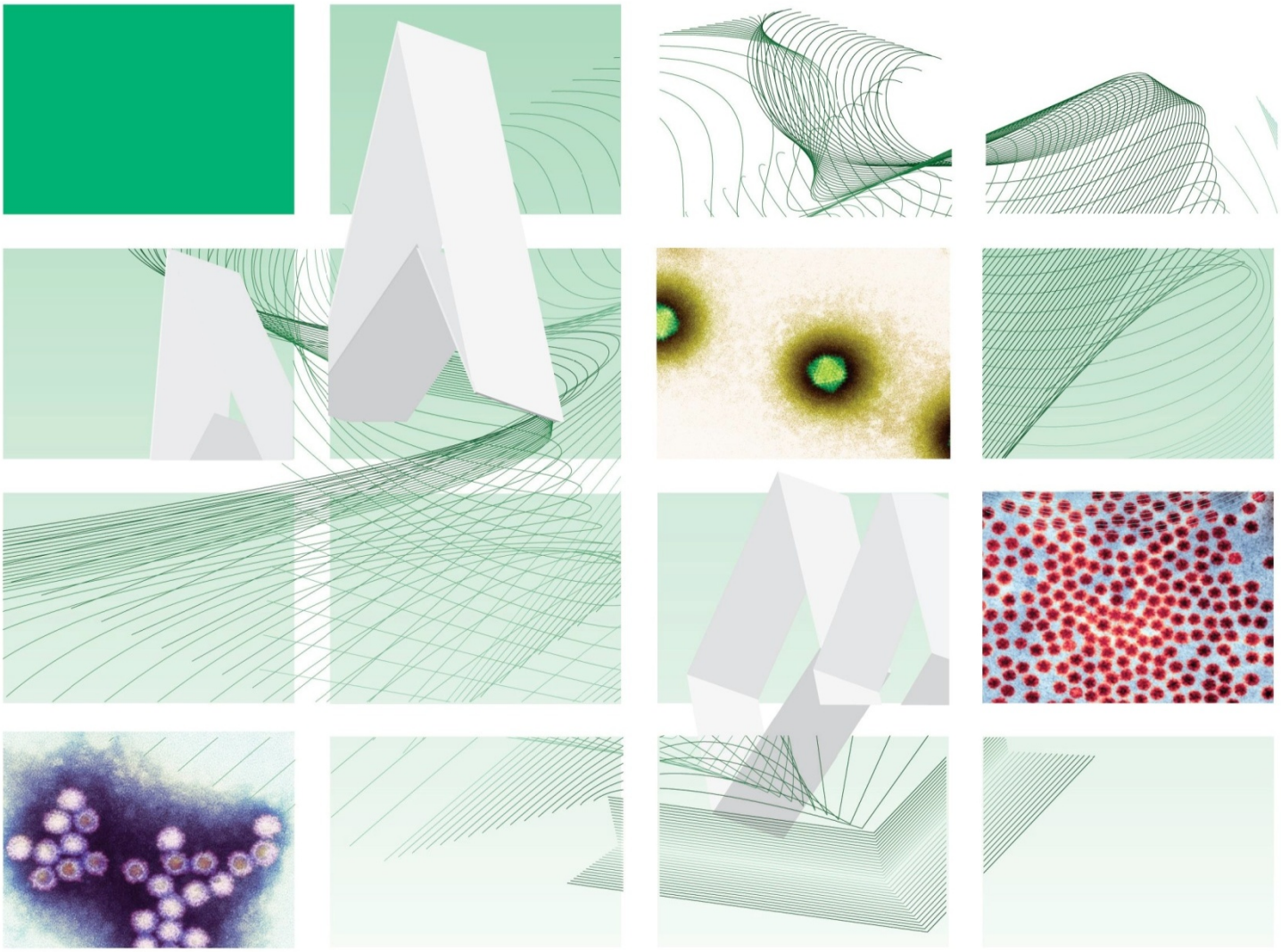
Public Health England

NHS

UK Standards for Microbiology Investigations

Electron Microscopy: Ammonium Sulphate Precipitation



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Virology | V 15 | Issue no: 5.3 | Issue date: 10.04.14 | 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.	5/10.04.14
Issue no. discarded.	5.2
Insert Issue no.	5.3
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 and notification references have been reviewed and updated. Scientific content remains unchanged.

Amendment No/Date.	4/20.04.12
Issue no. discarded.	5.1
Insert Issue no.	5.2
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.

Suggested Citation for this Document

Public Health England. (2014). Electron Microscopy: Ammonium Sulphate Precipitation. UK Standards for Microbiology Investigations. V 15 Issue 5.3. http://www.hpa.org.uk/SMI/pdf.

Scope of Document

This SMI describes a procedure for the concentration of viruses from faecal emulsions prepared from samples from outbreaks of gastroenteritis[1].

This SMI should be used in conjunction with other SMIs.

Introduction

Background

This procedure utilises the principle of precipitation of proteins by ammonium sulphate. The procedure has been used on many viruses with success. The method does not work well with enteroviruses. The technique cannot be used with samples containing large amounts of protein such as serum. The procedure preserves delicate virus morphology by allowing pelleting at comparatively low g-forces.

It is strongly recommended that ammonium sulphate precipitation, ultracentrifugation (V 14 - Electron Microscopy: Differential Centrifugation (Double-Spin Method)), or

Immune capture (V 16 - Investigation of Clinical Specimens by Electron Microscopy Using Solid Phase Immune Electron Microscopy) is used for all faecal specimens from outbreaks of gastroenteritis.

