

NATIONAL STANDARD METHOD

# X AND V FACTOR TEST

BSOP TP 38

Issued by Standards Unit, Department for Evaluations, Standards and Training  
Centre for Infections







## X AND V FACTOR TEST

Issue no: 2.1 Issue date: 27.07.10 Issued by: Standards Unit, Department for Evaluations, Standards and Training Page no: 1 of 9  
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# STATUS OF NATIONAL STANDARD METHODS

National Standard Methods, which include standard operating procedures (SOPs), algorithms and guidance notes, promote high quality practices and help to assure the comparability of diagnostic information obtained in different laboratories. This in turn facilitates standardisation of surveillance underpinned by research, development and audit and promotes public health and patient confidence in their healthcare services. The methods are well referenced and represent a good minimum standard for clinical and public health microbiology. However, in using National Standard Methods, laboratories should take account of local requirements and may need to undertake additional investigations. The methods also provide a reference point for method development.

National Standard Methods are developed, reviewed and updated through an open and wide consultation process where the views of all participants are considered and the resulting documents reflect the majority agreement of contributors.

Representatives of several professional organisations, including those whose logos appear on the front cover, are members of the working groups which develop National Standard Methods. Inclusion of an organisation's logo on the front cover implies support for the objectives and process of preparing standard methods. The representatives participate in the development of the National Standard Methods but their views are not necessarily those of the entire organisation of which they are a member. The current list of participating organisations can be obtained by emailing [standards@hpa.org.uk](mailto:standards@hpa.org.uk).

The performance of standard methods depends on the quality of reagents, equipment, commercial and in-house test procedures. Laboratories should ensure that these have been validated and shown to be fit for purpose. Internal and external quality assurance procedures should also be in place.

Whereas every care has been taken in the preparation of this publication, the Health Protection Agency or any supporting organisation cannot be responsible for the accuracy of any statement or representation made or the consequences arising from the use of or alteration to any information contained in it. These procedures are intended solely as a general resource for practising professionals in the field, operating in the UK, and specialist advice should be obtained where necessary. If you make any changes to this publication, it must be made clear where changes have been made to the original document. The Health Protection Agency (HPA) should at all times be acknowledged.

The HPA is an independent organisation dedicated to protecting people's health. It brings together the expertise formerly in a number of official organisations. More information about the HPA can be found at [www.hpa.org.uk](http://www.hpa.org.uk).

The HPA aims to be a fully 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<sup>1</sup>.

More details can be found on the website at [www.evaluations-standards.org.uk](http://www.evaluations-standards.org.uk). Contributions to the development of the documents can be made by contacting [standards@hpa.org.uk](mailto:standards@hpa.org.uk).

The reader is informed that all taxonomy in this document was correct at time of issue.

*Please note the references are now formatted using Reference Manager software. If you alter or delete text without Reference Manager installed on your computer, the references will not be updated automatically.*

## **Suggested citation for this document:**

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# AMENDMENT PROCEDURE

<b>Controlled document reference</b>	<b>BSOP TP 38</b>
<b>Controlled document title</b>	<b>X and V Factor Test</b>

Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from [standards@hpa.org.uk](mailto:standards@hpa.org.uk).

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
3/ 27.07.10	2	2.1	6	<b>4.1</b>	Table for X and V factor test method amended.

## X AND V FACTOR TEST

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# X AND V FACTOR TEST

## SCOPE OF DOCUMENT

This National Standard Method (NSM) describes the differentiation of *Haemophilus* species by the X and V test.

## 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)<sup>2</sup>.

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 *H. influenzae* as *H. parainfluenzae*.

More accurate results are obtained with the porphyrin synthesis test ([BSOP 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 as long as the X and V factors are set up first.

### X AND V FACTOR TEST

# 1 SAFETY CONSIDERATIONS<sup>3-9</sup>

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

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 manufacturers' instructions.

Commercial X, V and XV discs.

