

NATIONAL STANDARD METHOD

*UNDER REVIEW*

# 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

*Association of Medical Microbiologists*  
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ISOLATION OF VIRUSES ASSOCIATED WITH INFECTIONS OF THE EYE: KERATOCONJUNCTIVITIS

Issue no: 2 Issue date: 09.02.07 Issued by: Standards Unit, Evaluations and Standards Laboratory Page no: 1 of 12  
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## **Suggested citation for this document:**

Health Protection Agency (2007). *Isolation of viruses associated with infections of the eye: keratoconjunctivitis*. National Standard Method VSOP 21 Issue 2. [http://www.hpa-standardmethods.org.uk/pdf\\_sops.asp](http://www.hpa-standardmethods.org.uk/pdf_sops.asp).

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# AMENDMENT PROCEDURE

<b>Controlled document reference</b>	<b>VSOP 21</b>
<b>Controlled document title</b>	<b>Isolation of viruses associated with infections of the eye: keratoconjunctivitis</b>

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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
2/ 09/02/07	1.1	2	1	<b>Front Page</b>	Title amended
			5	<b>Title</b>	Title amended
			5	<b>Introduction</b>	Influenza and Measles added

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# 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<sup>2</sup>.

## 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 37. 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.

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# 1 SAFETY CONSIDERATIONS<sup>3-12</sup>

## 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<sup>13,14</sup>

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

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## 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<sup>15</sup>. 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 culture<sup>16,17</sup>. 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 document<sup>18,19</sup>.

### 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<sup>20</sup>.

#### 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.

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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 CO<sub>2</sub> 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

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## 6 QUALITY ASSURANCE

A quality system should be in place to ensure that appropriate internal and external quality assessment and quality control procedures are maintained<sup>21,22</sup>.

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 purpose<sup>23</sup>. 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 REPORTING TO THE HPA (LOCAL AND REGIONAL SERVICES AND CDSC CENTRE FOR INFECTIONS)

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

## 10 ACKNOWLEDGMENT AND CONTACTS

This National Standard Method was initiated and developed by the Virology Working Group on Standards and Quality ([http://www.hpa-standardmethods.org.uk/wg\\_virology.asp](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, Evaluations and Standards Laboratory, Centre for Infections, Health Protection Agency London.

For further information please contact us at:

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