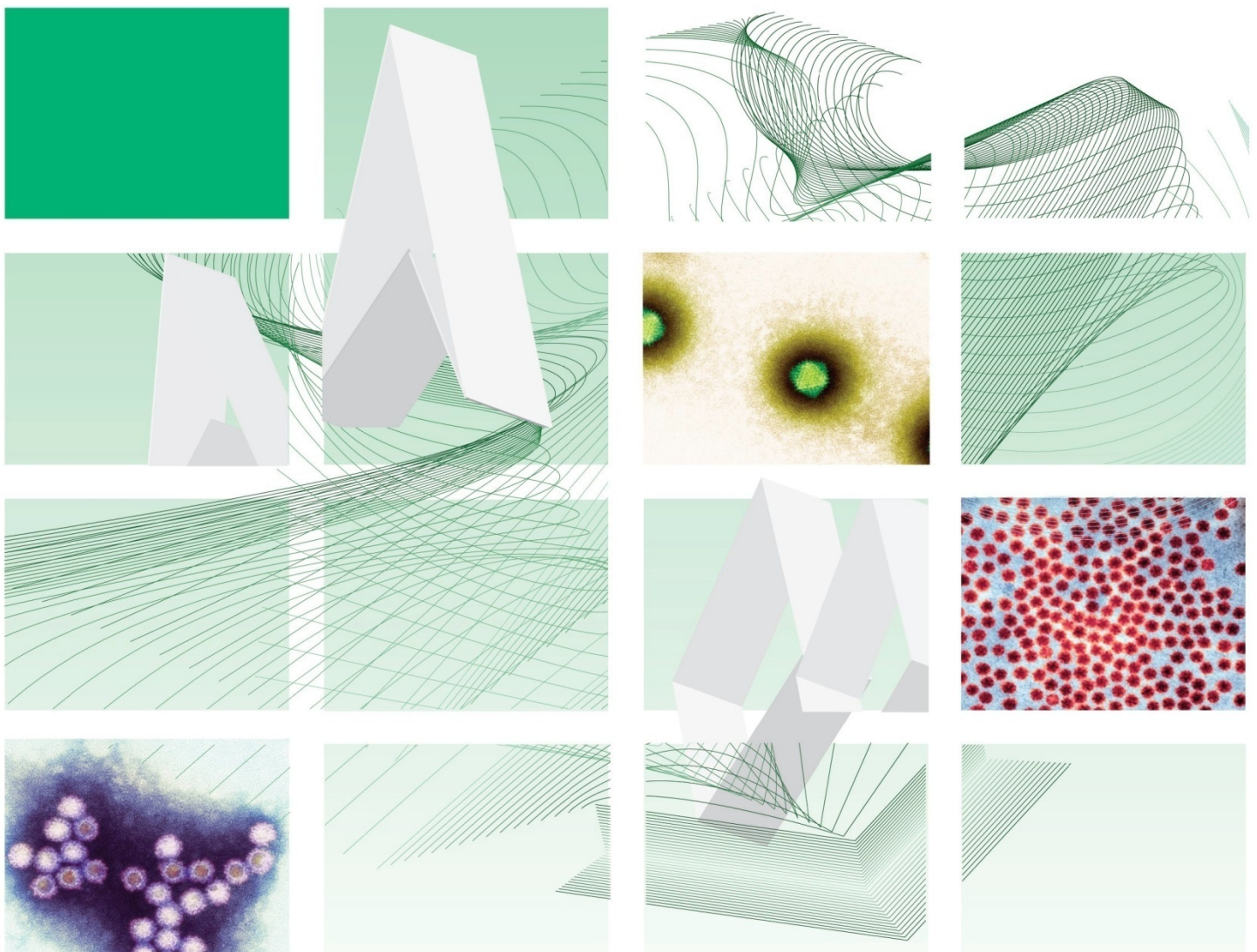




UK Standards for Microbiology Investigations

Preparation of Coated Grids for Electron Microscopy



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.

For further information please contact us at:

Standards Unit
Microbiology Services
Public Health England
61 Colindale Avenue
London NW9 5EQ

E-mail: standards@phe.gov.uk

Website: <http://www.hpa.org.uk/SMI>

UK Standards for Microbiology Investigations are produced in association with:



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NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation.

