

NATIONAL STANDARD METHOD

**PREPARATION OF COATED
GRIDS FOR ELECTRON
MICROSCOPY**

VSOP 12

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



UK Clinical Virology Network

Association of Medical Microbiologists
Association of Medical Microbiologists
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PREPARATION OF COATED GRIDS FOR ELECTRON MICROSCOPY

Issue number: 5.1 Issue date: 29.08.06 Issued by: Standards Unit, Evaluations and Standards Laboratory Page 1 of 11

Reference no: VSOP 12i5.1

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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.

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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.

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.

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PREPARATION OF COATED GRIDS FOR ELECTRON MICROSCOPY

Issue number: 5.1 Issue date: 29.08.06 Issued by: Standards Unit, Evaluations and Standards Laboratory Page 2 of 11

Reference no: VSOP 12i5.1

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

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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
1.4 CHEMICAL HANDLING.....	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 GRID PREPARATION.....	7
5.4 IDENTIFICATION.....	9
6 QUALITY ASSURANCE.....	9
6.1 ASSESSMENT OF PREPARATION.....	9
6.2 INTERNAL AND EXTERNAL QUALITY ASSURANCE.....	9
7 LIMITATIONS.....	9
8 REPORTING PROCEDURE.....	10
8.1 REPORTS.....	10
8.2 REPORTING TIME.....	10
9 REPORTING TO THE HPA (LOCAL AND REGIONAL SERVICES AND CDSC CENTRE FOR INFECTIONS).....	10
10 ACKNOWLEDGEMENTS AND CONTACTS.....	10
REFERENCES.....	11

PREPARATION OF COATED GRIDS FOR ELECTRON MICROSCOPY

Issue number: 5.1 Issue date: 29.08.06 Issued by: Standards Unit, Evaluations and Standards Laboratory Page 3 of 11

Reference no: VSOP 12i5.1

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

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AMENDMENT PROCEDURE

Controlled document reference	VSOP 12
Controlled document title	Preparation of coated grids for electron microscopy

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
7/ 29.08.06	5	5.1	5	1.3 Specimen Processing	Text transferred into new section: 1.4 Chemical handling
			7	5.2 Culture and Investigation	Text transferred into new section: 5.3 Grid Preparation.
			9	5.3 Identification	Bullet points amended and “blow” substituted with “huff” Section re-numbered as 5.4

PREPARATION OF COATED GRIDS FOR ELECTRON MICROSCOPY

Issue number: 5.1 Issue date: 29.08.06 Issued by: Standards Unit, Evaluations and Standards Laboratory Page 4 of 11

Reference no: VSOP 12i5.1

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

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PREPARATION OF COATED GRIDS FOR ELECTRON MICROSCOPY

SCOPE OF DOCUMENT

This National Standard Method (NSM) 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.

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 B98².

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 (VSOP 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.

PREPARATION OF COATED GRIDS FOR ELECTRON MICROSCOPY

Issue number: 5.1 Issue date: 29.08.06 Issued by: Standards Unit, Evaluations and Standards Laboratory Page 5 of 11

Reference no: VSOP 12i5.1

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1 SAFETY CONSIDERATIONS³⁻¹²

1.1 SPECIMEN COLLECTION

N/A

1.2 SPECIMEN TRANSPORT AND STORAGE

N/A

1.3 SPECIMEN PROCESSING

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

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

N/A

2.3 ADEQUATE QUANTITY AND APPROPRIATE NUMBER OF SPECIMENS

N/A

3 SPECIMEN TRANSPORT AND STORAGE

3.1 TIME BETWEEN SPECIMEN COLLECTION AND PROCESSING

N/A

3.2 SPECIAL CONSIDERATIONS TO MINIMISE DETERIORATION

N/A

PREPARATION OF COATED GRIDS FOR ELECTRON MICROSCOPY

Issue number: 5.1 Issue date: 29.08.06 Issued by: Standards Unit, Evaluations and Standards Laboratory Page 6 of 11

Reference no: VSOP 12i5.1

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

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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 5 cm
- Fibre-free tissue
- Wide container for holding water
- Vacuum-coating unit

4.2 REAGENTS

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

5 SPECIMEN PROCESSING/PROCEDURE

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 0.25 – 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 long-term (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 ethylene dichloride 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 6 wipes on each side of the glass will usually suffice

PREPARATION OF COATED GRIDS FOR ELECTRON MICROSCOPY

Issue number: 5.1 Issue date: 29.08.06 Issued by: Standards Unit, Evaluations and Standards Laboratory Page 7 of 11

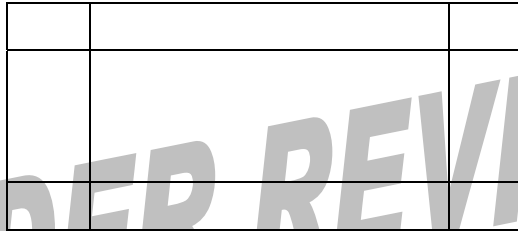
Reference no: VSOP 12i5.1

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- 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

PREPARATION OF COATED GRIDS FOR ELECTRON MICROSCOPY

Issue number: 5.1 Issue date: 29.08.06 Issued by: Standards Unit, Evaluations and Standards Laboratory Page 8 of 11

Reference no: VSOP 12i5.1

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- 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 (VSOP 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 (VSOP 13 - Investigation of clinical specimens by electron microscopy using the floatation (direct) method) or double-spin method (VSOP 14 - Investigation of clinical specimens by electron microscopy using concentration by differential centrifugation (double-spin method)):
- Prepare a high titre suspension of adenovirus or other 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 from 10 repeat grids from the standard suspension
- Check counts per unit area from each new grid batch and reject batches that give counts 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.

PREPARATION OF COATED GRIDS FOR ELECTRON MICROSCOPY

Issue number: 5.1 Issue date: 29.08.06 Issued by: Standards Unit, Evaluations and Standards Laboratory Page 9 of 11

Reference no: VSOP 12i5.1

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

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8 REPORTING PROCEDURE

8.1 REPORTS

N/A

8.2 REPORTING TIME

N/A

9 REPORTING TO THE HPA (LOCAL AND REGIONAL SERVICES AND CDSC CENTRE FOR INFECTIONS)

N/A

10 ACKNOWLEDGEMENTS 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). 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.

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PREPARATION OF COATED GRIDS FOR ELECTRON MICROSCOPY

Issue number: 5.1 Issue date: 29.08.06 Issued by: Standards Unit, Evaluations and Standards Laboratory Page 10 of 11

Reference no: VSOP 12i5.1

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PREPARATION OF COATED GRIDS FOR ELECTRON MICROSCOPY

Issue number: 5.1 Issue date: 29.08.06 Issued by: Standards Unit, Evaluations and Standards Laboratory Page 11 of 11

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