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

# 3 QUALITY CONTROL ORGANISMS<sup>10</sup>

**X and V factor**                      *Haemophilus influenzae* NCTC 10479

**V factor only**                      *Haemophilus parainfluenzae* NCTC 10665

# 4 PROCEDURE AND RESULTS

## 4.1 X AND V FACTOR TEST METHOD<sup>2</sup>

- 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 3.5 cm 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<sup>11</sup>
- Examine the plates in a good light source for growth around the discs and interpret according to the table below

Factor	<i>H. influenzae</i>	<i>H. parainfluenzae</i>
XV	+	-
X	-	-
V	-	+

### X AND V FACTOR TEST

## 5 ACKNOWLEDGEMENTS AND CONTACTS

This National Standard Method has been developed, reviewed and revised by the National Standard Methods Working Group for Clinical Bacteriology ([http://www.hpa-standardmethods.org.uk/wg\\_bacteriology.asp](http://www.hpa-standardmethods.org.uk/wg_bacteriology.asp)). The contributions of many individuals in clinical bacteriology laboratories and specialist organisations who have provided information and comment during the development of this document, and final editing by the Medical Editor are acknowledged.

The National Standard Methods are issued by Standards Unit, Department for Evaluations, Standards and Training, Centre for Infections, Health Protection Agency, London.

For further information please contact us at:

Standards Unit  
Department for Evaluations, Standards and Training  
Centre for Infections  
Health Protection Agency  
Colindale  
London  
NW9 5EQ

E-mail: [standards@hpa.org.uk](mailto:standards@hpa.org.uk)

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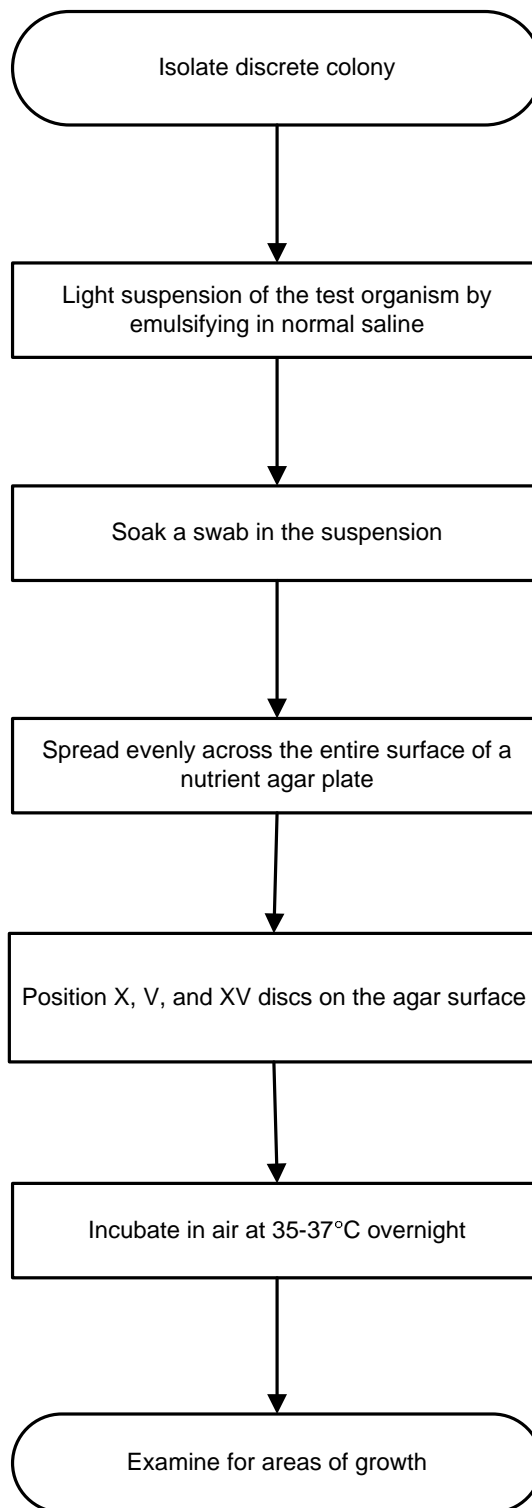
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# APPENDIX



## X AND V FACTOR TEST

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