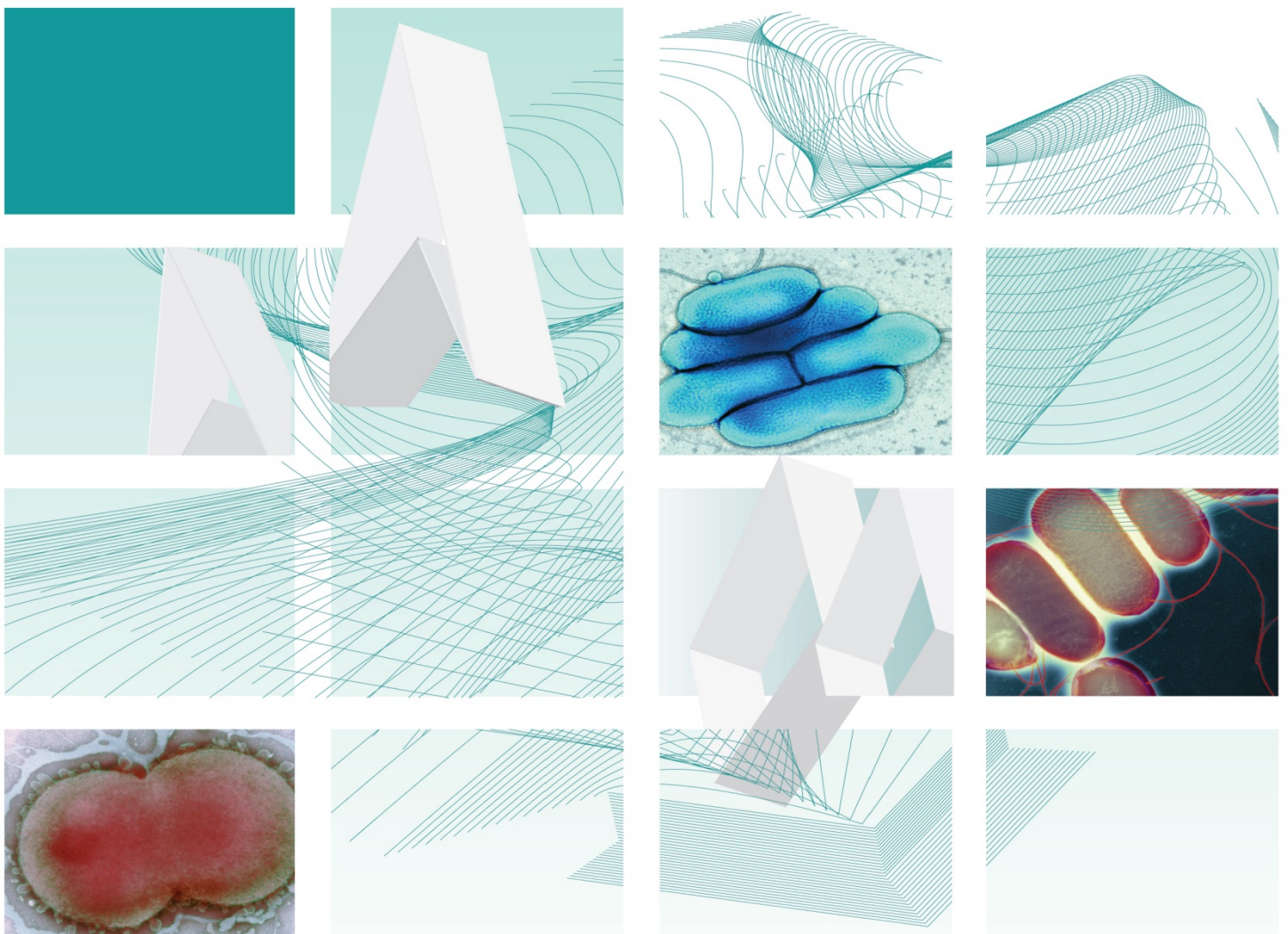




UK Standards for Microbiology Investigations

X and V factor test



"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011**."

