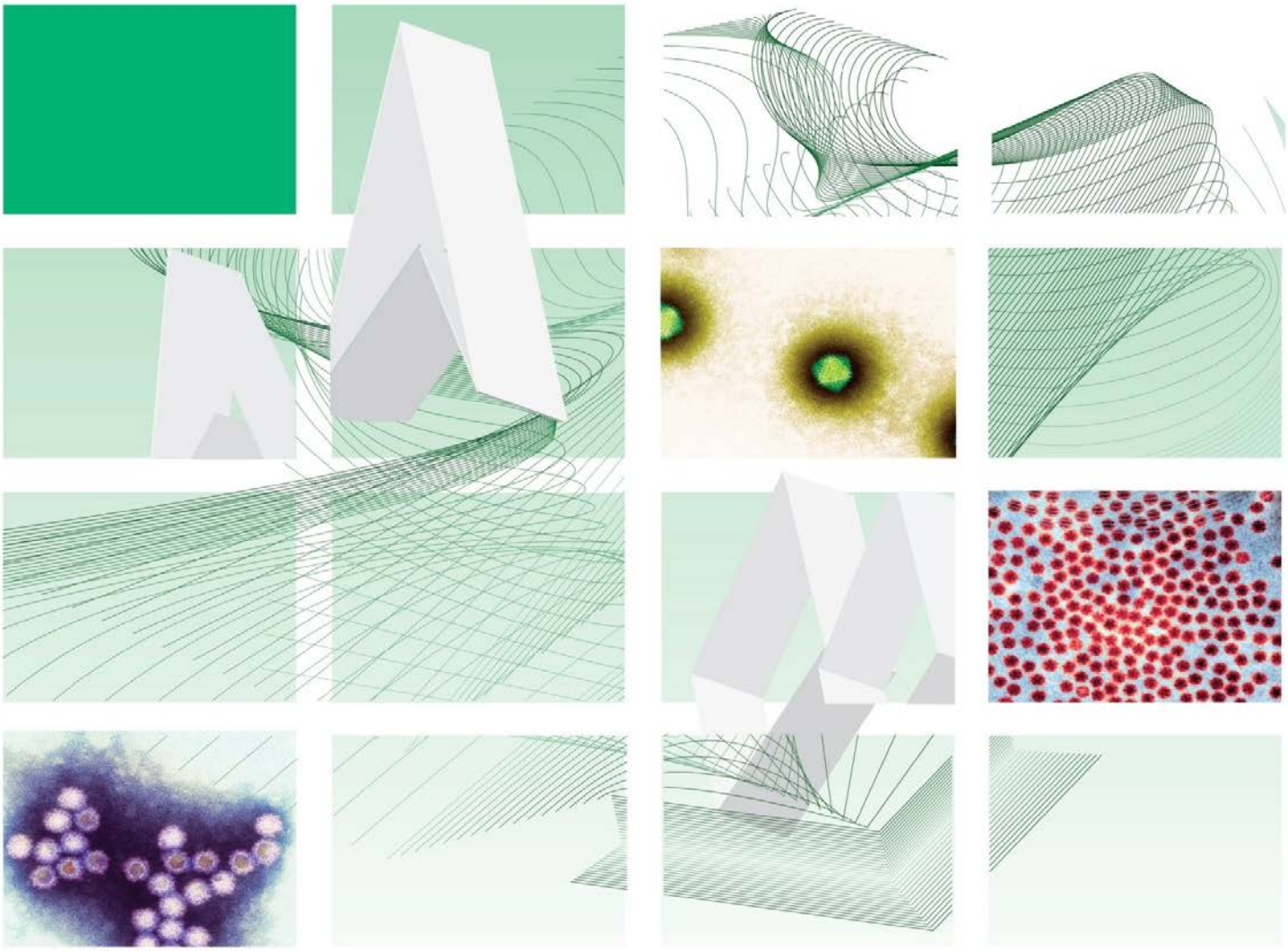
Public Health England

NHS

UK Standards for Microbiology Investigations

Isolation of Viruses Associated with Infections of the Eye: Keratoconjunctivitis



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Virology | V 21 | Issue no: 3.2 | Issue date: 09.10.13 | Page: 1 of 16

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.	5/09.10.13
Issue no. discarded.	3.1
Insert Issue no.	3.2
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 references have been reviewed and updated. Scientific content remains unchanged.

