

NATIONAL STANDARD METHOD

ISOLATION OF VIRUSES ASSOCIATED WITH INFECTIONS OF THE EYE: KERATOCONJUNCTIVITIS

VSOP 21

Issued by Standards Unit, Evaluations and Standards Laboratory
Centre for Infections



UK Clinical Virology Network

ISOLATION OF VIRUSES ASSOCIATED WITH INFECTIONS OF THE EYE: KERATOCONJUNCTIVITIS

Issue no: 3 Issue date: 11.06.10 Issued by: Standards Unit, Department of Evaluations, Standards and Training Page no: 1 of 12
VSOP 21i3

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

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.

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.

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 conditions¹.

More details can be found on the website at www.evaluations-standards.org.uk. Contributions to the development of the documents can be made by contacting 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:

Health Protection Agency (2010). *Isolation of viruses associated with infections of the eye: keratoconjunctivitis*. National Standard Method VSOP 21 Issue 3. http://www.hpa-standardmethods.org.uk/pdf_sops.asp.

ISOLATION OF VIRUSES ASSOCIATED WITH INFECTIONS OF THE EYE: KERATOCONJUNCTIVITIS

Issue no: 3 Issue date: 11.06.10 Issued by: Standards Unit, Department of Evaluations, Standards and Training Page no: 2 of 12
VSOP 21i3

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

INDEX

STATUS OF NATIONAL STANDARD METHODS.....	2
INDEX	3
AMENDMENT PROCEDURE	4
SCOPE OF DOCUMENT	5
INTRODUCTION.....	5
1 SAFETY CONSIDERATIONS.....	6
1.1 SPECIMEN COLLECTION.....	6
1.2 SPECIMEN TRANSPORT AND STORAGE	6
1.3 SPECIMEN PROCESSING.....	6
2 SPECIMEN COLLECTION.....	6
2.1 OPTIMAL TIME OF SPECIMEN COLLECTION	6
2.2 CORRECT SPECIMEN TYPE AND METHOD OF COLLECTION	6
2.3 ADEQUATE QUANTITY AND APPROPRIATE NUMBER OF SPECIMENS	6
3 SPECIMEN TRANSPORT AND STORAGE.....	6
3.1 TIME BETWEEN SPECIMEN COLLECTION AND PROCESSING.....	6
3.2 SPECIAL CONSIDERATIONS TO MINIMISE DETERIORATION	6
4 EQUIPMENT AND REAGENTS	7
4.1 EQUIPMENT.....	7
4.2 REAGENTS.....	7
5 SPECIMEN PROCESSING / PROCEDURE.....	7
5.1 TEST SELECTION	7
5.2 CULTURE AND INVESTIGATION	7
5.3 IDENTIFICATION	8
6 QUALITY ASSURANCE	8
7 LIMITATIONS.....	9
8 REPORTING PROCEDURE	9
8.1 REPORTS.....	9
9 NOTIFICATION TO THE HPA	9
10 ACKNOWLEDGMENT AND CONTACTS.....	10
REFERENCES.....	11

ISOLATION OF VIRUSES ASSOCIATED WITH INFECTIONS OF THE EYE: KERATOCONJUNCTIVITIS

Issue no: 3 Issue date: 11.06.10 Issued by: Standards Unit, Department of Evaluations, Standards and Training Page no: 3 of 12
VSOP 21i3

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

AMENDMENT PROCEDURE

Controlled document reference	VSOP 21
Controlled document title	Isolation of Viruses associated with Infections of The Eye: Keratoconjunctivitis

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.