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.	11/08.04.14
Issue no. discarded.	6.2
Insert Issue no.	6.3
Section(s) involved	Amendment
Whole document.	<p>Document has been transferred to a new template to reflect the Health Protection Agency's transition to Public Health England.</p> <p>Front page has been redesigned.</p> <p>Status page has been renamed as Scope and Purpose and updated as appropriate.</p> <p>Professional body logos have been reviewed and updated.</p> <p>Standard safety and notification references have been reviewed and updated.</p> <p>Scientific content remains unchanged.</p>

Amendment No/Date.	10/19.04.12
Issue no. discarded.	6.1
Insert Issue no.	6.2
Section(s) involved	Amendment
Whole document.	Amendment to template.

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). Preparation of Coated Grids for Electron Microscopy. UK Standards for Microbiology Investigations. V 12 Issue 6.3.
<http://www.hpa.org.uk/SMI/pdf>.

Scope of Document

The SMI describes a method for the preparation of formvar support films to be used in the investigation of clinical specimens by electron microscopy. The method may be easily adapted for other support films.

This SMI should be used in conjunction with other SMIs.

Introduction

Background

Electron microscope grids require that a hydrophilic plastic support film is applied to them to allow the attachment of viral particles. Many support films have been used with success, and the user should select a suitable support film for the purpose required. Support material that has been used includes Collodion, Formvar, Pioloform and Butvar B98¹.

Coated grids may be stabilised by applying a thin coating of carbon to the support film surface. This has the effect of producing a more uniformly hydrophilic surface for virus attachment and a more robust support film. Carbon coating is essential for grids that are to be used for immune capture ([V 16 - Investigation of Clinical Specimens by Electron Microscopy using Solid Phase Immune Electron Microscopy](#)).

Many different procedures exist for preparing support material coated grids. The procedure given here is only intended as a guide. Operators should develop their own procedures based around the equipment available to them.

Technical Information/Limitations

N/A

1 Safety Considerations²⁻¹⁸

1.1 Specimen Collection^{2,3}

N/A

1.2 Specimen Transport and Storage²⁻⁷

N/A

1.3 Specimen Processing²⁻¹⁸

N/A

1.4 Chemical Handling

- Formvar powder is an irritant and should be handled within a contained environment. The powder is very light and therefore should not be handled within a microbiological safety cabinet
- Chloroform, ethylene dichloride and amyl acetate solvents are toxic by inhalation and contact. Use within a well-ventilated environment and dispose of according to local chemical disposal rules

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

2 Specimen Collection

2.1 Type of Specimens

N/A

2.2 Optimal Time of Specimen Collection

N/A

2.3 Correct Specimen Type and Method of Collection

N/A

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

N/A

3.2 Special Considerations to Minimise Deterioration

N/A

4 Equipment and Reagents

4.1 Equipment

- Glass microscope slides
- Coplin jar
- Fine-point forceps (curved points are easier to use than straight points)
- Strips of clean filter paper about 2.5 x 5cm
- Fibre-free tissue (eg Velin tissue)
- Wide container for holding water
- Vacuum-coating unit
- Electron microscope grids

4.2 Reagents

- Formvar powder (polyvinyl formol) or an alternative
- Chloroform, amyl acetate or ethylene dichloride
- Carbon evaporation source

5 Specimen Processing/Procedure^{2,3}

5.1 Test Selection

N/A

5.2 Culture and Investigation

N/A

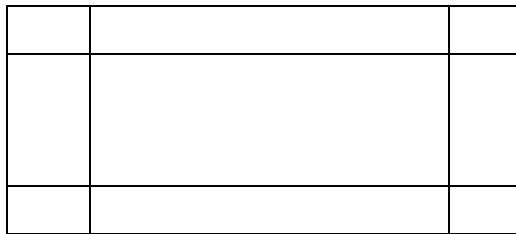
5.3 Grid Preparation

Preparation of Formvar coated grids

An apparatus is available for 'casting' support films onto electron microscope grids. This may be obtained from specialist electron microscopy accessory suppliers. The method given below describes a manual method using equipment that is readily available in a laboratory.

- Dissolve the support material in solvent to make a final concentration of between 0.2–0.5%. It is imperative that the formvar powder is completely dissolved. It is good practice to use the formvar solution within seven days of preparation, as the quality of the formvar films produced degrades with the age of the solution. The solution should be discarded after this time. Concentration depends on use and can be varied. Alternatively a stock solution of 2% formvar can be made and stored for a long period of time (preferably in a dessicator). This avoids the need to work with powdered formvar on each occasion. The stock remains stable for several months. Dilute the stock in selected solvent as required

