Public Health England

NHS

UK Standards for Microbiology Investigations

X and V Factor Test

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Issued by the Standards Unit, Microbiology Services, PHE

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.	7/17.03.14
Issue no. discarded.	2.4
Insert Issue no.	2.5
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.	6/21.10.11
Issue no. discarded.	2.3
Insert Issue no.	2.4
Section(s) involved	**Amendment**
Whole document.	Document presented in a new format.
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

Whilst every care has been taken in the preparation of SMIs, PHE 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.

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.

SMIs are Crown copyright which should be acknowledged where appropriate.

Suggested Citation for this Document

Public Health England. (2014). X and V Factor Test. UK Standards for Microbiology Investigations. TP 38 Issue 2.5. http://www.hpa.org.uk/SMI/pdf.

Scope of Document

This UK Standard for Microbiology Investigation (SMI) describes the differentiation of *Haemophilus* species by the X and V test.

This SMI should be used in conjunction with other 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, haemin or other iron-containing porphyrins. These are required for growth because X-dependent strains are unable to convert d-aminolaevulinic acid to protoporphyrin.

V factor comprises nicotinamide adenine dinucleotide (NAD) or nicotinamide adenine dinucleotide phosphate (NADP)[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.

Technical Information/Limitations

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.

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

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.

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 Synthesis (ALA) Test).

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 Considerations[2-18]

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

All work likely to generate aerosols must be performed 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 Reagents and Equipment

Discrete bacterial colonies growing on solid medium.

Test agar.

Blood agar/Nutrient base as recommended by manufacturer's' instructions.

Commercial X, V and XV discs.

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

3 Quality Control Organisms[19]

X and V factor

Haemophilus influenzae NCTC 11931

V factor only

Haemophilus parainfluenzae NCTC 10665

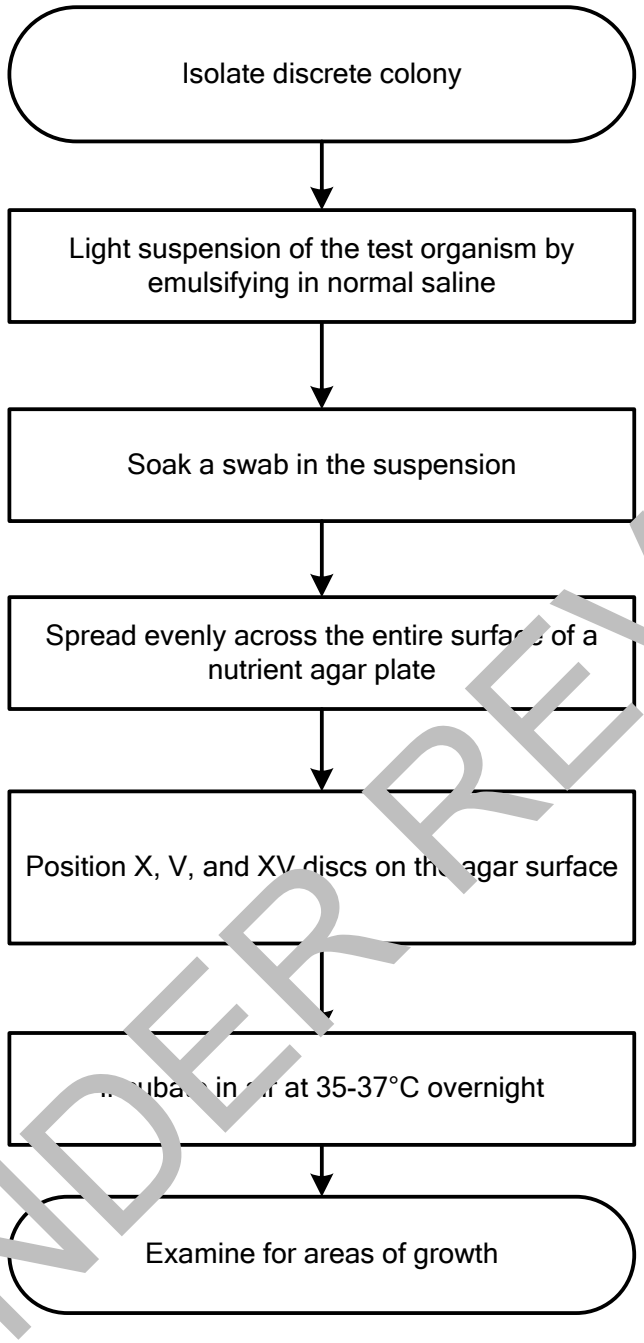
4 Procedure and Results

4.1 X and V Factor Test Method[1]

- 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 nutrient agar plate
- Position X, V and XV discs on the agar surface. Ensure the discs are a minimum of 3.5cm apart in an equilateral triangle configuration (to prevent diffusion from the discs giving false results) or follow manufacturer's instructions
- Incubate in humid air at 35-37°C overnight[20]
- Examine the plates in a good light source for growth around the discs and interpret according to the table below

Factor	*H. influenzae*	*H. parainfluenzae*
XV	+	+
X	-	-
V	-	+

Appendix: X and V Factor Test



Note:

X and V factor	Haemophilus influenzae NCTC 11931
V factor only	Haemophilus parainfluenzae NCTC 10665

This flowchart is for guidance only.

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