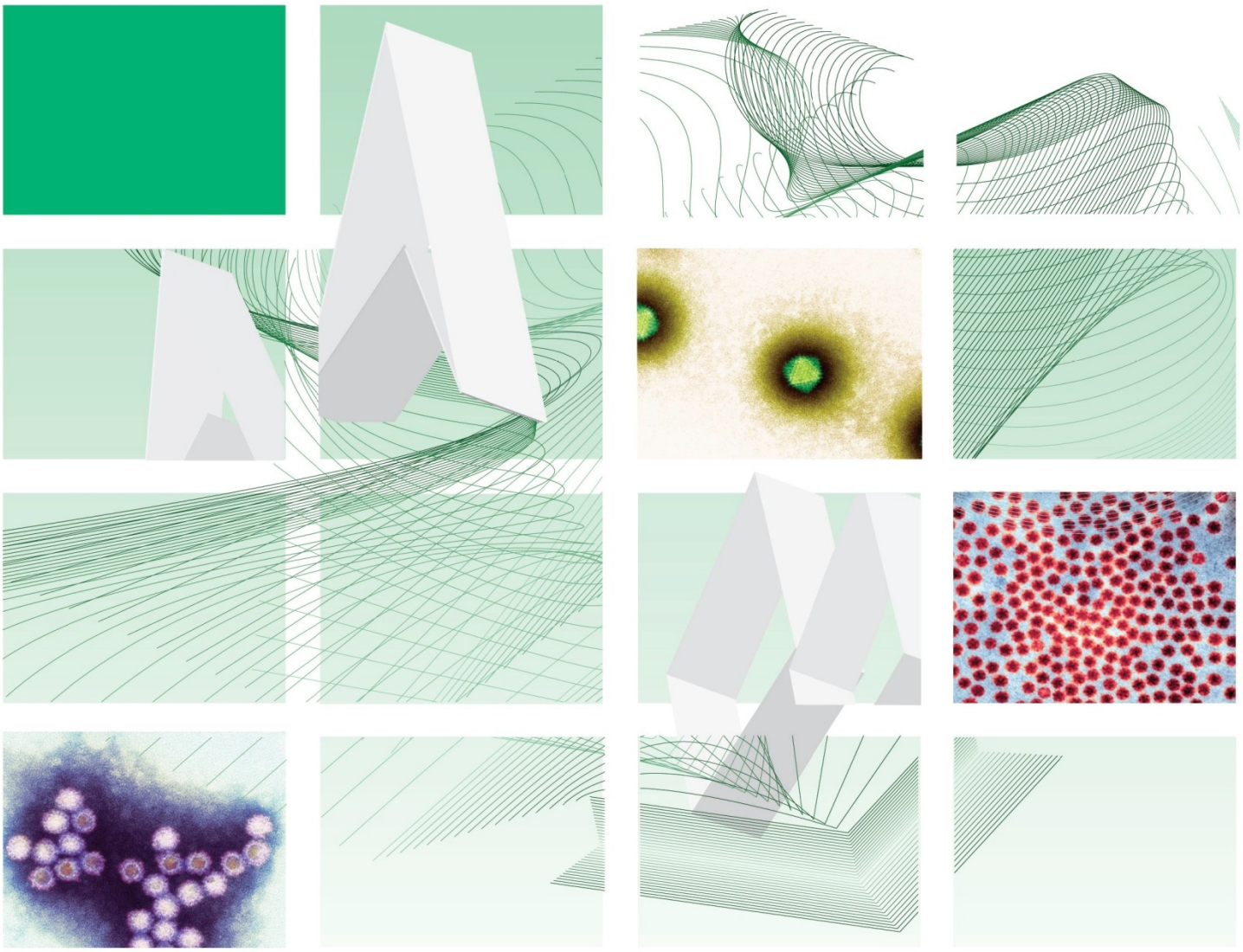
Public Health England

NHS

UK Standards for Microbiology Investigations

Procedure for the Care and Propagation of Cell Cultures for Virus Isolation



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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.	4/14.10.13
Issue no. discarded.	2.2
Insert Issue no.	2.3
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 references have been reviewed and updated. Scientific content remains unchanged.

Amendment No/Date.	3/20.04.12
Issue no. discarded.	2.1
Insert Issue no.	2.2
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.

