

UK Standards for Microbiology Investigations

X and V Factor Test



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:



UK Standards for Microbiology Investigations[[1]](#footnote-1)#: Status

Users of SMIs

Three groups of users have been identified for whom SMIs are especially relevant:

* SMIs are primarily intended as a general resource for practising professionals in the field operating in the field of laboratory medicine in the UK. Specialist advice should be obtained where necessary.
* SMIs provide clinicians with information about the standard of laboratory services they should expect for the investigation of infection in their patients and the documents provide information that aids the electronic ordering of appropriate tests from hospital wards.
* SMIs also provide commissioners of healthcare services with the 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 essential laboratory methodologies which underpin quality, for example assay validation, quality assurance, and understanding uncertainty of measurement.

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 interventions, surveillance, and research and development activities. SMIs align advice on testing strategies with the UK diagnostic and public health agendas.

Involvement of Professional Organisations

The development of SMIs is undertaken within PHE in partnership with the NHS, Public Health Wales and with professional organisations.

The list of participating organisations may be found at <http://www.hpa.org.uk/SMI/Partnerships>. Inclusion of an organisation’s logo in an SMI implies support for the objectives and process of preparing SMIs. Representatives of professional organisations are members of the steering committee and working groups which develop SMIs, although the views of participants are not necessarily those of the entire organisation they represent.

SMIs are developed, reviewed and updated through a wide consultation process. The resulting documents reflect the majority view of contributors. SMIs are freely available to view at <http://www.hpa.org.uk/SMI> as controlled documents in Adobe PDF format.

Quality Assurance

The process for the development of SMIs is certified to ISO 9001:2008.

NHS Evidence has accredited the process used by PHE to produce SMIs. Accreditation is valid for three years from July 2011. The accreditation is applicable to all guidance produced since October 2009 using the processes described in PHE’s Standard Operating Procedure SW3026 (2009) version 6.

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 well referenced 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. SMIs should be used in conjunction with other SMIs.

UK microbiology laboratories that do not use SMIs should be able to demonstrate at least equivalence in their testing methodologies.

The performance of SMIs depends on well trained staff and the quality of reagents and equipment used. 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.

Whilst every care has been taken in the preparation of SMIs, PHE, its successor organisation(s) and any supporting organisation, 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 SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

SMIs are the copyright of PHE which should be acknowledged where appropriate.

Microbial taxonomy is up to date at the time of full review.

Equality and Information Governance

An Equality Impact Assessment on SMIs is available at <http://www.hpa.org.uk/SMI>.

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.

Suggested Citation for this Document:

Public Health England. (YYYY <tab+enter>) X and V Factor Test. UK Standards for Microbiology Investigations. TP 38 Issue xxx. [www.hpa.org.uk/SMI/pdf](http://www.hpa.org.uk/SMI/pdf).

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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.

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| Amendment No/Date. | 7/dd.mm.yy <tab+enter> |
| Issue no. discarded. | 2.4 |
| Insert Issue no. | xxx |
| **Section(s) involved.** | **Amendment.** |
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| Amendment No/Date. | 6/21.10.11 |
| Issue no. discarded. | 2.3 |
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| **Section(s) involved.** | **Amendment.** |
| Whole document. | Document presented in a new format. |
| References. | Some references updated. |

Scope of Document

This UK Standard for Microbiology Investigation (SMI) describes the differentiation of *Haemophilus* species by the X and V test. Because similarities exist in growth factor requirements of *Haemophilus* species, it is not recommended that this procedure be the sole criterion for species identification.

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 place 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 V.

**Commercial Identification Kits**

Commercial manufacturers of X and V discs do not specify the concentration of the factors. Acceptance of a batch of discs must 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**

Care must be taken to avoid carryover of blood from the medium when ‘picking’ colonies, which will lead to erroneous results.

No nutrient agar is entirely deficient in X factor and the disc test may be erroneous in up to 20% of cases, usually identifying *Haemophilus influenzae* as *Haemophilus parainfluenzae.*

More accurate results are obtained with the porphyrin synthesis test ([TP 29 – Porphyrin test](http://www.hpa.org.uk/SMI/pdf/Testprocedures)).

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

1 Safety Considerations2-10

*Haemophilus influenzae* is a Hazard Group 2 organism, and, and in some cases the nature of the work may dictate full Containment Level 3 conditions. All laboratories should handle specimens as if potentially high risk.

*H. influenzae* can cause serious invasive disease, especially in young children. Invasive disease is usually caused by encapsulated strains of the organism.

Vaccination against influenza is available; guidance is given in the DH Green Book11. Influenza vaccination is recommended for healthcare workers directly involved in patient care, who should be offered influenza immunisation on an annual basis.

Laboratory acquired infections have been reported12. The organism infects primarily by the respiratory route (inhalation), autoinoculation or ingestion in laboratory workers13.

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 and reagents documented in this SMI.

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

Compliance with postal and transport regulations is essential.

2 Reagents and Equipment

Discrete bacterial colonies growing on solid medium.

Normal saline or Distilled water

Sterile swabs

Test agar plate - Blood agar/Nutrient agar base as recommended by manufacturers’ instructions

Commercially available discs impregnated with X, V and XV factors.

Bacteriological straight wire/loop (preferably nichrome) or disposable alternative.

3 Quality Control Organisms

X and V factor

*Haemophilus influenzae* NCTC 11931.

V factor only

*Haemophilus parainfluenzae* NCTC 10665.

**X factor only**

*Haemophilus haemoglobinophilus*  NCTC 8540

**Note**: These strains have not been validated by NCTC to give this result.

4 Procedure and Results

4.1 X and V Factor Test Method1,14

* 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 growth around the discs and interpret the test agar plates according to the table below


*H. influenzae*  *H. parainfluenzae*

(Growth around XV disc only) (Growth around V and XV discs)

**Interpretation**

Organisms that require only X Factor will grow only in the area of the X and XV Factor discs. Organisms that require only V Factor will grow only in the areas of the V and the XV Factor discs. If both X and V Factors are required, the organism will grow only in the area of the XV Factor disc. Below is a summary of X, V and XV Factor 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 MacFaddin1 |

Appendix: X and V Factor Test Flowchart



The flowchart is for guidance only.

References

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14. Jones AM. Haemophilus influenzae and H. parainfluenzae: the influence of media and CO2 on differentiation using X, V and XV discs. Med Lab Sci 1982;39:189-91.

1. # UK Standards for Microbiology Investigations were formerly known as National Standard Methods.

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. [↑](#footnote-ref-1)