- Decant the solution into a Coplin jar and cover with the lid
- Fill a clean basin with distilled water, or clean tap water, and 'dust' the surface of the water by drawing a piece of fibre-free tissue across the water
- Clean a glass microscope slide with a fibre-free tissue. The slide should be clean but not polished. About six wipes on each side of the glass will usually suffice
- Place the cleaned microscope slide into the formvar solution and leave it there for a few seconds
- Withdraw the slide from the solution with a steady movement. The speed of removal effects the final thickness of the support film
- Allow the film to completely dry, at room temperature, in a dust-free environment
- Scribe the film with the pattern shown below



- Immediately 'huff' on the scribed surface in order to produce a fine layer of condensation and introduce the slide, scribed surface up, at an angle of about 30° to the water. The formvar film should float off the slide onto the water
- Remove any damaged pieces of formvar film from the surface of the water
- Assess the thickness of the film by viewing against a light. Films that reflect silver light are of acceptable thickness, films reflecting gold to purple light are generally too thick, films reflecting grey are too thin
- Carefully apply new electron microscope grids, dull side down to the surface of the floating film, until the film is covered. Individual grids must not overlap
- Remove the patch of grids from the surface of the water by picking it up with a strip of filter paper. Press down and through the water surface, turning the filter paper over with a confident and continuous motion
- Allow the patch to dry in a dust-free environment at room temperature

Note: Readers should be aware that this is a procedure that requires practice. The initial failure rate is high.

Carbon coating of plastic coated grids (optional)

There are many different designs of vacuum coating unit available. Likewise the evaporation sources vary with each machine. Carbon evaporation sources suitable for the equipment available must be used.

- Load the coating head of the vacuum coating unit with a single strip of carbon fibre or equivalent

- Place three or four dry patches of grids into a vacuum coating unit, and evacuate the unit to a vacuum less than 10^{-2} mbar/PA
- Evaporate the carbon to produce a light grey/brown coating over the grids. The precise settings and time to achieve the desired coating is a matter of trial and error for each machine
- Remove the coated grids from the unit and label each patch
- If the coating unit has a glow-discharge, fitting it can be advantageous to glow-discharge the grids. This is not a mandatory procedure but will produce a uniformly hydrophilic surface. Glow discharge for up to 30 seconds with air. Extended times or repeated glow discharge may strip the coating, or result in a highly charged grid surface which will adsorb too much material. Glow discharge may be done before or after carbon coating
- Store the grids in a dust free environment at room temperature

5.4 Identification

N/A

6 Quality Assurance

6.1 Assessment of Preparation

Quality assessment of coated grids

Generally it is not necessary to quality assess coated grids for routine use. However, if grids are intended for specialist use, such as solid phase immune electron microscopy (SPIEM), every patch of grids must be tested for their suitability. The following is an appropriate procedure:

- Allow the new grids to stabilise for at least 48 hours in the working environment in which they will be used
- Test two non-adjacent grids from each patch using the SPIEM procedure ([V 16 - Investigation of Clinical Specimens by Electron Microscopy using Solid Phase Immune Electron Microscopy](#)) with the normal positive control. If the number of virus particles seen on either grid from each patch differs noticeably from the routine positive control then all grids from that patch must be discarded
- A suggested method to test the quality of support films for use in the floatation method ([V 13 - Investigation of Clinical Specimens by Electron Microscopy using the Floatation \(Direct\) Method](#)) or double-spin method ([V 14 - Investigation of Clinical Specimens by Electron Microscopy using Concentration by Differential Centrifugation \(Double-Spin Method\)](#)):
- Prepare a high titre suspension of adenovirus, or another stable virus, from a faecal extract in standard diluent. These can remain stable at 4°C for many months, if not years
- Establish mean counts per-unit-area of support film using 10 repeat grids from the standard suspension

- Check counts per-unit-area from each new grid batch, and reject batches that give counts of more than 2SD below the previously established mean

The results of the quality control exercise for Formvar/carbon coated grids should be recorded.

6.2 Internal and External Quality Assurance

N/A

7 Limitations

Quality of the prepared grids depends on the skill and experience of the operator. Quality may also be affected by atmospheric humidity.

8 Reporting Procedure

8.1 Reports

N/A

8.2 Reporting Time

N/A

9 Notification to PHE^{19,20} or Equivalent in the Devolved Administrations²¹⁻²⁴

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 Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAs) and Creutzfeldt–Jakob disease (CJD) under ‘Notification Duties of Registered Medical Practitioners’: it is not noted under ‘Notification Duties of Diagnostic Laboratories’.

<http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/HealthProtectionRegulations/>

Other arrangements exist in [Scotland](#)^{21,22}, [Wales](#)²³ and [Northern Ireland](#)²⁴.

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