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Suggested Citation for this Document

Public Health England. (2013). Procedure for the Care and Propagation of Cell Cultures for Virus Isolation. UK Standards for Microbiology Investigations. V 39 Issue 2.3. http://www.hpa.org.uk/SMI/pdf.

Scope of Document

The SMI describes the production of cell cultures for virus isolation/detection.

The cell lines included in the SMI are by no means exclusive, or exhaustive, but are among those most commonly used for virus isolation/detection. The most important consideration when selecting cell lines for this purpose is their susceptibility to the virus(es) under investigation.

Cell lines most commonly used for virus isolation and their individual requirements[1]		
Cell Line	**Dissociating Agents**	**Virus Sensitivity**
RhMK Uncharacterised primary or secondary cells. Origin Rhesus monkey kidney. Mixed cell types present. No longer available in the UK from April 2006 for ethical reasons.	Trypsin/Versene	Influenza viruses Parainfluenza viruses Enteroviruses. Polioviruses Adenoviruses Mumps virus Measles virus
MRC-5 Well characterised semi-continuous cell line. Origin human foetal lung. Fibroblastic in character	Trypsin/Versene	Herpes simplex virus Adenoviruses Respiratory syncytial virus Varicella Zoster virus Cytomegalovirus Enteroviruses Polioviruses Rhinoviruses
HEp-2 Well characterised continuous cell line. Origin human carcinoma of larynx. Epithelial in character.	Versene	Adenoviruses Respiratory syncytial virus Herpes simplex Enteroviruses Polioviruses
VERO Well characterised continuous cell line. Origin African green monkey lung. Epithelial in character	Trypsin/Versene	Herpes simplex virus Enteroviruses Polioviruses Adenoviruses Measles virus
PLC-PRF5 Well-characterised continuous cell line. Origin human hepatoma. Hepatitis B virus surface antigen expressed.	Trypsin/Versene	Influenza viruses Parainfluenza viruses Adenoviruses Enteroviruses

		Polioviruses Respiratory syncytial virus
MDCK Well-characterised continuous cell line. Origin – Dog Kidney Epithelial in Character	Trypsin/Versene **Note:** Cells may require prolonged treatment for dissociation	Influenza viruses Parainfluenza viruses

***** The cell line has been shown to grow the listed viruses but may not be the cell line of choice for those viruses.

This SMI should be used in conjunction with other SMIs.

Introduction

Background

The process by which mammalian cells are removed from tissues and grown under controlled conditions in a laboratory is referred to as cell culture. Once established a cell culture is capable of self sustenance through growth and replication until limited by a variable such as nutrient depletion. Therefore all cultures will need periodic medium changes ("feeding") followed eventually by subculture when the cells are proliferating. Intervals between sub-cultures can vary from one cell line to another depending on the rate of growth and metabolism. When all the available substrate is used or when the cell concentration exceeds the capacity of the medium either the frequency of medium changing must increase or the culture must be divided.

The usual practice in sub-culturing an adherent cell line involves removal of the medium and dissociation of the cells in the monolayer with the proteolytic enzyme trypsin, the chelating agent versene (EDTA) or a mixture of both[2].

Exceptionally, some cell monolayers cannot be dissociated with trypsin and require the action of alternative proteases such as acutase, pronase, dispase or collagenase[3-5].

A major problem in cell culture production is the maintenance of sterility of components and freedom of cell lines from contamination. Bacteria especially mycoplasma, viruses, fungi or other cell types can contaminate cell cultures. The contamination can originate in the tissue used to initiate the cell culture, the components of media, the operator or from the environment.

Technical Information/Limitations

N/A

1 Safety Considerations[6-22]

1.1 Specimen Collection[6,7]

N/A

1.2 Specimen Transport and Storage[6-11]

N/A

1.3 Specimen Processing[6-22]

Local COSHH and general risk assessments (GRA) must be carried out.

Some cell cultures and the chemicals used during their culture are potentially hazardous and must be handled with caution. When there is doubt in regard to the risks implicated in handling a reagent or cell line, refer to the appropriate local COSHH assessment for further information. All staff involved in handling cell cultures must be made aware of the hazards and risks involved.

Although carefully selected, controlled, and monitored animals are used, cell lines derived from them may contain viruses that are pathogenic to humans. Cells from animals of unknown or uncertain origin must not be used for virus isolation.

