

NATIONAL STANDARD METHOD

# ELECTRON MICROSCOPY: FLOTATION (DIRECT) METHOD

VSOP 13

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



UK Clinical Virology Network

## ELECTRON MICROSCOPY: FLOTATION (DIRECT) METHOD

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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.

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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](http://www.hpa.org.uk).

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More details can be found on the website at [www.evaluations-standards.org.uk](http://www.evaluations-standards.org.uk). Contributions to the development of the documents can be made by contacting [standards@hpa.org.uk](mailto: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:**

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# AMENDMENT PROCEDURE

Controlled document reference	VSOP 13
Controlled document title	Electron Microscopy: Flotation (Direct) Method

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](mailto: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
8/ 11.06.10	5	6	All  10	<b>All</b>  <b>9 Notification to the HPA</b>	Section renamed and reference inserted Document and references reviewed

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# ELECTRON MICROSCOPY: FLOTATION (DIRECT) METHOD

## SCOPE OF DOCUMENT

This National Standard Method (NSM) provides a rapid method for the direct examination of clinical specimens that are expected to contain very large numbers of virus particles.

## INTRODUCTION

### Background

This method does not use virus concentration prior to the preparation of grids. It is therefore useful only in situations where the virus is expected to be present in high titre, for example, faecal samples from children under 5 years of age with acute diarrhoea or vesicle fluids or crusts collected from vesicular lesions. It is a rapid technique and may successfully demonstrate viruses present in the sample. It is generally accepted that  $10^6$  -  $10^9$  virus particles per gram of starting material are required to give a reliable result using this technique<sup>2</sup>. This procedure is not recommended for the examination of samples from outbreaks of gastroenteritis where a viral aetiology is suspected. Immune capture ([VSOP 16 – Electron Microscopy using solid phase immune electron microscopy](#)), Differential centrifugation ([VSOP 14 – Electron microscopy: Differential centrifugation \(double-spin method\)](#)) or Ammonium sulphate precipitation ([VSOP 15 – Electron microscopy: Ammonium sulphate precipitation](#)) are recommended for the examination of samples from outbreaks of gastroenteritis where a viral aetiology is suspected.

**Note:** This procedure must not be used on samples suspected of containing smallpox or any other category 4 organism.

# SAFETY CONSIDERATIONS<sup>3-13</sup>

## 1.1 SPECIMEN COLLECTION

Appropriate hazard labelling according to local policy.

## 1.2 SPECIMEN TRANSPORT AND STORAGE

Sterile leakproof container in a sealed plastic bag.

## 1.3 SPECIMEN PROCESSING

Vesicular lesions may occur as a result of many different viral agents (some of which are category 4 pathogens). Where a Hazard Group 4 pathogen is suspected (such as smallpox), this NSM must not be used. Material from other vesicular lesions may be handled under Hazard Group 2 or Hazard Group 3 containment facilities, as appropriate.

Staff who are pregnant and susceptible to varicella/zoster should not handle vesicular material.

Bacteria, viruses, fungi and parasites, encountered unexpectedly, can cause severe and sometimes fatal disease. Guidance is given in the Health Protection Agency immunisation policy. Processing must be carried out by trained laboratory personnel in a properly equipped laboratory and under the supervision of a qualified microbiologist. Disposable gloves should be worn during all procedures.

All enteric viruses are in Hazard Group 2 and should be processed in a suitable area with due care. Processing in a microbiological safety cabinet is not mandatory but is advisable. Some specimens may contain pathogenic bacteria or parasites. It is recommended that faecal samples with bloody diarrhoea or haemolytic uraemic syndrome (*Escherichia coli* O157) are not processed until bacteriology results are known.

Refer to current guidance on the safe handling of all Hazard Group 2 and Hazard Group 3 organisms documented in this NSM.

Prepared grids may be infectious prior to examination in the EM. Dispose of used grids by autoclaving.

Use caution when handling forceps.

Forceps should be decontaminated after use.

Phosphotungstic acid is corrosive. Gloves and eye protection should be worn when making the non-pH adjusted solution.

It is recommended that any grids prepared from body fluids from patients who are HIV or HBV positive are inactivated with a drop of fixative (see appendix).

