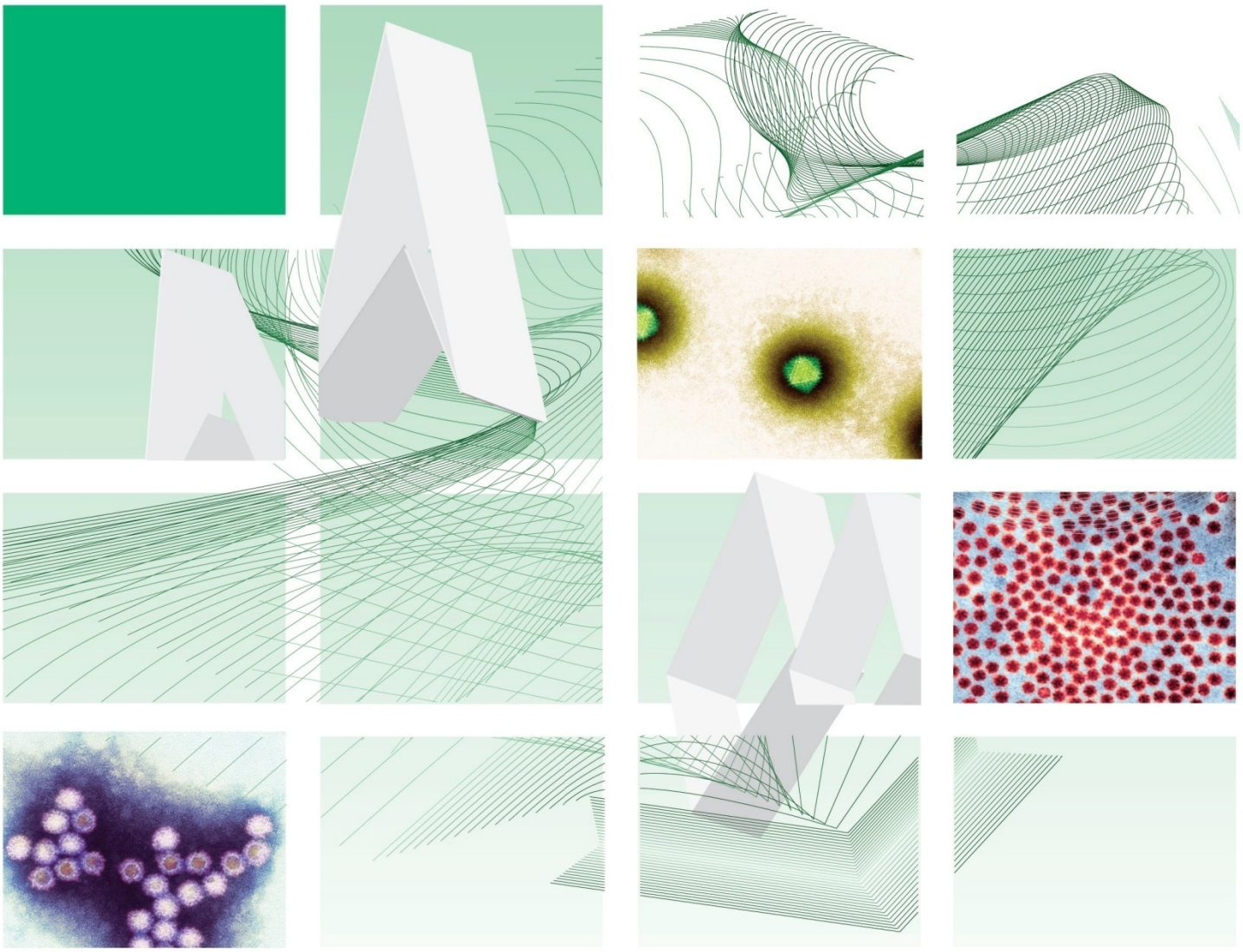
Public Health England

NHS

UK Standards for Microbiology Investigations

Electron Microscopy Using Solid Phase Immune Electron Microscopy



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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.	5/11.04.14
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 Using Solid Phase Immune Electron Microscopy. UK Standards for Microbiology Investigations. V 16 Issue 5.3. http://www.hpa.org.uk/SMI/pdf.