Continuous cell lines may have been derived from carcinomas or transformed by a viral agent. Although the risk to laboratory staff may be statistically small, they should be handled with the same precautions as animal derived cells.

Cell lines which may be hazardous (see appropriate COSHH references) should be processed in a class II safety cabinet and the operator should wear gloves and a gown at all times.

Cell lines must not be disposed of as general waste but using the same methods as for pathogenic microorganisms. This involves either autoclaving and/or incineration of all containers and instruments used. All liquid waste should be disposed of into Hypochlorite solution (2,500ppm Chlorine) or other suitable disinfectant solution.

In attempting to isolate viruses such as herpes simplex from HIV-positive patients there is the danger that if cells such as MRC 5 are used which have CD4 receptors HIV might also replicate if incubation exceeds three days. Hence any manipulations should take place in Containment Level 3, or tubes not opened after inoculation.

2 Specimen Collection

2.1 Type of Specimens

N/A

2.2 Optimal Time of Specimen Collection

N/A

2.3 Correct Specimen Type and Method of Collection

N/A

2.4 Adequate Quantity and Appropriate Number of Specimens

N/A

3 Specimen Transport and Storage[6,7]

3.1 Time between Specimen Collection and Processing

N/A

3.2 Special Considerations to Minimise Deterioration

Compliance with current postal and transportation regulations is essential.

For transport by road or rail, cell cultures should be wrapped in absorbent protective materials and packed in strong containers filled with polystyrene chips or other appropriate shock-absorbing material. For transport by air, see the current appropriate regulations.

Special consideration must be made to minimise deterioration. Prolonged exposure to temperatures below 10°C and above 35°C must be avoided. The delivery system used should guarantee delivery within 24hr.

4 Equipment and Reagents

4.1 Equipment

4.1.1 Incubators

Incubation can be performed in sealed flasks in a regular dry incubator or hot room capable of maintaining temperatures within the range 30–37°C some vessels eg dishes or multiwell plates require a controlled atmosphere with high humidity and increased CO_2 levels.

CO_2 levels should be checked daily and the incubator recalibrated every six months. The temperature should be checked daily using a calibrated thermometer or thermocouple.

The interiors of incubators should be cleaned at regular intervals to prevent a build-up of microorganisms, using alcohol or a mild phenolic disinfectant followed by cleaning with a mild detergent solution.

4.1.2 Cabinets

Class II safety cabinets should be used for manipulation of hazardous cell lines. Laminar flow cabinets (vertical flow) may be used for manipulating reagents and media but only after local risk assessments have been carried out. All cabinets should meet all the relevant current standards.

To prevent cross contamination between cell lines and build-up of microorganisms, cabinet interiors should be cleaned when the cell line being manipulated changes and at the end of each work period. Alcohol (Industrial methylated spirit) or a phenolic disinfectant should be used.

The airflow should be checked weekly using a calibrated anemometer. Readings should be taken at each corner and the centre of the front opening; airflows must comply with the manufacturer's and national safety recommendations.

Biological tests may be carried out to check the performance of the filters on a monthly basis. These tests should consist of exposing bacterial and fungal culture plates at several positions across the work surface of the cabinet for at least an hour whilst the cabinet is operating, to detect the presence of any bacterial or fungal contamination due to a failure of the filters.

Cabinets should be checked at 6 monthly intervals by an appropriately qualified engineer and a certificate of satisfactory performance issued. Records of any failure of cabinets and the reason must be maintained.

4.1.3 Culture vessels

Disposable plastic tissue culture grade vessels are preferred but suitable neutral glass alternatives may be used.

Glass vessels must be cleaned using a cell culture compatible cleaning agent, followed by thorough rinsing in tap water (x6) and deionised water (x3). Alternatively, a suitable automatic washer may be used which has a rinse programme compatible with cell culture requirements, a cleaning agent compatible with cell culture must be used.

4.1.4 Other equipment

Pipettes

In order to prevent contamination, use only those which have an incorporated membrane filter.

Centrifuges

These must comply with the current relevant safety standards.

Microscopes

Both inverted and standard microscopes are required.

Racks for cell culture tubes

These should be designed to hold the tubes in a slightly inclined position, which permits attachment and growth of cells towards the bottom of the tubes and ensures covering of the monolayer with the nutrient medium. Preferably, they should be capable of withstanding sterilisation by autoclaving.

