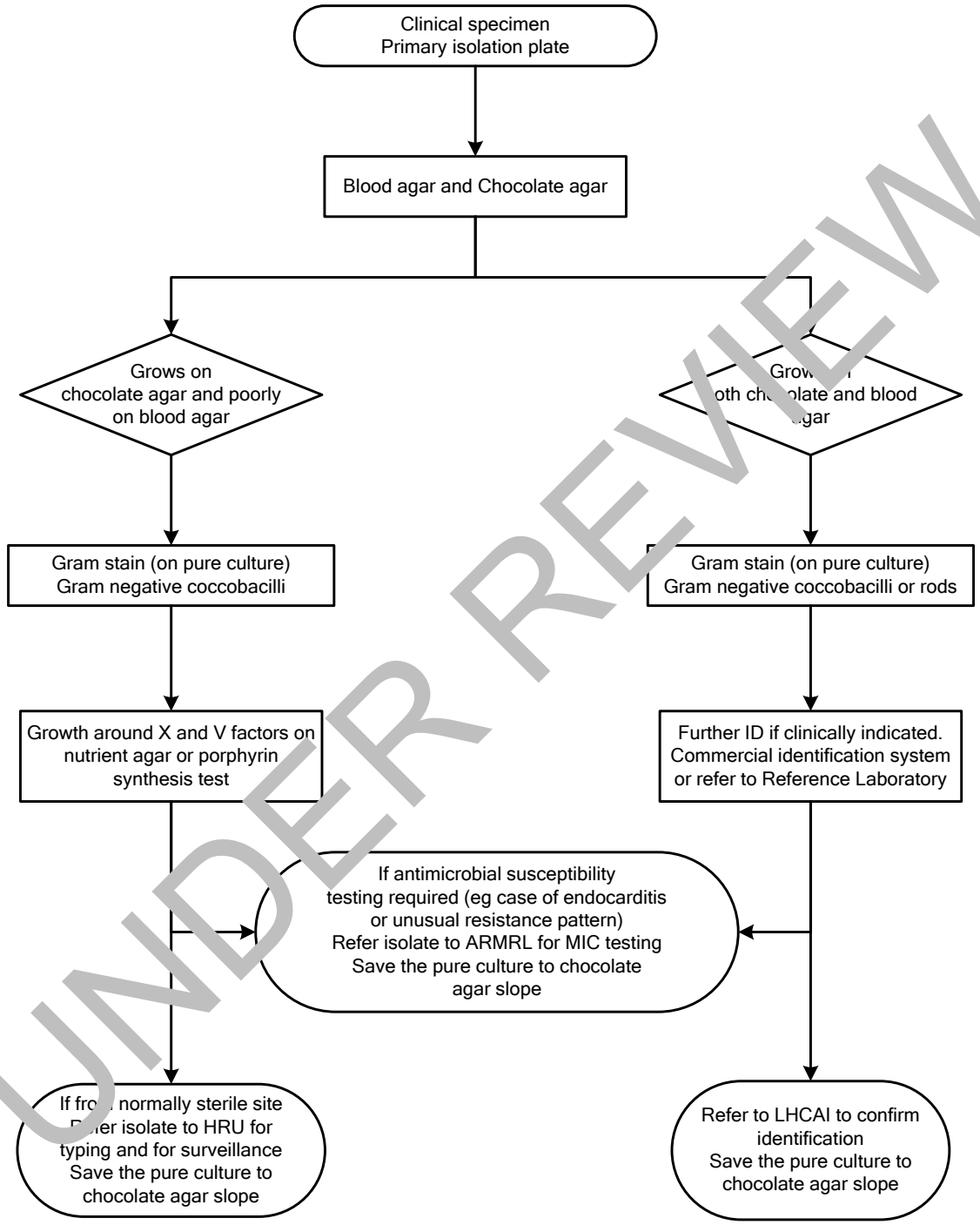
3.5 Confirmation

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

3.6 Storage and Referral

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

4 Identification Flowcharts: *Haemophilus* species, HACEK group



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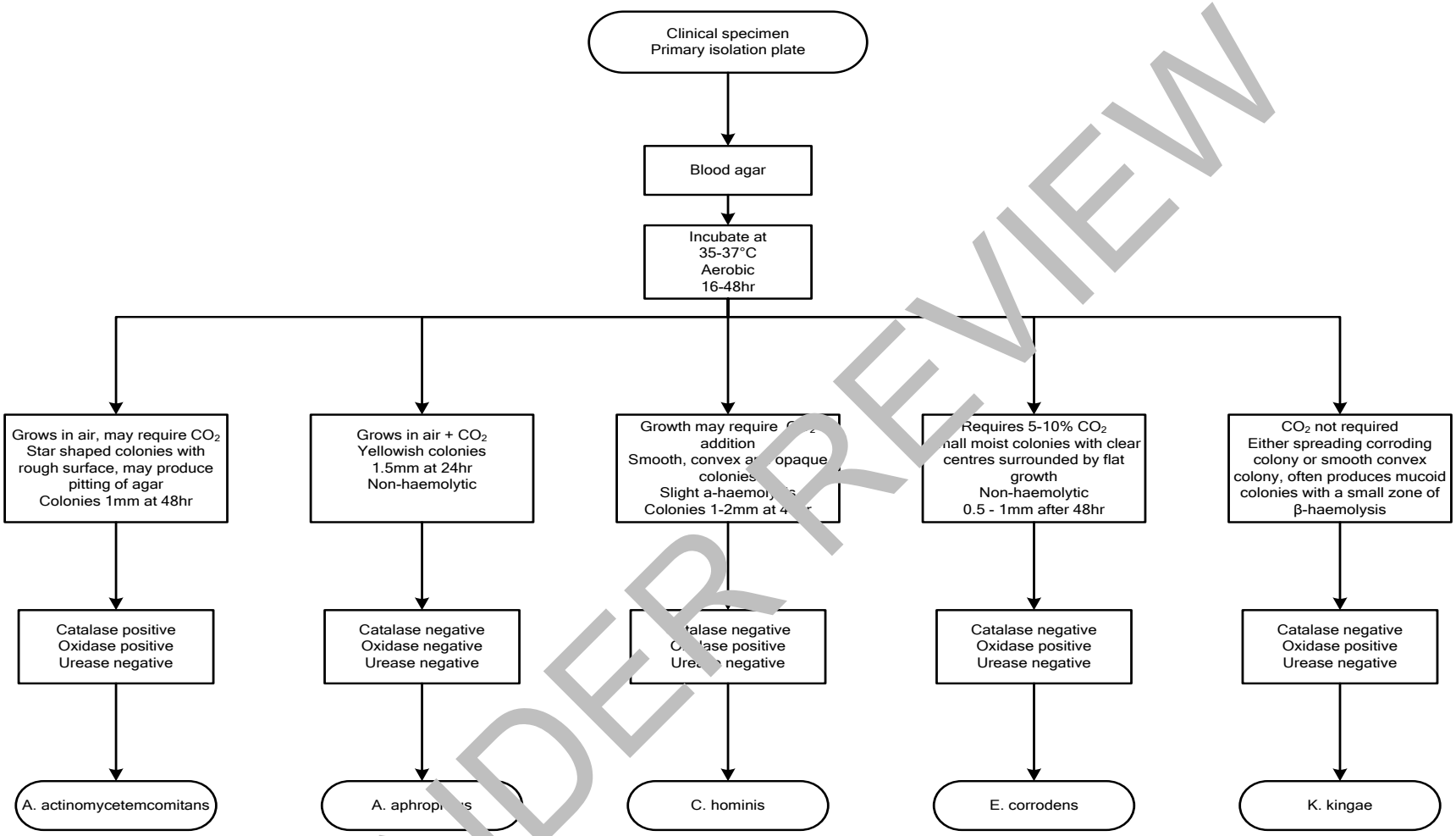

This flowchart is for guidance only

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 lecithinase activity, motility, penicillin susceptibility and 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 presumed or confirmed *Haemophilus* species or other member of the HACEK group of organisms when the request card 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[48]

Refer to current guidelines on CDSC and COSURV reporting.

5.6 Infection Control Team

N/A

6 Referrals

6.1 Reference Laboratory

Contact appropriate devolved nation 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 Systemic Infection Laboratory
Microbiology Services Division

Public Health England
61 Colindale Avenue
London
NW9 5EQ

http://www.hpa.org.uk/cfi/rsil/rsiluser.pdf

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 Division
Public Health England
61 Colindale Avenue
London
NW9 5EQ

http://www.hpa.org.uk/cfi/lhcai/req_single_isolate.pdf

Telephone: +44 (0) 208 327 6511

Haemophilus ducreyi

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

Sexually Transmitted Bacteria Reference Laboratory

Microbiology Services Division

Public Health England

61 Colindale Avenue

London

NW9 5EQ

http://www.hpa.org.uk/cfi/sbrl/default.htm

Tel: +44 (0) 20 8327 6461

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

England and Wales
http://www.hpa.org.uk/webw/HPAweb&Page&HPAwebAutoListName/Page/1158313434370?p=1158313434370

Scotland

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

Northern Ireland

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

7 Notification to PHE[48,49] or Equivalent in the Devolved Administrations[50-53]

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'.

Other arrangements exist in Scotland[50,51], Wales[52] and Northern Ireland[53].

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