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/ 11/06/10	2	3	All 9	All 9 Notification to the HPA	Section renamed and reference inserted Document and references reviewed

ISOLATION OF VIRUSES ASSOCIATED WITH INFECTIONS OF THE EYE: KERATOCONJUNCTIVITIS

Issue no: 3 Issue date: 11.06.10 Issued by: Standards Unit, Department of Evaluations, Standards and Training Page no: 4 of 12
VSOP 21i3

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

ISOLATION OF VIRUSES ASSOCIATED WITH INFECTIONS OF THE EYE: KERATOCONJUNCTIVITIS

Types of specimens:Conjunctival swabs
Corneal swabs
Corneal scrape

SCOPE OF DOCUMENT

This National Standard Method (NSM) describes the detection and isolation of viruses in material from the conjunctiva and cornea of the eye. Detection of viruses within the eye (eg CMV and VZV retinitis) is not described in this NSM. For more detailed information on cell culture refer to [VSOP 39 – Procedure for the propagation of cell cultures for virus isolation](#).

Parasites and bacteria, including *Chlamydia trachomatis*, are dealt with in other NSMs.

INTRODUCTION

The most common viral infections of the external surfaces of the eye and conjunctiva are adenoviruses and herpes simplex virus type 1. Occasionally varicella zoster virus may infect the eye, usually as a consequence of shingles affecting the facial dermatome covering the eye and scalp that may lead to visual impairment. The clinical presentation of varicella zoster infection is usually obvious. Molluscum contagiosum lesions around the eye can also be associated with conjunctivitis and is usually a clinical diagnosis.

Adenoviruses cause a range of clinical ocular disease. Most strains isolated are serotypes 3 and 4. Outbreaks of potentially more serious infection may be caused by adenovirus type 8, 19 and 37². Community acquired infection with adenovirus is common and adenovirus also causes cross-infection in eye departments usually due to inadequate sterilisation of equipment or the multiple patient use of eye drops. Where laboratories are able to type strains of adenovirus there is a much better ability to detect cross infection problems.

HSV infection initially presents as a superficial dendritic ulcer of the corneal epithelium. However recurrent HSV episodes may cause permanent damage as deeper layers of the corneal stroma are involved. Ulceration and corneal scarring may lead to sight impairment. HSV infection of the eye is almost always due to HSV type 1.

Conjunctivitis is a feature of measles in the prodromal phase before the rash appears, in association with upper respiratory symptoms and fever. Conjunctivitis may also occur in rubella infection.

Haemorrhagic conjunctivitis due to infection with Enterovirus type 70 or Coxsackie A24 has been reported chiefly in Asia and Africa. To date these have not caused outbreaks in the United Kingdom.

Influenza A can cause conjunctivitis. This is a particular feature of avian H7N7 influenza affecting humans, so multiple cases of conjunctivitis among those working with poultry should raise the suspicion of avian influenza. Another avian disease, Newcastle disease, can also cause conjunctivitis occasionally in humans.

Although treatment of viral infections is often non-specific, diagnosis assists the control of inappropriate treatment that could lead to more serious clinical sequelae, eg the application of steroids during infection with HSV allows the virus to multiply more rapidly. The prompt use of aciclovir has been demonstrated to reduce HSV recurrence.

ISOLATION OF VIRUSES ASSOCIATED WITH INFECTIONS OF THE EYE: KERATOCONJUNCTIVITIS

Issue no: 3 Issue date: 11.06.10 Issued by: Standards Unit, Department of Evaluations, Standards and Training Page no: 5 of 12
VSOP 21i3

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

1 SAFETY CONSIDERATIONS³⁻¹²

1.1 SPECIMEN COLLECTION

Appropriate hazard labelling according to local policy. Duplicate specimens may be required for the exclusion of other microbial pathogens.

1.2 SPECIMEN TRANSPORT AND STORAGE

Compliance with current postal and transportation regulations is essential.

A suitable virus transport system must be used and the specimen placed in a sealed plastic bag.

1.3 SPECIMEN PROCESSING

- Viruses associated with infections of the eye are in Hazard Group 2; refer to current guidance on the safe handling of Hazard Group 2 organisms
- Laboratory procedures that may give rise to infectious aerosols, eg vortexing swabs, must be conducted in a microbiological safety cabinet and the operator should wear gloves. Chance contact of infected gloved hand with the operator's eye must be avoided as laboratory acquired infection would be a likely outcome
- Safety considerations also need to be assessed in the type and handling of the cell lines used in this method. Some cells are from foetal material eg HEK, MRC-5, others comprise of human transformed cells eg HEp2, Graham 293 cells and A549^{13,14}

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

2 SPECIMEN COLLECTION

2.1 OPTIMAL TIME OF SPECIMEN COLLECTION

N/A

2.2 CORRECT SPECIMEN TYPE AND METHOD OF COLLECTION

Specimens should be placed into Virus Transport Medium (VTM) immediately after collection. Samples collected after the application of fluorescent dye to the patient's eye do not appear to affect the isolation of virus by cell culture.