4.2 Reagents

Unless otherwise stated, all reagents are sterile and all procedures/manipulations must be performed aseptically.

Reagents and media must only be obtained from reputable sources.

Samples of every batch of reagents, solutions, and media produced "in house", or commercial reagents aliquoted prior to use, must be tested for sterility after being dispensed into their containers.

4.2.1 Basal media

A wide range of media may be used but those stated below have been found to produce satisfactory growth in the cell lines listed in this UK Standard for Microbiology Investigation.

To ensure that a more consistent, quality controlled product is used, basal media should be purchased ready for use from an accredited supplier (appropriate ISO or similar). Batches should be tested for suitability prior to use and large volumes of approved batches reserved.

Minimal essential medium-Earles salt buffered (EMEM)

In order to reduce the number of reagents to be added, it is suggested that medium is purchased ready for use, thereby reducing the risk of contamination. Medium should be purchased which already contains sodium bicarbonate, L-glutamine, and a pH indicator.

4.2.2 Complete media

For propagation and maintenance of cells, the following complete media may be produced by making the listed additions aseptically to the basal medium:

EMEM growth medium **To EMEM add:**		
Penicillin	final concentration	50iu/mL
Streptomycin		100µg/mL
Amphotericin B		2.5µg/mL
Newborn calf serum[2]		10%
EMEM maintenance medium **To EMEM add:**		
Penicillin	final concentration	50iu/mL
Streptomycin		100µg/mL
Amphotericin B		2.5µg/mL
Foetal calf serum[2]		2%

4.2.3 Sera

In routine use both newborn and foetal bovine sera have been found suitable for cultivation of the cells listed in this SMI. However, enhanced sera, offering reduced

batch to batch variation, and offering equal or improved performance. All serum used must be tested for suitability prior to purchase. It is advisable to reserve a large batch and test for suitability prior to confirming purchase. If serum is received part frozen it is advisable to allow it to thaw completely before refreezing.

Do not use heat inactivated serum as it is not suitable for cultivation of some cell lines.

4.2.4 Supplementary reagents

Antibiotics

Concentrations of antibiotics stated are those found in textbooks[3].

Note: Antibiotics can cause allergic reactions.

Phosphate buffered saline (PBS)

Dulbecco A solution

(without calcium and magnesium) pH 7.3.

Versene

Dissolve 10g, ethylenediaminetetra-acetic acid disodium salt (Analar grade) in 1000mL, of distilled water. Distribute into bijou bottles and sterilise by autoclaving at 115°C for 10 mins. Store at 2-8°C. For use, add 0.4mL to 20mL of PBS.

5 Specimen Processing/Procedure[6,7]

5.1 Test Selection

A suitable reliable identification system, such as colour coding of tubes, should be used to indicate the type of cell line contained within a culture vessel. Vessels should be labelled with the seeding date.

Cells must be obtained from a reputable supplier who can supply a full history and certification of freedom from contaminants; ECACC or similar. Cells may be purchased either in suspension or as a monolayer in culture flasks.

Avoid pouring liquids from or into containers, pipetting should be used wherever practicable.

5.1.1 Flasks

Follow manufacturer's instructions for the treatment of the culture. Otherwise, the following method may be used.

On arrival, examine flasks to assess the cell growth and condition. On receipt record as per local requirements.

If cells are satisfactory, add sufficient growth medium, if needed, and then incubate at 37°C.

When cell growth reaches confluence, the flasks are ready for use or further passage.

At this stage, the medium may be changed to maintenance medium until the flasks are required. Otherwise they may be subcultured directly into tubes (see below), passaged (if suitable cell line), or disassociated and frozen in liquid nitrogen (see Appendix).

5.1.2 Suspension

1. Dilute the suspension as instructed by the supplier or dilute to a suitable concentration.
2. Add the diluted suspension to cell culture flasks or if required directly into tubes. The amount added to each flask is dependent upon the flask size but should be sufficient to cover the entire growth area to a depth of 3-5mm.
3. Incubate at 37°C.
4. When cell growth is confluent, the flasks are ready for use.

At this stage, the medium may be changed to maintenance medium until the flasks are required. Otherwise they may be subcultured directly into tubes (see below), passaged (if suitable cell line), or disassociated and frozen in liquid nitrogen.