Acknowledgments

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

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

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Logos correct at time of publishing.

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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.

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

Amendment number/date	9/08.05.19
Issue number discarded	3
Insert issue number	4
Anticipated next review date*	08.05.22
Section(s) involved	Amendment
Whole document.	Document updated. References updated with grades. Flowchart updated to include commercial alternatives.
Quality control organisms.	Alternative bacterial NCTC strain (NCTC 12975) tested and validated for this test and EUCAST susceptibility tests.

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

UK SMI[#]: scope and purpose

Users of UK SMIs

Primarily, UK SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. UK SMIs also 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. The documents also 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 UK SMIs

UK 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. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of UK 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

UK 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 UK SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing UK SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop UK 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. UK SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the UK SMI working groups to produce UK SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of UK SMIs is certified to ISO 9001:2008. UK SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. UK SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level

[#] 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.

of complex laboratory investigation possible. In using UK SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. UK SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. UK SMIs also provide a reference point for method development. The performance of UK 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 UK SMI working groups are committed to patient and public involvement in the development of UK SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting UK 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 UK SMIs is subject to PHE Equality objectives <https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity>.

The UK 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

While every care has been taken in the preparation of UK SMIs, PHE and the partner organisations, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an UK SMI or any information contained therein. If alterations are made by an end user to an UK SMI for local use, it must be made clear where in the document the alterations have been made and by whom such alterations have been made and also acknowledged that PHE and the partner organisations shall bear no liability for such alterations. For the further avoidance of doubt, as UK SMIs have been developed for application within the UK, any application outside the UK shall be at the user's risk.

The evidence base and microbial taxonomy for the UK SMI is as complete as possible at the date 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.

UK SMIs are Crown copyright which should be acknowledged where appropriate.

Suggested citation for this document

Public Health England. (2019). X and V Factor Test. UK Standards for Microbiology Investigations. TP 38 Issue 4. <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>

Scope of document

The UK SMI describes the differentiation of *Haemophilus* species by the X and V test. Because similarities exist in growth factor requirements of *Haemophilus* species, in critical situations, it is not recommended that this procedure be the sole criterion for species identification.

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

Introduction

Species of the genus *Haemophilus* require either or both of two factors X and V for growth and can be used to differentiate the species. Both factors are present in blood.

X factor comprises protoporphyrin IX, also called haemin or other iron-containing porphyrins. These are required for growth because X-dependent strains are unable to convert d-aminolaevulinic acid to protoporphyrin. They are heat stable.

V factor comprises nicotinamide adenine dinucleotide (NAD) or nicotinamide adenine dinucleotide phosphate (NADP). They are heat labile¹.

The factors are incorporated in filter paper discs which are placed on a blood free medium previously inoculated with the organism under test. After incubation, the presence or absence of growth around the discs is recorded. The presence of growth around the disc but not elsewhere on the plate indicates a requirement for that particular factor.

Technical information/limitations

Erroneous results

V factor diffuses more readily than X factor. If the discs are placed too close together, V factor may diffuse towards the X factor disc, leading to growth apparently due to X factor rather than V¹.

Quality control of commercial identification kits

Commercial manufacturers of X and V factor discs do not specify the concentration of the factors. Acceptance of a batch of discs should be based on an 'in use' performance test with a range of *Haemophilus* species rather than an assay of content.

Each batch or shipment of XV factor discs should be checked with a positive control, and the X factor and V factor discs are tested with both known positive and negative controls before routine use in the laboratory to ensure quality control.

Agar media

The use of chocolate agar is preferable for X and V factor testing rather than blood agar or blood containing medium because of the risk of carry-over 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*².

Manufacturers' instructions should be followed when performing this test.

More accurate results are obtained with the porphyrin synthesis test ([TP 29 – porphyrin test](#)).

The swab used for setting up the plate for X and V factors can also be used for setting up antibiotic plates providing the X and V factors are set up first.

Incubation

The X and V factor tests could sometimes give false V dependent results if incubated in CO₂².