2.3 ADEQUATE QUANTITY AND APPROPRIATE NUMBER OF SPECIMENS

N/A

3 SPECIMEN TRANSPORT AND STORAGE

3.1 TIME BETWEEN SPECIMEN COLLECTION AND PROCESSING

Specimens should be transported to the laboratory and processed as soon as possible.

3.2 SPECIAL CONSIDERATIONS TO MINIMISE DETERIORATION

Specimens that may be delayed should be refrigerated prior to transportation to the laboratory

ISOLATION OF VIRUSES ASSOCIATED WITH INFECTIONS OF THE EYE: KERATOCONJUNCTIVITIS

4 EQUIPMENT AND REAGENTS

4.1 EQUIPMENT

N/A

4.2 REAGENTS

N/A

5 SPECIMEN PROCESSING / PROCEDURE

5.1 TEST SELECTION

Conventional virus culture and examination of cytopathic effect may be used both for adenoviruses and HSV. However an alternative method for adenovirus detection is the use of a shell vial culture (see section 4.3) system although it may be less sensitive than conventional culture¹⁵. Detection of HSV and adenovirus from eye material using direct immunofluorescence or EIA techniques are sub optimal. These viruses usually require amplification in culture prior to performing these techniques. Molecular methods of detection are also available but are not described in this document¹⁶.

5.2 CULTURE AND INVESTIGATION

5.2.1 CONVENTIONAL CULTURE METHOD

Specimen processing

The swab should be agitated to release maximum material into the virus transport medium. This should be carried out within a microbiological safety cabinet.

Choice of cell culture

Different cells selected have to be susceptible to infection with HSV and adenovirus. It is therefore recommended that two tubes of different cell types should be chosen or if this is not possible two tubes of the same cell line. MRC-5 or VERO cells are susceptible to infection with HSV culture and MRC-5, HEK, Graham 293, A549, PLC, HEp2 or HeLa cells are susceptible to infection with adenovirus.

Isolation

Inoculate 0.2 mL of vortexed VTM containing clinical material into each of two cell culture tubes containing the selected lines. The cells should be incubated at 35°C – 37°C, with or without rolling, for at least ten days. Some strains of adenovirus may need longer incubation by this method. Cell cultures should be examined at 24 hours and 48 hours, then every other day for the appearance of cytopathic changes characteristic of HSV or adenovirus.

Identification

Confirm cytopathic effect using direct or indirect immunofluorescence using group-specific monoclonal antibodies. Sero-typing may be performed using type-specific monoclonal antibodies. Sero-typing of adenovirus isolates may also be achieved using a viral neutralisation technique.

5.2.2 SHELL VIAL CULTURE

Prepare shell vial monolayers of a cell type listed for adenoviruses as in section 5.2.1. Ideally cells should be about 80% confluent when used.

Select a shell vial, label with specimen number and date.

ISOLATION OF VIRUSES ASSOCIATED WITH INFECTIONS OF THE EYE: KERATOCONJUNCTIVITIS

Issue no: 3 Issue date: 11.06.10 Issued by: Standards Unit, Department of Evaluations, Standards and Training Page no: 7 of 12
VSOP 21i3

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

Decant medium from shell vial, into 2% hypochlorite solution.
Vortex specimen and inoculate vial with 0.5 mL of specimen.

Centrifuge shell vial at 2500 g for 1 hour at 30°C.

Following centrifugation, add 1 mL maintenance medium.

Incubate vial at 37°C in a CO₂ incubator for 3 days.

Fix and stain for adenovirus (see section 5.2.3).