5.2 Culture and Investigation

These instructions are for a 75cm^2 flask, volumes should be adjusted to suit the size of the vessel if different.

1. Select a flask that has achieved confluent growth.
2. Remove the medium and rinse with two changes of approx. 35mL of PBS. Drain the second change completely.
3. Add 15mL of dissociating mixture and rinse all the internal surfaces of the flask carefully, then remove the mixture but do not drain completely.
4. Incubate the flask at 37°C until the cells detach, the time will vary according to cell type.
5. Resuspend the detached cells in 18mL of growth medium.
6. Determine the cell concentration using a counting chamber (modified Fuchs-Rosenthal or similar).
7. Dilute the cells in growth medium to produce the required amount of suspension at a concentration of approx. 1.0x105/mL, (with experience this concentration may be adjusted to achieve the optimum outcome for individual cell lines). With continuous cell lines, any undiluted cells may be returned to the original vessel with sufficient growth medium to continue the culture for future use.
8. Mark a line and/or the date on the side of the tubes; this will form a reference so that the tube is always placed in the incubator with the line at the top.
9. Seed diluted cells into tissue culture tubes in suitable volumes.
10. Incubate at 37°C at a slight angle from horizontal (approx. 5°).
11. When tubes have formed a monolayer which is approximately 80% confluent they are ready to use and should be changed to maintenance medium.

5.2.1 Disaggregation[23]

Remove cell culture medium from the culture vessel by aspiration and discard, rinse the monolayer with Ca^{++} and Mg^{++} free salt solution (such as 0.1M PBS pH 7.2) to remove all traces of serum. Remove the salt solution by aspiration.

Repeat the step outlined above (these steps are important as they remove traces of serum that might otherwise inhibit the action of Trypsin).

Dispense pre-warmed (to 37°C) 0.25% Trypsinor Trypsin/Versene solution into the cell culture vessel to completely cover the monolayer of cells (5mL/75 cm^2 flask) and incubate at 37°C for 1 to 2 minutes.

Remove the Trypsin or Trypsin/Versene solution by aspiration and return the closed culture vessel to the incubator.

The coated cells are allowed to incubate until they round up and detach from the surface. Progress can be checked by examination with an inverted microscope. The time required to remove cells from the surface is dependent on the cell type, population density, potency of Trypsin and time since last subculture. Trypsin causes cellular damage and the time of exposure should be kept to a minimum.

When the trypsinisation process is complete, the cells will be in suspension and appear rounded.

It is advisable to add a medium containing serum to the cell suspension as soon as possible to inhibit further enzyme activity which may damage cells.

Cells can be re-suspended by gently pipetting the cell suspension. Further dilution can be made, if required, for cell (viable) counts or sub-culturing.

If serum free medium is used at this stage a trypsin inhibitor will need to be used.

A suspension of single cells (not clumped) is desirable at sub-culture to ensure an accurate cell count and uniform growth on reseeding.

5.2.2 Aftercare

Tubes should normally be maintained at 37°C until required.

If it is necessary to hold tubes for more than 4-5 days, continuous and semi-continuous cell lines may be moved to 33°C until needed. Primary cells may not tolerate being moved to 33°C; doing so should be based on experience with each cell type.

Change cells onto fresh maintenance medium every 3-4 days. When cells are passaged this should be recorded on the localized record sheet.

5.2.3 Prevention of contamination

Laboratory layout

Facilities available may depend on space and economic importance of cell culture system.

Areas to consider:

Designated room for cell culture production.

Design of room for ease of cleaning.

Use of laminar flow cabinet.

Use of UV light for sterilizing cabinet.

Prevention of a large number of staff having access to the area.

Reduction of risk by discarding continuous cell cultures at regular intervals and bringing in new cell cultures from an approved source.

Aseptic technique

All staff must be trained in basic aseptic technique.

Use of disinfectants such as hypochlorite and 70% alcohol.

Good hand washing practice.

Use of disposable gloves where appropriate.

Use of Bunsen burner for flaming surfaces.

Disinfection of work areas when there is a change of batch number in the cell lines used.

Use of antibiotics

It is preferable and in some cases essential that laboratories use antibiotic-free medium for virus culture. However, due to the risks of contamination, most laboratories use some antibiotics. First line antibiotics include Penicillin 100iu/µL, Streptomycin 100µg/mL. The additional use of other antibiotics may depend on the isolation of the contaminating organism and a sensitivity test being performed to find the most suitable antibiotic.