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet.

Compliance with packing, postal and transport regulations is essential.

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

## 2 SPECIMEN COLLECTION

### 2.1 OPTIMAL TIME OF SPECIMEN COLLECTION

Successful demonstration of virus particles by electron microscopy requires specimens that are taken during the acute phase of the illness. For faecal specimens, best results are obtained with specimens collected within 48 hours of the onset of symptoms.

### 2.2 CORRECT SPECIMEN TYPE AND METHOD OF COLLECTION

#### Faecal specimens

A minimum of 1 g of faeces is required which should be collected directly into a clean container.

#### Vesicular lesions

The preferred method for the collection of vesicle fluids is to puncture the vesicle and express the fluid directly onto a clean glass microscope slide and allow it to dry. The slide should then be marked to indicate the surface used and the position of the dried sample and dispatched to the laboratory in a plastic slide carrier. Vesicle crusts may be removed and sent for examination in a small sealed bottle. Swabs, or samples in transport medium are not acceptable for electron microscopy, but may be useful for cell culture.

**Note:** Vesicle fluids should not be submitted to the laboratory in hypodermic syringes or capillary tubes: it is difficult to recover the sample and could be hazardous to laboratory staff.

### 2.3 ADEQUATE QUANTITY AND APPROPRIATE NUMBER OF SPECIMENS

See above.

## 3 SPECIMEN TRANSPORT AND STORAGE

### 3.1 TIME BETWEEN SPECIMEN COLLECTION AND PROCESSING

Specimens should be transported and processed as soon as possible.

### 3.2 SPECIAL CONSIDERATIONS TO MINIMISE DETERIORATION

Specimens should be stored at +4°C if processing cannot be performed within 24 hours.

**Note:** Faecal specimens must not be frozen.

## 4 EQUIPMENT AND REAGENTS

### 4.1 EQUIPMENT

- Fine-point jeweller's forceps eg Dumont forceps number 5. A separate pair of forceps is required for each sample processed. Forceps must be decontaminated after use
- Coated electron microscope grids ([VSOP 12 – Preparation of coated grids for electron microscopy](#))
- Hydrophobic surface eg 'BenchKote', Parafilm or a Petri dish
- Strips of clean filter paper about 1 cm x 2.5 cm in size
- Clean 5 mL bottles or plastic tubes

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- Clean glass microscope slides
- Disposable transfer pipettes
- Griffiths tube homogeniser or similar (optional)

## 4.2 REAGENTS

- Negative stain: The negative stain that is most widely used for viruses in electron microscopy is phosphotungstic acid. Alternatives are available (eg methylamine tungstate)
- Specimen diluent: Deionised or glass distilled water, sterile phosphate-buffered-saline (PBS) and sterile single-strength minimal essential medium have all been used as a sample diluent. The authors of this SOP could find no observable difference in virus morphology or sample presentation whichever diluent was used. There are, however, unpublished observations that storage in PBS has a deleterious effect on the morphology of some viruses. It is good practice to incorporate broad-spectrum antibiotics and a fungicide into the sample diluent
- Deionised or glass distilled water

# 5 SPECIMEN PROCESSING

## 5.1 TEST SELECTION

N/A

## 5.2 CULTURE AND INVESTIGATION

N/A

## 5.3 PREPARATION OF CLINICAL SPECIMENS

### Faecal emulsions

- Dilute faecal specimens to 10 - 20% weight/volume in the specimen diluent. The faecal extract must be thoroughly emulsified. If samples are mixed by shaking, or preferably vortexing the emulsion must be enclosed within a sealed bottle, which must remain unopened for at least 30 minutes to allow aerosols to settle. Bottles should be opened in a laboratory safety cabinet
- Clarify the suspension by either settling or by centrifuging (up to 3,000x g for up to 20 minutes)

### Vesicle fluids/crusts

- Rehydrate vesicle fluid that has been dried onto a microscope slide with a drop of deionised or glass distilled water. The rehydrated vesicle fluid can be used for grid preparation without further processing.
- Vesicle crusts may be softened and disrupted in a small drop of deionised or glass distilled water on a clean glass microscope slide using the points of the fine-pointed forceps. Alternatively, vesicle crusts may be ground in a Griffiths tube or similar homogeniser. The resulting 'homogenate' may be used for grid preparation without further processing.