Scope of Document

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

This procedure has been used with success to detect noroviruses (Norwalk-like viruses) from faeces, vomit samples from outbreaks of non-bacterial gastroenteritis and parvovirus B19 from biopsy material in cases of hydrops foetalis[2,3]. In the case of noroviruses, multiple serotypes exist and it should be borne in mind that a capture antibody from a single serum may only be active against some of these serotypes.

The technique may be easily adapted to use either IgG or IgM as the capturing antibody[4].

This SMI should be used in conjunction with other SMIs.

Introduction

The assay uses a solid phase immune capture system. Protein A is coated onto a formvar-carbon coated EM grid. Alternatively, coat with an anti-IgG or anti-IgM. The coated grid is then coated with human convalescent serum as the capturing antibody. Finally the virus is captured onto this grid. The procedure produces an increase in sensitivity over the direct floatation method (V 13 - Electron Microscopy: Flotation (Direct) Method) in excess of 1,000 fold. Grids produced using this procedure are also substantially free from background debris. This makes for easier observation of otherwise indistinct virus particles.

Technical Information/Limitations

Specimen Containers[5,6]

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[5-21]

1.1 Specimen Collection[5,6]

Appropriate hazard labelling according to local policy.

1.2 Specimen Transport and Storage[5,6,8-10,22]

Sterile leakproof container in a sealed plastic bag.

1.3 Specimen Processing[5,6,8-22]

- 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 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
- The sodium azide used as a preservative can form highly unstable copper azide when in contact with copper pipes. This compound is explosive. A liquid containing sodium azide must be discarded into chemical disposal containers

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

2 Specimen Collection

2.1 Type of specimens

Faecal specimens

2.2 Optimal Time of Specimen Collection

Faecal specimens

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 48hr 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 sterile container.

Biopsy specimens

Biopsy material should be removed into a sterile clear bottle. The material must not be fixed. Separate specimens should be removed into appropriate fixative for histological investigation if required. Sufficient material should be removed to ensure a reasonable amount of the lesion is examined. Approximately 1mm^3 of lesion is required.

2.4 Adequate Quantity and Appropriate Number of Specimens

See above.

3 Specimen Transport and Storage[5,6]

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 24hr.

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 specimen processed. Forceps must be decontaminated after use

- Carbon/formvar coated electron microscope grids (V 12 - Preparation of Coated Grids for Electron Microscopy)
- Hydrophobic surface, eg a Petri dish, Benchkote or Parafilm
- Strips of clean filter paper about 1cm x 2.5cm in size
- Clean 5mL bottles or plastic tubes
- Disposable transfer pipettes
- Humid chamber

4.2 Reagents

- 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
- Deionised or glass distilled water
- 0.08% sodium azide in phosphate buffered saline
- Protein A (working dilution 1µg/mL) or anti-IgG or anti-IgM
- Suitable capturing antibody (see Appendix)
- Positive control sample (see Appendix)

5 Specimen Processing/Procedure[5,6]

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 specimens are mixed by shaking, or preferably vortexing, the homogenate must be enclosed within a sealed bottle which must remain unopened for at least 30 minutes to allow aerosols to settle

- Clarify the homogenate by either settling or by centrifuging (up to 3000 xg for up to 30 minutes)

Biopsy material/Foetal organs

- Disrupt biopsy material or tissue specimens in about 1mL of deionised or glass distilled water in a Griffith's tube homogeniser. The material must be gently homogenised
- Clarify the suspension by centrifuging (up to 3,000 xg for up to 30 minutes) in a sealed-bucket rotor

