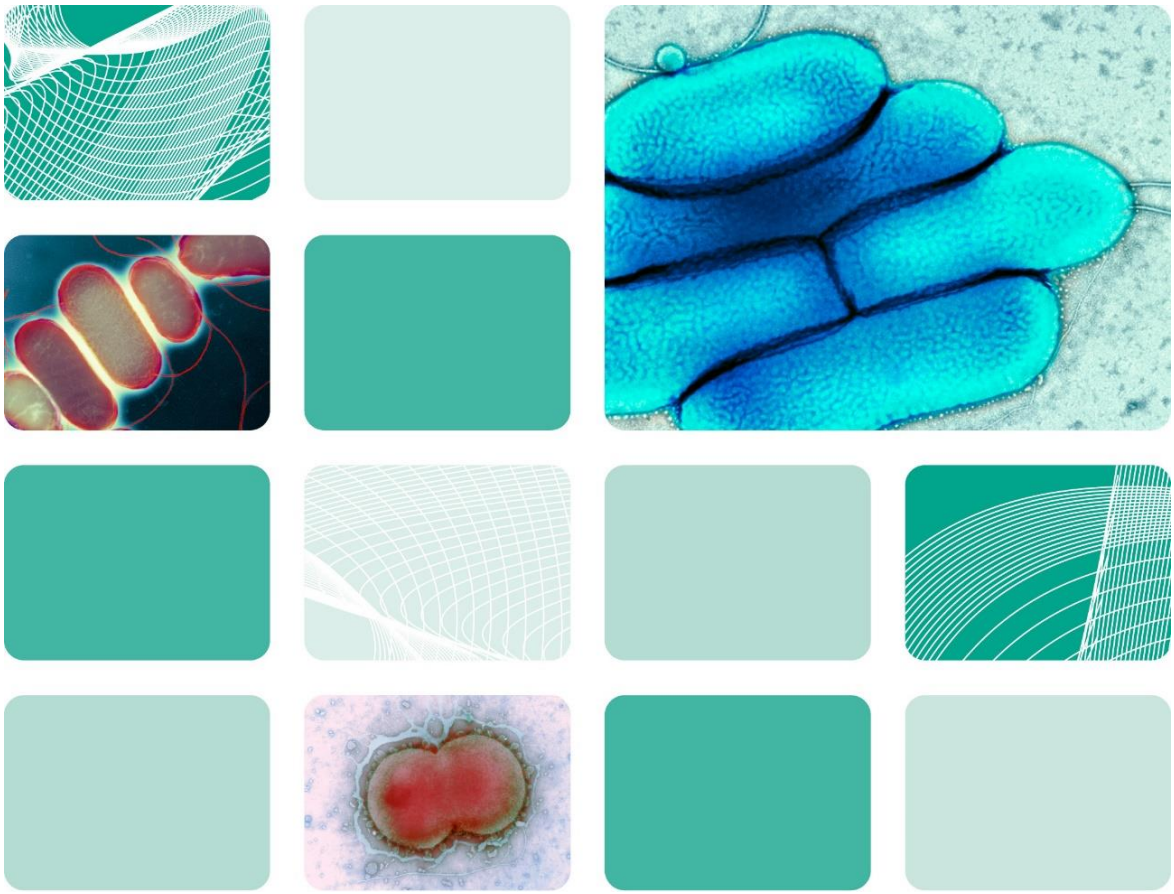
UK Health Security Agency

UK Standards for Microbiology Investigations

X and V factor test



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Test Procedures | TP 38 | Issue number: 4.1 | Issue date: 16.10.24 | Page: 1 of 12

Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of UKHSA working in partnership with the partner organisations whose logos are displayed below and listed on the UK SMI website. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a 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.

UK SMIs are produced in association with:

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Contents

Amendment table

Each UK SMI document has an individual record of amendments. The amendments are listed on this page. The amendment history is available from standards@ukhsa.gov.uk.

Any alterations to this document should be controlled in accordance with the local document control process.

Amendment number/date	10/16.10.24
Issue number discarded	4
Insert issue number	4.1
Section(s) involved	**Amendment**
Whole document.	Document transferred to new template. Minor changes to text for clarity. Table of results has moved to section 10
Technical information/limitations	Correction made for X and V factor test to be performed using a minimal nutrient agar, such as basic nutrient agar

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 5 years where appropriate

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 Standards for Microbiology Investigations (UK SMI) document describes the differentiation of *Haemophilus* species by the X and V test.

This procedure is not recommended to be the sole criterion for species identification because similarities exist in growth factor requirements of Haemophilus species. Test results of other biochemical tests and even molecular analysis may be required for complete identification

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

4 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 labile (1).

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.

5 Technical information/limitations

Erroneous results

V factor diffuses more readily than X factor. V factor may diffuse towards the X factor disc, leading to growth seemingly being due to X factor rather than V (1). To reduce this, place the discs at least 1cm apart from each other.

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 X and V factor test should be performed using a minimal nutrient agar, such as basic nutrient agar, but for which the X and V discs have been validated. Any trace of the X or V factors in the medium could influence the results usually identifying *H. influenzae* as *H. parainfluenzae*.

Manufacturers' instructions should be followed when performing this test.

More accurate results are obtained with the porphyrin synthesis test. Please refer to UK SMI 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 CO_2 (2).

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 factor (1).

6 Safety considerations

The section covers specific safety considerations (3-21) related to this UK SMI, and should be read in conjunction with the general safety considerations.

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 (22). The organism infects primarily by the respiratory route (inhalation), autoinoculation or ingestion in laboratory workers (23).