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## 5.4 PREPARATION AND STAINING OF GRIDS

- Spot the prepared sample onto a hydrophobic surface pre-labelled with the specimen number. The drops of faecal emulsion must be at least 1 cm apart to minimise the risk of sample-to-sample contamination.
- Place a grid coated with a suitable support film onto the surface of the drop using clean forceps and incubate at room temperature. The precise adsorption time varies depending on the number of virus particles expected in the preparation and the amount of 'debris' present in the sample and is best determined by experiment and experience.
- Pick up the grid and blot to remove excess fluid by touching a piece of filter paper to the edge of the grid. Then transfer the grid to a drop of a suitable negative stain. After approximately 5 seconds the grid is blotted to remove all but a thin film of stain and air dried, support film side up, on a clean piece of filter paper. An optional washing stage in which the inoculated grid is transferred onto a drop of sterile deionised water for a few seconds may precede the staining if required.
- Alternatively, equal volumes of stain and sample can be mixed and the grid applied as above.
- Examine the grid in the electron microscope using a screen magnification of approximately x50,000

## 5.5 IDENTIFICATION

Viruses are identified by their characteristic morphology.

# 6 QUALITY ASSURANCE

## 6.1 ASSESSMENT OF PREPARATION

Grids should be examined for at least 5 minutes before being considered 'negative'. Grids with damaged support films should be repeated as insufficient grid surface will not permit the minimum required examination. Grids that are over-stained or have too much adherent material to be easily readable should be repeated with a more dilute or re-clarified extract.

## 6.2 INTERNAL AND EXTERNAL QUALITY ASSURANCE

Laboratories should participate in any external quality assurance schemes that may become available for electron microscopy<sup>14</sup>.

# 7 LIMITATIONS

Successful detection of viruses depends on the skill and experience of the microscopist, collection of specimens at the appropriate time, transport, storage and processing and the provision of adequate/suitable clinical information. Viruses will not be detected unless there are sufficient numbers in the sample, which is usually considered to be  $10^6$  -  $10^9$  particles/mL sample.

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 samples should be reported as 'NO VIRUS PARTICLES SEEN'

Positive samples should be reported as eg 'HERPESVIRUS PARTICLES PRESENT'

Some workers may wish to provide an estimate of the amount of virus present. Accurate quantitative assessment requires the application of specialist procedures but a well-trying scheme that gives consistent results is as follows:

- +++ equates to one or more virus particles in each of four randomly selected fields.
- ++ equates to 10 or more virus particles seen in each of four grid-squares examined.
- + equates to between 1 and 10 virus particles seen in each of four grid-squares examined.
- +/- equates to between 1 and 10 virus particles seen in total.

### 8.2 REPORTING TIME

Urgent requests	Telephone as soon as available
Written reports	Normally available the next working day or in accordance with local reporting policy

## 9 NOTIFICATION TO THE HPA<sup>15,16</sup>

Any positive result should be reported to the HPA or equivalent in accordance with published guidelines.

## 10 ACKNOWLEDGEMENTS AND CONTACTS

This National Standard Method has been developed, reviewed and revised by Dr Hazel Appleton of the Electron Microscopy Unit Centre for Infections and Dr Alan Curry HPA Manchester and the Standard Methods Working Group for Virology ([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, Department for Evaluations, Standards and Training, Centre for Infections, Health Protection Agency, London.

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# APPENDIX

**Phosphotungstic Acid** - this is used at a concentration of between 1% and 3% by weight in deionised or glass distilled water. The exact concentration used is a matter for personal preference. The pH is adjusted to approximately 6.5 (+/-0.1) by the addition of sodium hydroxide or potassium hydroxide. The stain is relatively stable at room temperature but the pH must be checked regularly.

**Fixation of grids prior to negative staining** - transfer the inoculated grid from the specimen to a drop of fixative (eg buffered formalin). Leave at room temperature for at least 2 minutes. Stain with a drop of phosphotungstic acid. This procedure may adversely affect the morphology of some common viruses.

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