Issues with the HACEK group of organisms (apart from *Haemophilus* species)

Eikenella corrodens are X-dependent as they exhibit growth around the X disc when tested, which is a useful diagnostic test. The other organisms may be X and V-dependent or may require only either X or V factor¹.

1 Safety considerations³⁻²⁰

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 reported²¹. The organism infects primarily by the respiratory route (inhalation), autoinoculation or ingestion in laboratory workers²².

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

2 Reagents and equipment

Discrete bacterial colonies growing on solid medium

Normal saline or distilled water

Sterile swabs

Test agar plate as recommended by manufacturers' instructions

Commercially available discs/strips impregnated with X, V and XV factors

Bacteriological straight wire/loop or disposable alternative

3 Quality control organisms

X and V factor

Haemophilus influenzae NCTC 11931 or NCTC 12975

V factor only

Haemophilus parainfluenzae NCTC 10665

X factor only

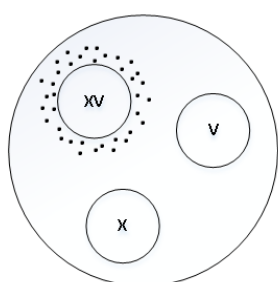
Haemophilus haemoglobinophilus NCTC 8540

Note: These strains have been validated by NCTC to give this result.

4 Procedure and results

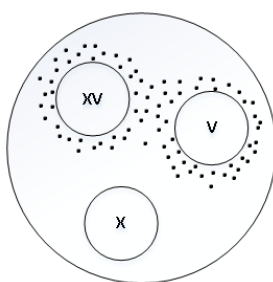
4.1 X and V factor test method^{1,2}

- make a light suspension of the test organism by touching one or more morphologically similar colonies with a straight wire and emulsifying in normal saline or distilled water
- soak a swab in the suspension and spread evenly across the entire surface of a test agar plate. This allows for maximum growth
- allow a few minutes for agar surface to dry
- place X, V and XV discs on the agar surface in area of inoculum. Ensure the discs are a minimum of 1cm apart in an equilateral triangle configuration (to prevent diffusion from the discs giving false results) or follow manufacturer's instructions
- gently press down on discs so that they adhere to agar surface
- incubate in 3-5% CO₂ at 35-37°C overnight
- examine the plates in a good light source for visible growth between and around the discs. Interpret the test agar plates according to the table below



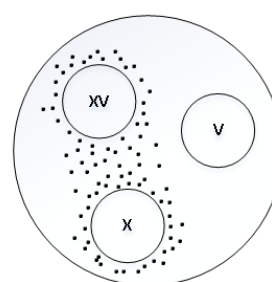
H. influenzae

(Growth around XV disc only)



H. parainfluenzae

(Growth around V and XV discs)



H. haemoglobinophilus

(Growth around X and XV discs)

Interpretation

Organisms that require only X factor will grow only around the X and XV factor discs.

Organisms that require only V factor will grow only around the V and the XV factor discs.