5.4 Immune (IgG) Capture Procedure

- Do not allow the grid to dry out until the final stage
- Dilute the protein A to a working strength of 1-25µg/mL in PBS + 0.08% sodium azide or dilute the alternative capture antibody to its working dilution (approximately 1:400 for commercial anti-IgG and anti-IgM)
- Incubate formvar/carbon coated EM grid on drop of diluted protein A/capture antibody for 10 minutes at room temperature in a humid chamber
- Blot off excess fluid and wash the grid on one drop of deionised, glass distilled water or PBS
- Blot off excess fluid and coat the grid on a drop of suitably diluted human convalescent antiserum (see appendix), in PBS + 0.08% sodium azide for one hour at room temperature in a humid chamber
- Blot off excess fluid and wash the grid on one drop of deionised, glass distilled water or PBS
- Blot excess fluid from the grid, and capture the virus from the specimen on the grid by floating the grid onto a drop of specimen for two hours at room temperature in a humid chamber
- Wash the grid under a gentle stream of running deionised, glass distilled water or PBS from a laboratory wash-bottle for about five seconds
- Blot off excess water and place grid on a drop of negative stain for a few seconds
- Blot the grid dry and examine using the EM at an on-screen magnification of at least x50,000

5.5 Identification

Viruses are identified by their characteristic morphology.

6 Quality Assurance

6.1 Assessment of Preparation

Grids should be scanned for at least five minutes (minimum of four different grid-squares) before being considered 'negative'. Grids with damaged support films, where insufficient grid surface remains to permit the minimum required examination, should

be repeated. 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.

A positive control specimen must be included with each batch of samples processed. This specimen should be ++ positive. Test batches, where the positive control fails to reach this level of positivity, must be discarded.

6.2 Internal and External Quality Assurance[23,24]

Laboratories should participate in any external quality assurance schemes that may become available for electron microscopy[23,24].

7 Limitations[25]

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 not 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 ‘Noroviruses 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[26,27] or Equivalent in the Devolved Administrations[28-31]

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[28,29], Wales[30] and Northern Ireland[31].

Appendix

Phosphotungstic Acid - this is used at a concentration of 1%-3% by weight in deionised 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 ready-to-use 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 formaldehyde). Leave at room temperature for at least two minutes. Stain as above on a drop of phosphotungstic acid. This procedure may adversely affect the morphology of some common viruses.

Selection and Standardisation of Capture Antibody -

- **For noroviruses** solicit a selection of sera (those from old people are usually best) from the serology laboratory. A suitable blood sample would be one that permits the removal of at least 2mL of serum, is not from a patient who is immunocompromised patient and is not within a recognised risk group for HIV or Hepatitis.

  Note: sera may only be tested for HIV/Hepatitis B & C with the consent of the patient. For parvovirus select sera that give strongly positive results in B19 IgG ELISA tests

- Dilute each serum 1:2000 in PBS + 0.08% sodium azide. Test each serum at this single dilution against the positive control faecal emulsion or known positive B19 specimen. Discard any sera that produce a result of <+++ (ie titre <1:2,000)
- Double-dilute positive sera to end-point (when result drops to +). Use these sera at two dilutions below the end-point, eg a serum that gave a + result at 1:16,000 should be used at 1:4,000
- Aliquot in 100µL volumes in sealed Eppendorf tubes and store at -20°C.
- Record the sample provenance
- Working-strength antibody should be diluted in PBS + 0.08% sodium azide and stored at +4°C

Selection of Positive Control - Routine faecal samples that are +++ positive, and are in sufficient quantity (≥5gms), should be stored at +4°C under liquid paraffin for use as positive controls. Serum samples containing parvovirus B19 detectable by direct electron microscopy (V 13 - Electron Microscopy: Flotation (Direct) Method) should be aliquoted in small volumes in sealed Eppendorf tubes and stored at –20°C.

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