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.

7 Reagents and equipment

- Normal saline or distilled water
- Sterile swabs
- Test agar plate, follow manufacturers' instructions
- Commercially available discs/strips impregnated with X, V and XV factors
- Bacteriological straight wire/loop or disposable alternative

8 Quality control organisms

X and V factor

Haemophilus influenzae NCTC 11931 or NCTC 12975

V factor only

Haemophilus parainfluenzae NCTC 10665

X factor only

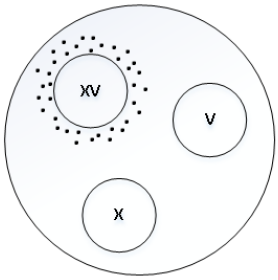
Haemophilus haemoglobinophilus NCTC 8540

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

9 Procedure and results

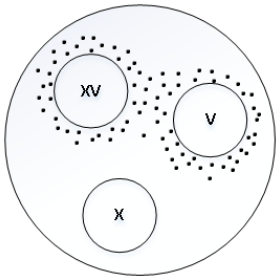
Procedure for X and V factor test method (1,2):

- 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% CO_2 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



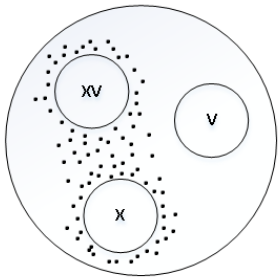
H. influenzae

(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.

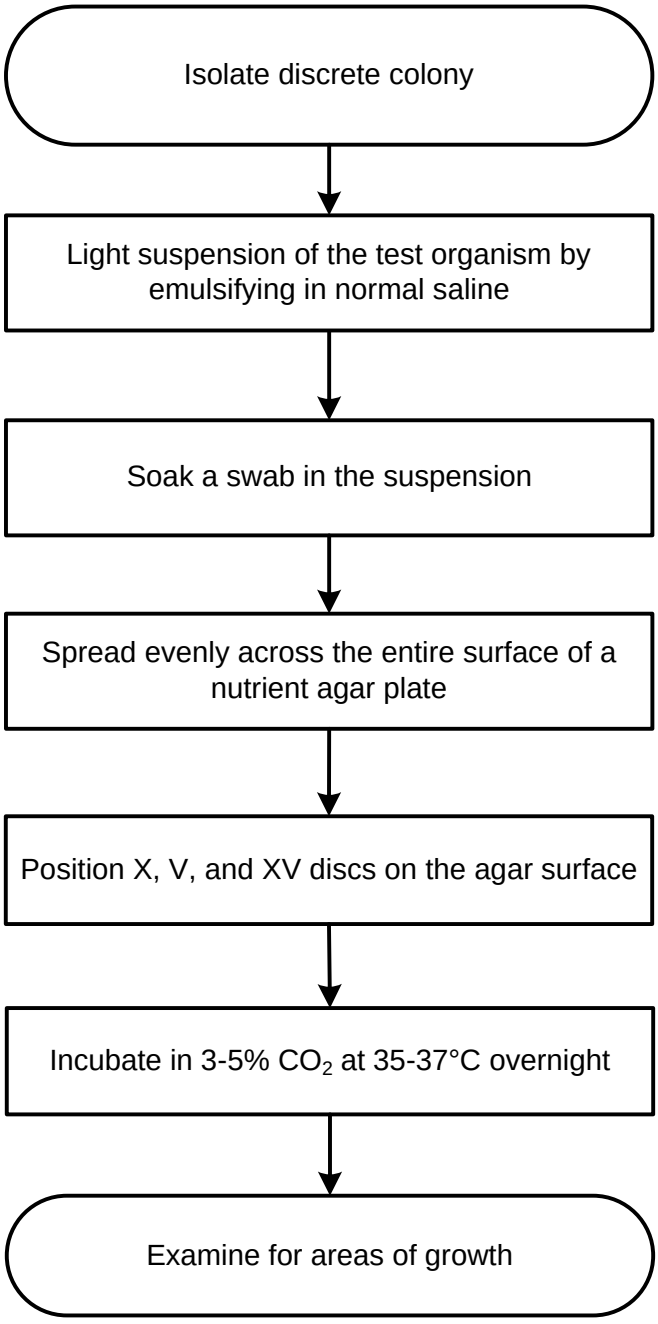
Organisms that require only V factor will grow only around the V and the XV factor discs.

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

For a summary of X, V and XV factor results, please see section 10.

10 Algorithm: X and V factor test

An accessible text description of this flowchart is provided with this document



Note:

X and V factor	*Haemophilus influenzae* NCTC 11931 or NCTC12975
V factor only	*Haemophilus parainfluenzae* NCTC 10665
X factor only	*Haemophilus haemoglobinophilus* NCTC 8540

Please note that sometimes the X and V factor tests could give false V dependent results if incubated in CO_2.

11 Table: Summary of X and V factor test results

***Haemophilus* species**	**Growth around discs**		
	X	**V**	**XV**
H. influenzae	-	-	+
H. parainfluenzae	-	+	+
H. haemoglobinophilus	+	-	+
*H. aegyptius**	-	-	+
H. haemolyticus	-	-	+
H. pittmaniae	-	+	+
H. parahaemolyticus	-	+	+
H. paraphrohaemolyticus	-	+	+
H. ducreyi	+	-	+
H. sputorum	-	+	+
**H. aegyptius* is indistinguishable from *H. influenzae* biotype III in normal laboratory tests. Adapted from MacFaddin (1)			

References

An explanation of the reference assessment used is available in the scientific information section.

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