5.2.4 Detection of contamination

Detection of bacterial or fungal contamination in media

Sterility testing of components of the medium and completed growth and maintenance media should be carried out. A small number of aliquots of the components or the complete medium should be inoculated into media suitable for growing bacteria or fungi, incubated and examined daily for a designated time period. Nutrient broth is suitable for the detection of bacterial contamination. Sabouraud agar is suitable for detection of fungal contamination. All cultures should be incubated at 37°C and room temperature.

Any component that is found to be contaminated should be discarded. (In exceptional circumstances it may be filtered/re-sterilized and re-tested)[24].

Detection of bacterial and fungal contamination in cell culture

Bacterial (except mycoplasma) and fungal contamination are generally visible in cell culture bottles. Low grade contamination can be detected by inoculating the cell suspension into bacterial or fungal culture media.

Unless the contaminated cells are particularly valuable, they should be discarded. Treatment of an infected cell culture with antibiotics can be attempted but may not be successful.

Detection of mycoplasma in cell culture

Mycoplasma species are difficult to detect visually in cell culture and the first indication of infection is often poor growth of the cells and poor appearance.

Mycoplasma can be detected by growth on specific culture media or with specific staining techniques on the cells. (Mycoplasma testing of media prior to use is difficult and components should be sourced with a Mycoplasma free guarantee).

Detection of viral contamination

The components of the cell culture medium are not routinely tested for viruses except by some commercial sources.

Cell cultures may contain latent viruses, some of which can be detected by light microscopy, electron microscopy or haemadsorption. Some cell lines when obtained from a commercial source will be guaranteed free of certain latent viruses.

Detection of contamination of cells by other cell lines

This contamination may occur prior to receipt in the laboratory or during cell manipulation. Testing of cell lines for purity is not routinely carried out and may only be vital in research work. Detection may be by simple microscopy. More complex methods of detecting extraneous cells involve genetic analysis of the cells, isoenzyme electrophoresis and immunofluorescence[25].

5.3 Identification

N/A

6 Quality Assurance

6.1 Assessment of Preparation

A quality system should be in place to ensure that appropriate internal and external quality assessment and quality control procedures are maintained (Q 2 – Quality Assurance in the Diagnostic Virology and Serology Laboratory)[26].

It is essential that laboratories have evidence of adequate validation of methods, equipment and commercial and in-house test procedures demonstrating that they are fit for the purpose[27].

All cell lines must be checked for virus susceptibility when a new stock is obtained and at the intervals stated below. New stock should be obtained certified free from bacteria, mycoplasma, fungi, and yeasts.

Continuous cell lines may be maintained in medium containing Kanamycin (100µg/mL) for 4 weeks to remove contamination with mycoplasma[2]. Cultures suspected of contamination with mycoplasma should be checked using fluorescence microscopy.

Tubes should be retained from every production batch to check for: -

- Satisfactory morphology, growth, and longevity by microscopic examination
- Freedom from contamination with viruses, bacteria, yeasts, and/or fungi

6.1.1 Growth

Cell growth should be assessed by microscopic examination. Cells should achieve semi-confluent growth within 48hr depending upon cell type.

Morphology should be consistent with the cell type; any variation should be noted. Cells displaying inconsistent or wide variation in morphology should be discarded and a new batch brought into use.

6.1.2 Sterility

All reagents produced "in-house" must be checked for sterility by culture in a liquid bacterial growth medium (LabLemco broth or similar).

6.1.3 Susceptibility

A suitable panel of viruses should be tested, eg virus panel:

- Adenovirus
- Herpes Simplex virus
- Respiratory syncytial virus
- Rhinovirus
- Influenza A virus (obtain current strain from Respiratory virus reference Laboratory)

Preparation and storage of virus control panel

Susceptibility testing

Cell Line	Frequency	Test Virus	Comments
MRC-5	On receipt and following thawing from liquid Nitrogen	HSV, RSV	
RhMK	Primary culture and following thawing from liquid Nitrogen	Polio Influenza A	Check haemadsorption
MDCK	On receipt and following thawing from liquid Nitrogen	Influenza A	Check haemadsorption
HEP-2	On receipt and following thawing from liquid Nitrogen	Adenovirus RSV	
VERO	On receipt and following thawing from liquid Nitrogen	HSV	
PLC-PRF5	On receipt and following thawing from liquid Nitrogen	Adenovirus Polio	

1. The virus controls should be inoculated into two tubes of the appropriate cell lines in the same manner as specimens and incubated at the required temperature.
2. Always include negative controls.
3. The cell lines should be observed daily for CPE and the results should be recorded.
4. If a virus control/cell line combination fails to give the expected result and the cause cannot be ascertained or corrected, the cell line should be discarded.

