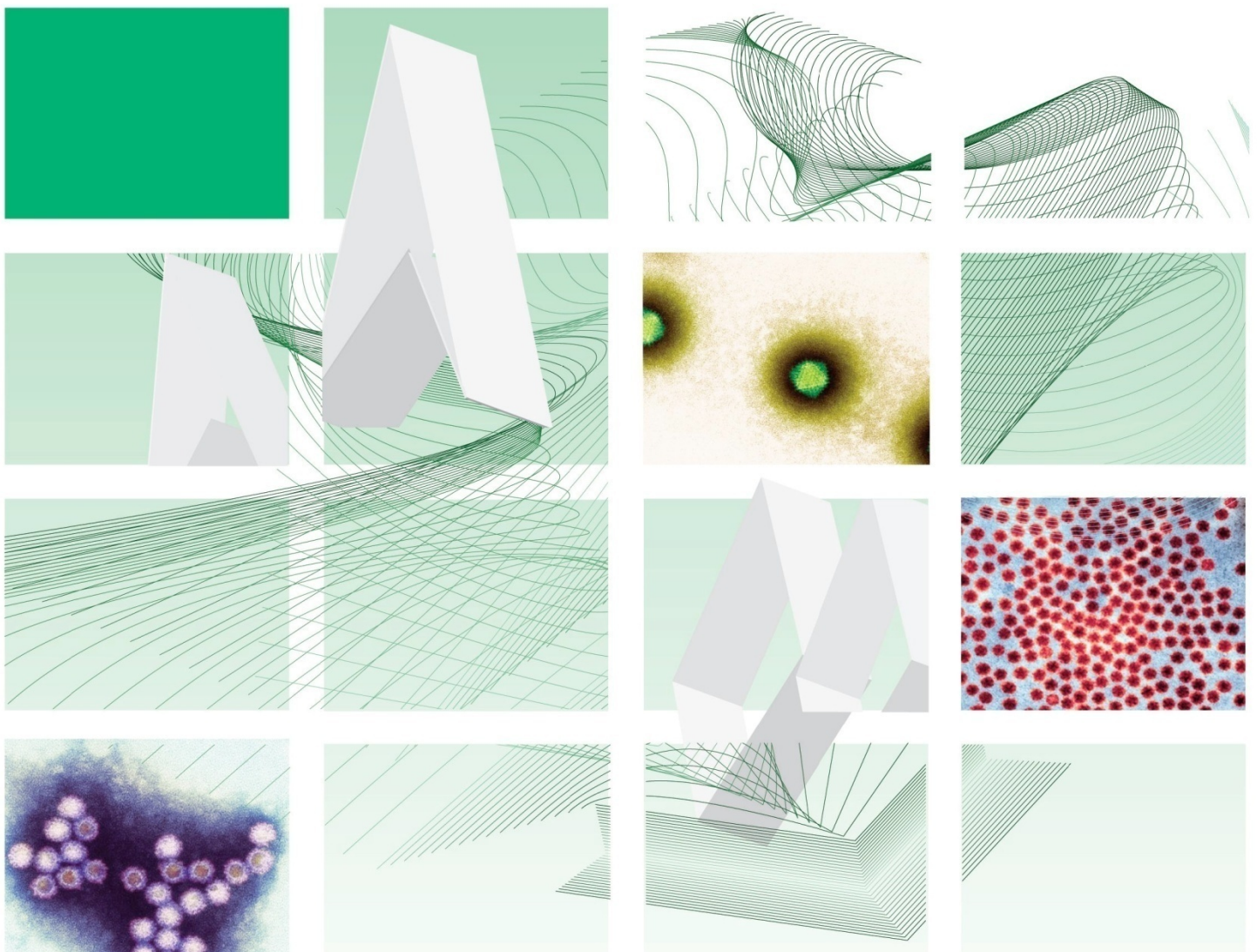




UK Standards for Microbiology Investigations

Electron Microscopy: Floatation (Direct) Method



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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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.	10/20.04.12
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). Electron Microscopy: Floatation (Direct) Method. UK Standards for Microbiology Investigations. V 13 Issue 6.3.
<http://www.hpa.org.uk/SMI/pdf>.

Scope of Document

The SMI provides a rapid method for the direct examination of clinical specimens that are expected to contain very large numbers of virus particles.

This SMI should be used in conjunction with other SMIs.

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 less than five 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 technique¹. This procedure is not recommended for the examination of samples from outbreaks of gastroenteritis where a viral aetiology is suspected. Immune capture ([V 16 – Electron Microscopy Using Solid Phase Immune Electron Microscopy](#)), differential centrifugation ([V 14 – Electron Microscopy: Differential Centrifugation \(Double-Spin Method\)](#)) or Ammonium sulphate precipitation ([V 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.

Technical Information/Limitations

Specimen Containers^{2,3}

SMIs use the term “CE marked leak proof container” to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: “The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes”.

1 Safety Considerations²⁻¹⁸

1.1 Specimen Collection^{2,3}

Appropriate hazard labelling according to local policy.

1.2 Specimen Transport and Storage²⁻⁷

Sterile leak-proof container in a sealed plastic bag.

1.3 Specimen Processing²⁻¹⁸

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 SMI 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 Public Health England 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 SMI.

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 Type of Specimens

Faecal specimen, vesicular lesions

2.2 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.

2.3 Correct Specimen Type and Method of Collection

Faecal specimens

A minimum of 1g 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, 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.

2.4 Adequate Quantity and Appropriate Number of Specimens

See above.

3 Specimen Transport and Storage^{2,3}

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 ([V 12 – Preparation of Coated Grids for Electron Microscopy](#))
- Hydrophobic surface, eg, 'BenchKote', Parafilm or a Petri dish
- Strips of clean filter paper about 1cm x 2.5cm in size
- Clean 5mL bottles or plastic tubes
- 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 SMI 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/Procedure^{2,3}

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.

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 1cm 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 five 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 microscopy¹⁹.

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.

The procedure(s) in this document 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 SMI 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 1-10 virus particles seen in each of four grid-squares examined.
- +/- equates to between 1-10 virus particles seen in total.

8.2 Reporting time

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

9 Notification to PHE^{20,21} or Equivalent in the Devolved Administrations²²⁻²⁵

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](#)^{22,23}, [Wales](#)²⁴ and [Northern Ireland](#)²⁵.

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 two minutes. Stain with a drop of phosphotungstic acid. This procedure may adversely affect the morphology of some common viruses.

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