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 Considerations[2-18]

1.1 Specimen Collection[2,3]

Appropriate hazard labelling according to local policy.

1.2 Specimen Transport and Storage[2-7]

Sterile leakproof container in a sealed plastic bag.

1.3 Specimen Processing[2-18]

- Bacteria, viruses, fungi and parasites, encountered unexpectedly, can cause severe and sometimes fatal disease. Laboratory acquired infections have been reported. 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
- Where the clinical features may indicate a Hazard Group 3 infection sample processing should only be carried out with appropriate containment conditions. It is recommended that faecal specimens from patients with bloody diarrhoea or haemolytic uraemic syndrome (*Escherichia coli* O157) are not processed until bacteriology results are available. Suspected Hazard Group 4 samples (eg deliberate release of smallpox) should only be processed for electron microscopy after inactivation
- Other samples may be handled as Hazard Group 2. Processing in a microbiological safety cabinet is not mandatory but is advisable. Some specimens may contain pathogenic bacteria or parasites
- Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet
- Refer to current guidance on the safe handling of all Hazard Group 2 organisms documented in this SMI
- Prepared grids may be infectious prior to examination in the EM. Dispose of used grids by autoclaving
- Compliance with packing, postal and transport regulations is essential.
- Use caution when handling forceps. Forceps should be decontaminated after use by autoclaving

1.3.1 Ultracentrifugation

Ultracentrifugation presents particular safety hazards associated with the high rotational forces generated. Staff should be made aware of the dangers and instructed in their use. A service company or the manufacturer should regularly maintain the centrifuges and rotors. Laboratories should have their own maintenance protocols, in accordance with the manufacturer's recommendations.

1.3.2 Chemical handling

- 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)

- Phosphotungstic acid is corrosive. Gloves and eye protection should be worn when making the non-pH adjusted solution
- Do not discard ammonium sulphate into hypochlorite disinfectant, as large quantities of chlorine gas will be evolved

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

2 Specimen Collection

2.1 Type of Specimens

Faecal emulsions

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. Best results are obtained with specimens collected within 48 hours of the onset of symptoms.

2.3 Correct Specimen Type and Method of Collection

N/A

2.4 Adequate Quantity and Appropriate Number of Specimens

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

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

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

Faecal specimens must not be frozen.

4 Equipment and Reagents

4.1 Equipment

- Bench centrifuge capable of producing 2,000 to 3,000x g
- Preparative ultracentrifuge and rotor capable of producing at least 25,000x g with a minimum sample volume of 1mL
- 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

4.2 Reagents

- Analar Ammonium sulphate
- Negative stain: The negative stain that is most widely used for viruses in electron microscopy is phosphotungstic acid, but alternative stains are available
- 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 specimen diluent. The authors of this SMI could find no observable difference in virus morphology or specimen preservation 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
- Sterile deionised or glass distilled water

5 Specimen Processing/Procedure[2,3]

5.1 Test Selection

N/A

5.2 Culture and Investigation

5.2.1 Preparation of clinical specimens

- Dilute faecal specimens to 5-10% weight/volume in 2.25mL of the specimen diluent. The faecal extract must be thoroughly emulsified. If specimens 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
- Clarify the suspension by centrifuging the emulsion at (2,000 to 3,000 x g for up to 30 minutes) in a sealed-bucket rotor

5.2.2 Concentration stage

- Add 1.5g of solid ammonium sulphate and 2mL of specimen diluent to a pre-numbered, clean 5mL bottle
- Transfer the clarified suspension to the appropriately numbered 5mL bottle containing the ammonium sulphate and specimen diluent (final volume of liquid

in the bottle is about 4mL). Cap the bottle and mix until the crystals of ammonium sulphate are completely dissolved. Leave the mixture to stand at room temperature for a minimum of four minutes. The specimens may be left at +4°C overnight if more convenient

- Transfer the mixture to a pre-numbered, clean centrifuge tube and centrifuge at about 10,000 x g for 10 minutes. If a centrifuge capable of delivering this g-force is not available the precipitate can be pelleted in a bench centrifuge with a sealed-bucket rotor running at 3,000 x g for one hour
- Tip off the supernatant into an empty 50mL bottle for subsequent autoclaving and disposal. Do not discard into hypochlorite disinfectant, as large quantities of chlorine gas will be generated. Re-suspend the deposit in three drops of deionised water or glass distilled water

5.2.3 Concentration Stage

- Spot the concentrated specimens onto a hydrophobic surface pre-labelled with the specimen number. The specimen drops must be at least 1cm apart to minimise the risk of cross-contamination
- Place a coated grid support film down, onto the surface of each drop, using clean forceps and incubate, covered, 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. Transfer the grid onto a drop of a suitable negative stain. After staining (staining time is variable and depends on experience, but should be a minimum of about five 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 deionised or glass distilled water for a few seconds, may precede the staining if required
- Alternatively, equal volumes of stain and sample may be mixed then applied to the grid as above
- Examine the grid in the electron microscope, using a screen magnification of approximately x50,000

5.4 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[19,20]

Laboratories should participate in any external quality assurance schemes that may become available for electron microscopy.

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, “Virus particles not seen.”

Positive samples should be reported as, eg “Astrovirus 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-tried 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 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 results are available.
Written reports local	Normally available the next working day or in accordance with reporting policy.

9 Notification to PHE[21,22] or Equivalent in the Devolved Administrations[23-26]

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 (HCAIs) 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[23,24], Wales[25] and Northern Ireland[26].

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