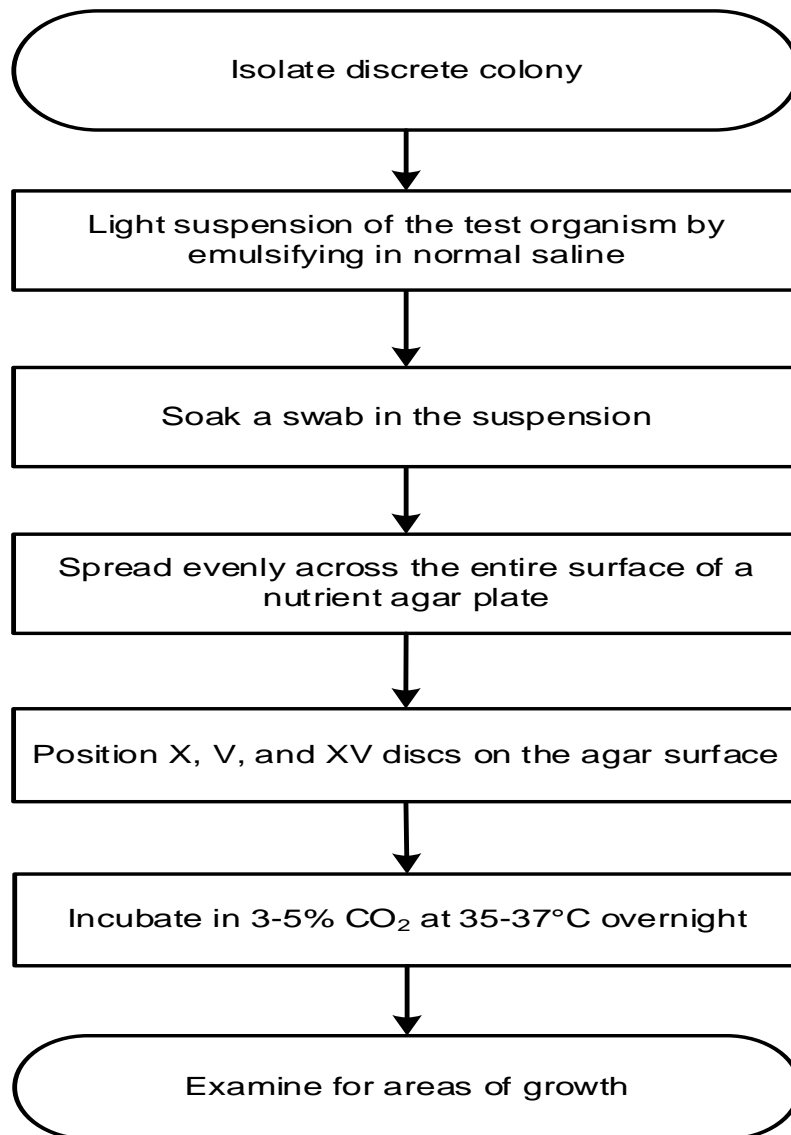
If both X and V factors are required, the organism will grow only around the XV factor disc.

Below is a summary of X, V and XV factor results.

<i>Haemophilus</i> species	Growth around discs		
	X	V	XV
<i>H. influenzae</i>	-	-	+
<i>H. parainfluenzae</i>	-	+	+

<i>H. haemoglobinophilus</i>	+	-	+
<i>H. aegyptius</i> *	-	-	+
<i>H. haemolyticus</i>	-	-	+
<i>H. pittmaniae</i>	-	+	+
<i>H. parahaemolyticus</i>	-	+	+
<i>H. paraphrohaemolyticus</i>	-	+	+
<i>H. ducreyi</i>	+	-	+
<i>H. sputorum</i>	-	+	+
* <i>H. aegyptius</i> is indistinguishable from <i>H. influenzae</i> biotype III in normal laboratory tests. Adapted from MacFaddin ¹			

Appendix: X and V factor test

**Note:**

X and V factor
V factor only
X factor only

Haemophilus influenzae NCTC 11931 or NCTC12975
Haemophilus parainfluenzae NCTC 10665
Haemophilus haemoglobinophilus NCTC 8540

The flowchart is for guidance only.

References

Modified GRADE table used by UK SMLs when assessing references

Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) is a systematic approach to assessing references. A modified GRADE method is used in UK SMLs for appraising references for inclusion. Each reference is assessed and allocated a grade for strength of recommendation (A-D) and quality of the underlying evidence (I-VIII). A summary table which defines the grade is listed below and should be used in conjunction with the reference list.

Quality/certainty of evidence	Types of evidence
A Strongly recommended	I Evidence from randomised controlled trials, meta-analysis and systematic reviews
B* Recommended but other alternatives may be acceptable	II Evidence from non-randomised studies
	III Evidence from documents describing techniques, methods or protocols
C* Weakly recommended: seek alternatives	IV Non-analytical studies, eg case reports, reviews, case series
D Never recommended	V Expert opinion and wide acceptance as good practice but with no study evidence
	VI Required by legislation, code of practice or national standard/ guideline
	VII Letter /short communication /editorials /conference communication
	VIII Electronic citation

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