5.2.3 SHELL VIAL CULTURE – ADENOVIRUS IMMUNOFLUORESCENCE

Fixation

Decant medium from shell vial into fresh hypochlorite solution.

Add 1 mL 0.1 M phosphate buffered saline (PBS) pH 7.2 to the shell vial, swirl gently and decant into hypochlorite solution.

Add 1 mL fixative (50:50 acetone:methanol), swirl and decant into hypochlorite solution.

Add 1 mL fresh fixative and leave for 10 minutes.

Decant fixative as above.

Remove coverslip from vial and allow to dry.

Staining

Pipette 8 µL fluorescein conjugated monoclonal adenovirus antibody onto a clean labelled slide.

Place coverslip cell-side down onto reagent.

Place slide in a moist-box at 37°C for 30 minutes.

Wash coverslip in PBS for 5 minutes.

Rinse in distilled water, dry and mount, using a suitable mountant, cell-side down on a clean labelled slide. The mountant used must not auto-fluoresce and should preserve the fluorescence for the required storage time.

Examine under UV using a x25 objective.

Results

Positive cells occur singly, occasionally in small groups, and exhibit a bright apple-green nuclear or whole cell fluorescence. Extremely strongly positive specimens may produce a dull, diffuse fluorescence over the whole cell sheet which may be missed by the unwary and reported as negative.

Negative cells, if Evans Blue counter-stain is used, will appear as a dull red colour.

5.3 IDENTIFICATION

N/A

6 QUALITY ASSURANCE

A quality system should be in place to ensure that appropriate internal and external quality assessment and quality control procedures are maintained¹⁷.

ISOLATION OF VIRUSES ASSOCIATED WITH INFECTIONS OF THE EYE: KERATOCONJUNCTIVITIS

Issue no: 3 Issue date: 11.06.10 Issued by: Standards Unit, Department of Evaluations, Standards and Training Page no: 8 of 12
VSOP 21i3

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

It is essential that laboratories have evidence of adequate validation of methods, equipment and commercial and in-house test procedures demonstrating that they are fit for purpose¹⁸. Likewise it is important that the cell lines in use are shown to be susceptible to the viruses being looked for.

7 LIMITATIONS

Successful isolation of organisms depends on correct specimen collection, transport, storage and processing, the quality and range of cell lines used and the use of correct conditions for culture and the provision of adequate/suitable clinical information.

The procedure(s) in these documents aim to describe good microbiological standard methods for the specimen types specified. Other procedures may be required and professional interpretation by qualified staff is essential. Please note that knowledge of infectious diseases changes constantly and although this NSM is regularly reviewed it may not include emerging pathogens.

8 REPORTING PROCEDURE

8.1 REPORTS

Negative specimens should be reported as:

“Virus not isolated”. Negative shell vial results may be held back until the conventional tube culture result is available.

Positive specimens should be reported as one of the following:

“Herpes simplex virus isolated”

“Herpes simplex virus type 1 isolated”

“Adenovirus isolated”

“Adenovirus type xx isolated”

9 NOTIFICATION TO THE HPA^{19,20}

Any positive results from ocular sites should be reported to HPA or equivalent in accordance with published guidelines.

10 ACKNOWLEDGMENT AND CONTACTS

This National Standard Method was initiated and developed by the National Standard Methods Working Group for Clinical Virology (http://www.hpa-standardmethods.org.uk/wg_virology.asp). The contributions of many individuals in clinical virology 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, Health Protection Agency, London.