Amendment No/Date.	4/02.11.11
Issue no. discarded.	3
Insert Issue no.	3.1
Section(s) involved	**Amendment**
Whole document.	Document presented in a new format. Standard heading for Technical Information added.
Section 4.	Heading for 'equipment and reagents' removed.
Section 4.	Added.
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.

[#]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.

SMIs are developed, reviewed and updated through a wide consultation process.

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.

Suggested Citation for this Document

Public Health England. (2013). Isolation of Viruses Associated with Infections of the Eye: Keratoconjunctivitis. UK Standards for Microbiology Investigations. V 21 Issue 3.2. http://www.hpa.org.uk/SMI/pdf.

Scope of Document

The SMI 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 SMI. For more detailed information on cell culture refer to V 39 – Procedure for the Propagation of Cell Cultures for Virus Isolation.

This SMI should be used in conjunction with other SMIs.

Introduction

The most common viral infections of the external surfaces of the eye and conjunctiva are adenoviruses and herpes simplex virus type1. 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[1]. 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.

Technical Information/Limitations

N/A

1 Safety Considerations[2-18]

1.1 Specimen Collection[2,3]

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[2-7]

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[2-18]

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[19,20].

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

2 Specimen Collection

2.1 Type of Specimens

Conjunctival swabs, corneal swabs, corneal scrape

2.2 Optimal Time of Specimen Collection

N/A

2.3 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.

2.4 Adequate Quantity and Appropriate Number of Specimens

N/A

3 Specimen Transport and Storage[2,3]

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.

4 Specimen Processing/Procedure[2,3]

4.1 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 4.2.2) system although it may be less sensitive than conventional culture[21]. 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[22].

4.2 Culture and Investigation

4.2.1 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 0.2mL of vortexed VTM containing clinical material into each of two cell culture tubes containing the selected lines. The cells should be incubated at 35–37°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. Serotyping may be performed using type-specific

monoclonal antibodies. Serotyping of adenovirus isolates may also be achieved using a viral neutralisation technique.

4.2.2 Shell vial culture

Prepare shell vial monolayers of a cell type listed for adenoviruses as in section 4.2.1. Ideally cells should be about 80% confluent when used.

- Select a shell vial, label with specimen number and date
- Decant medium from shell vial, into 2% hypochlorite solution
- Vortex specimen and inoculate vial with 0.5mL of specimen
- Centrifuge shell vial at 2500g for 1 hour at 30°C
- Following centrifugation, add 1mL maintenance medium
- Incubate vial at 37°C in a CO_2 incubator for 3 days
- Fix and stain for adenovirus (see section 4.2.3)

4.2.3 Shell vial culture – Adenovirus Immunofluorescence

Fixation

Decant medium from shell vial into fresh hypochlorite solution.

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

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

Add 1mL fresh fixative and leave for 10 minutes.

Decant fixative as above.

Remove coverslip from vial and allow to dry.

Staining

Pipette 8µL 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. 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.

4.3 Identification

N/A

5 Quality Assurance

A quality system should be in place to ensure that appropriate internal and external quality assessment and quality control procedures are maintained[23].

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[24]. Likewise it is important that the cell lines in use are shown to be susceptible to the viruses being looked for.

6 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 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 SMI is regularly reviewed it may not include emerging pathogens.

7 Reporting Procedure

7.1 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”.

8 Notification to PHE[25,26] or Equivalent in the Devolved Administrations[27-30]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days. For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health Protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of HIV & STIs, HCAIs and CJD under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

Other arrangements exist in Scotland[27,28], Wales[29] and Northern Ireland[30].

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