6.2 Internal and External Quality Assurance

N/A

7 Limitations

Successful isolation of organisms depends on correct specimen collection, transport, storage and processing; the quality and range of cell lines used and the use of correct conditions for culture and the provision of adequate/suitable clinical information.

Susceptibility should be checked on acquisition and at regular intervals while in use.

Cells removed from liquid nitrogen should be checked for sensitivity prior to use.

8 Reporting Procedure

N/A

9 Notification to PHE[28,29] or Equivalent in the Devolved Administrations[30-33]

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 HIV & STIs, HCAIs and CJD under ‘Notification Duties of Registered Medical Practitioners’: it is not noted under ‘Notification Duties of Diagnostic Laboratories’.

Other arrangements exist in Scotland[30,31], Wales[32] and Northern Ireland[33].

Appendix: Storage of Cells in Liquid Nitrogen

Health and Safety

Note: Local risk assessments must be carried out on this procedure.

These procedures should only be carried out by trained or closely supervised staff.

The temperature of liquid nitrogen is -196°C and the vapour phase in the refrigerators varies between –120°C and –170°C. Therefore cryogenic work must be carried out with extreme caution and using protective equipment, especially gloves and goggles. Upon evaporation, the liquid produces a volume of gas 682 times greater than the original volume; the vapour is heavier than air. Therefore, liquid nitrogen should always be stored and handled in a well-ventilated area where an oxygen monitor and alarm is fitted.

Routine Procedures & Maintenance

The gas phase vessels must be checked every day.

The refrigerators must *never* be allowed to become empty or valuable and irreplaceable resources will be lost.

Materials and Equipment

1. Di-methyl sulphoxide (DMSO)

Note: DMSO is toxic and should be handled with care. It can be absorbed through the skin and may cause irritation and/or burns. It is teratogenic and an allergen. Wear gloves and if not working in a cabinet, eye protection should be worn

2. MEM growth medium as required for the individual cell line.
3. Cryotubes (plastic).
4. Freezing equipment to allow controlled cooling at 1°C per minute.

Methods

Actively growing cell cultures should be used for storage in liquid nitrogen. When cells are frozen and stored in liquid nitrogen, complete the appropriate “stock record sheet”. Record the storage bin ID, rack number and position on the correct record sheet, and the rack plan for each individual vial.

1. Harvest the cells using the relevant reagent(s). However, when harvesting cells, leave the full volume of reagent(s) in contact with the cells and follow the method below.
2. When all the cells are detached, pipette the suspension aseptically into a universal and centrifuge at 1500-2000*g* for 5 minutes.
3. While the cells are centrifuging, prepare a 10% solution of DMSO in the relevant growth medium for the cells.
4. Remove the supernatant fluid from the centrifuged cells and resuspend them in the DMSO\GM mixture. Use approximately 1.8mL of medium per ampoule required. The number of ampoules is decided by the number of flasks stripped and density of growth of the cells.

5. Dispense the suspension into Cryotubes. Label the tubes with the cell type, passage number (if required), tube number, and date.
6. Place the ampoules in freezing equipment to allow controlled cooling at 1°C per minute.
7. Remove the ampoules from the cooling equipment when the temperature reaches -60°C or below.
8. Enter the details of each batch on the correct record chart.

Removal from Storage

1. Locate the required ampoule and carefully remove it from the rack. When vials are removed from liquid nitrogen, cross out the vial removed on the rack plan and record the date of removal on the record sheet.
2. Rapidly thaw the ampoule in the 37°C water bath, without total immersion.
3. When thawed centrifuge the ampoule at 1500-2000*g* for 5 minute.
4. Remove the supernatant fluid and resuspend the cells in the appropriate growth medium.
5. Count the cells, dilute to the required concentration and seed into flasks or tubes as required.
6. Record the removal on the relevant record chart.

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