For further information please contact us at:

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

E-mail: standards@hpa.org.uk

ISOLATION OF VIRUSES ASSOCIATED WITH INFECTIONS OF THE EYE: KERATOCONJUNCTIVITIS

Issue no: 3 Issue date: 11.06.10 Issued by: Standards Unit, Department of Evaluations, Standards and Training Page no: 10 of 12
VSOP 21i3

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

REFERENCES

1. Department of Health NHS Executive: The Caldicott Committee. Report on the review of patient-identifiable information. London. December 1997.
2. Lenaerts L, De Clercq E, Naesens L. Clinical features and treatment of adenovirus infections. *Rev Med Virol* 2008;18:357-74.
3. Advisory Committee on Dangerous Pathogens. 2004 Approved List of Biological Agents. <http://www.hse.gov.uk/pubns/misc208.pdf>. p. 1-17.
4. Public Health Laboratory Service Standing Advisory Committee on Laboratory Safety. Safety Precautions: Notes for Guidance. 4th ed. London: Public Health Laboratory Service (PHLS); 1993.
5. Control of Substances Hazardous to Health Regulations 2002. General COSHH. Approved Code of Practice and Guidance, L5. Suffolk: HSE Books; 2002.
6. Health and Safety Executive. 5 steps to risk assessment: a step by step guide to a safer and healthier workplace, IND (G) 163 (REVL). Suffolk: HSE Books; 2002.
7. Health and Safety Executive. A guide to risk assessment requirements: common provisions in health and safety law, IND (G) 218 (L). Suffolk: HSE Books; 2002.
8. Health Services Advisory Committee. Safety in Health Service laboratories. Safe working and the prevention of infection in clinical laboratories and similar facilities. 2nd ed. Suffolk: HSE Books; 2003.
9. NHS Estates. Health Building Note 15. Facilities for pathology services. 2nd ed. London: Her Majesty's Stationary Office (HMSO); 2005.
10. BS EN 12469: 2000. Biotechnology - performance criteria for microbiological safety cabinets. London: British Standards Institution (BSI); 2000.
11. BS 5726: 1992. Microbiological safety cabinets. Part 2. Recommendations for information to be exchanged between purchaser, vendor and installer and recommendations for installation. London: British Standards Institution (BSI); 1992.
12. BS 5726: 1992. Microbiological safety cabinets. Part 4. Recommendations for selection, use and maintenance. London: British Standards Institution (BSI); 1992.
13. Yirrell DL, Roome AP, Darville JM, Ashley CR, Harbour J. Comparison of the continuous cell line 293 with human embryo kidney cells and human embryo fibroblast cells for the cultivation of ocular viruses. *J Clin Pathol* 1983;36:996-9.
14. Leonardi GP, Costello P, Harris P. Use of continuous human lung cell culture for adenovirus isolation. *Intervirology* 1995;38:352-5.
15. Kowalski RP, Karenchak LM, Romanowski EG, Gordon YJ. Evaluation of the shell vial technique for detection of ocular adenovirus. *Community Ophthalmologists of Pittsburgh, Pennsylvania. Ophthalmology* 1999;106:1324-7.
16. Kinchington PR, Turse SE, Kowalski RP, Gordon YJ. Use of polymerase chain amplification reaction for the detection of adenoviruses in ocular swab specimens. *Invest Ophthalmol Vis Sci* 1994;35:4126-34.

ISOLATION OF VIRUSES ASSOCIATED WITH INFECTIONS OF THE EYE: KERATOCONJUNCTIVITIS

Issue no: 3 Issue date: 11.06.10 Issued by: Standards Unit, Department of Evaluations, Standards and Training Page no: 11 of 12
VSOP 21i3

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

17. Curry A, Ashley CR. Quality assurance in electron microscopy. In: Snell JJS, Brown DFJ, Roberts C, editors. Quality Assurance Principles and Practice in the Microbiology Laboratory. London: Public Health Laboratory Service; 1999. p. 221-30.
18. Clinical Pathology Accreditation (UK) Ltd. Standards for the Medical Laboratory. Sheffield 2004. p. 1-56
19. Health Protection Agency. Laboratory Reporting to the Health Protection Agency: Guide for Diagnostic Laboratories. 2008.
20. Department of Health. Health Protection Legislation (England) Guidance 2010. http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_114510. p. 1-112.

ISOLATION OF VIRUSES ASSOCIATED WITH INFECTIONS OF THE EYE: KERATOCONJUNCTIVITIS

Issue no: 3 Issue date: 11.06.10 Issued by: Standards Unit, Department of Evaluations, Standards and Training Page no: 12 of 12
VSOP 21i3

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk