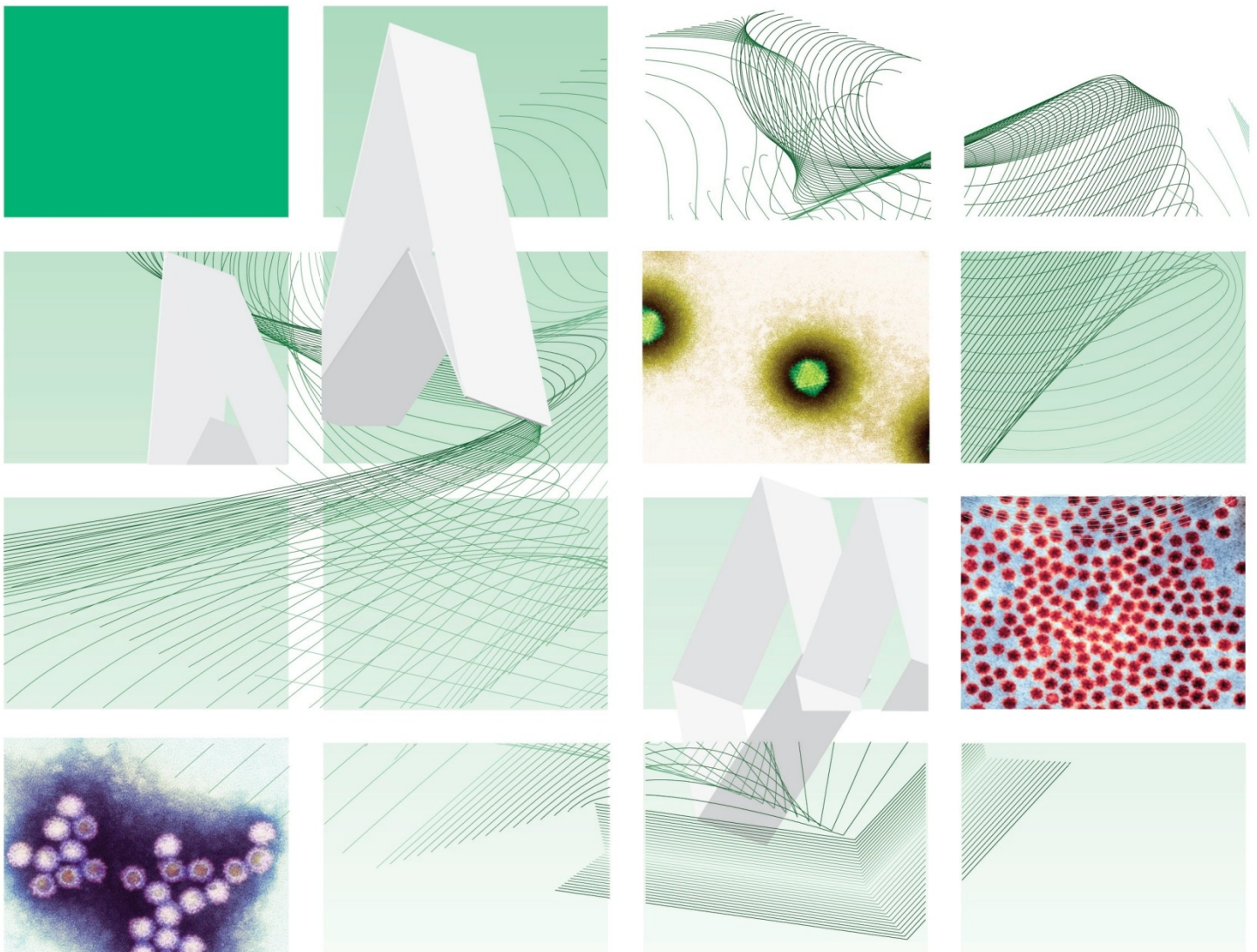




UK Standards for Microbiology Investigations

Isolation of Enteroviruses and Parechoviruses



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>).

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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.	6/10.10.13
Issue no. discarded.	3.2
Insert Issue no.	3.3
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 references have been reviewed and updated.</p> <p>Scientific content remains unchanged.</p>

Amendment No/Date.	5/20.04.12
Issue no. discarded.	3.1
Insert Issue no.	3.2
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

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<http://www.hpa.org.uk/SMI/pdf>.

Scope of Document

The SMI describes the isolation and identification of enteroviruses and parechoviruses from clinical material.

This SMI should be used in conjunction with other SMIs.

Introduction

Background

In the genera *Enterovirus* and *Parechovirus* in the family *Picornaviridae* there are over 60 serotypes that can infect humans. The human enteroviruses are classified into 5 species, the polioviruses and the human enterovirus groups A-D¹. Before the more recent molecular classification subgroups were based on serotype relationships and included polioviruses (3 serotypes), Coxsackie A and B (23 and 6 serotypes respectively), echoviruses (26 serotypes) and the newer numbered enteroviruses types 68-71. Two viruses formerly classified with the echoviruses, echovirus 22 and 23, were shown to comprise a separate genus within the *Picornaviridae* family, the genus *Parechovirus*; these viruses are now referred to as human parechovirus types 1 and 2. Discovery and characterisation of picornaviruses has progressed rapidly with the introduction of molecular testing and molecular typing schemes and there are now over 90 viruses classed as enteroviruses and 6 parechovirus types have been described. Throughout this document the term 'enterovirus' will be used generically to cover both enteroviruses and parechoviruses.

The enteroviruses can cause a wide spectrum of human illness, from mild non-specific fever and rash to upper respiratory tract infections, aseptic meningitis, and pleurodynia, through to life-threatening infections such as myocarditis, encephalitis and paralytic poliomyelitis. The majority of infections however are asymptomatic.

Diagnosis of enterovirus infection by isolation in cell culture is of diminishing importance, it was relatively quick and the isolate could be typed using antisera for epidemiological purposes. However PCR methods are being used increasingly. These are generally more sensitive and more rapid than cell culture methods and can detect infections with uncultivable enteroviruses^{2,3}.

Technical Information/Limitations

Specimen Containers^{4,5}

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 Considerations⁴⁻²⁰

1.1 Specimen Collection^{4,5}

Appropriate hazard labelling according to local policy should be used.

1.2 Specimen Transport and Storage⁴⁻⁹

Compliance with current postal and transport regulations is essential.

A suitable virus transport system must be used (where appropriate) and the specimen placed in a sealed plastic bag or pouch.

Appropriate hazard labelling according to local policy should be used.

1.3 Specimen Processing⁴⁻²⁰

- All enteroviruses are in Hazard Group 2. Laboratory procedures that may give rise to infectious aerosols must be conducted in a microbiological safety cabinet
- Vaccination against poliovirus is required; guidance is given in the Health Protection Agency immunisation policy
- Refer to current guidance on the safe handling of all Hazard Group 2 organisms documented in this UK Standard for Microbiology Investigation
- Please note that laboratories retaining wild poliovirus infectious or potentially infectious materials are recommended to operate under biosafety level 2/polio (BSL-2/polio)²¹

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

2 Specimen Collection

2.1 Type of Specimens

Throat swab, vesicle swab, eye swab, CSF, faeces, pericardial fluid, rectal swab, biopsy tissue, vesicle fluid, post mortem tissue

2.2 Optimal Timing of Specimen Collection^{4,5}

Swabs, scrapings, pericardial fluid and tissue should be taken as soon as possible after symptoms appear and put immediately into Virus Transport Medium (VTM). Faeces and CSF should be collected in CE marked leak proof containers sealed in plastic bags.

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^{4,5}

3.1 Time between Specimen Collection and Processing

Specimens should be processed as soon as possible. Ideally, transportation systems should ensure that specimens arrive in the laboratory within 24 hours.

3.2 Special Considerations to Minimise Deterioration

Specimens should be placed in a suitable VTM immediately after collection.

When there is a delay in processing, specimens should be refrigerated. If the delay is likely to exceed 24 hours, samples should be frozen at -70°C, or lower, and thawed immediately prior to processing. Repeated freezing and thawing should be avoided.

4 Specimen Processing/Procedure^{4,5}

4.1 Test Selection

In the past enterovirus isolation in cell culture was the most widely used method for diagnosis. PCR techniques now offer significant advantages in terms of sensitivity and turnaround time. Although PCR does not provide an isolate for typing specialist centres may still be able to infer the species type by gene sequencing of the original sample²².

4.2 Culture and Investigation

4.2.1 Sample preparation

Faeces

Faecal specimens are made into a 10-20% suspension in a balanced salts solution with antibiotics, then clarified by centrifugation at 1600-2000g for 10 minutes.

Tissues

Tissues are ground in a sterile mortar. Occasionally the addition of sterile sand and a small amount of medium is required.

After making a 10-20% suspension in VTM, clarification by centrifugation should occur at 1600-2000g for 10 minutes.

Swabs

Swabs should be agitated to release cellular material into the virus transport medium, taking care not to produce aerosols.

CSF and Pericardial fluid specimens

These are considered pure samples and require only minimum preparation.

4.2.2 Isolation

Many of the cell lines in routine use for virus isolation are susceptible to infection with most enteroviruses. Of the commonly used cell lines, primary rhesus monkey kidney (RMK) are the most susceptible but have not been available since April 2006 for ethical reasons. The human diploid cell lines eg MRC-5 have been found to be equally susceptible and to show cytopathic changes more rapidly than other cell lines²³. RD

(rhabdomyosarcoma) cells have also been found to be susceptible to many enteroviruses (exception being the Coxsackie B group) including a number of the Coxsackie A group which normally are only isolated in suckling mice²⁴. Multiple cell lines should be used in order to increase the yield and enhance the rapidity of enterovirus isolation.

Inoculate up to 0.2mL CSF, faecal extract or VTM containing the clinical material into each of the two cell lines. The cells should be incubated at 35–37°C, for up to 10 days. They should be examined at regular intervals for the appearance of cytopathic changes characteristic of enteroviruses.

4.3 Identification

4.3.1 Within the laboratory

The growth of enteroviruses in cell culture may be identified through the appearance of characteristic cytopathic changes and confirmed by immunofluorescence or neutralisation.

Serotyping of enteroviruses should be performed, principally to exclude poliovirus. This is commonly done using indirect immunofluorescence with type-specific monoclonal antibodies to determine the enterovirus group, echovirus, coxsackie B virus and poliovirus. Poliovirus should be further typed to determine serotype 1-3.

Further typing may be carried out if required by indirect immunofluorescence or neutralisation tests using the Lin, Benyesh, Melnick (LBM) combination neutralising serum pools or other commercial reagents²⁵.

Preparation and staining of samples for typing by immunofluorescence using commercial reagents should be carried out strictly in accordance with the manufacturer's instructions.

4.4 Referral to Reference Laboratories

All polioviruses and unidentified viruses for typing must be referred to PHE Colindale. It is important that surveillance continues to find and to differentiate vaccine and wild type polioviruse isolates. For further information on polioviruses refer to [P 1 – Surveillance of polio in the UK](#).

5 Quality Assurance

A quality system should be in place to ensure that appropriate internal and external quality assessment and quality control procedures are maintained. For further information on quality assurance refer to [Q 2 – Quality assurance in the diagnostic virology and serology laboratory](#).

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 purpose²⁶.

6 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.

Only cell lines proven to be susceptible to infection with enterovirus should be used. Susceptibility should be checked on acquisition and at regular intervals while in use. Cells removed from liquid nitrogen storage should be checked for sensitivity before use.

The procedure(s) in these documents aim to describe good microbiological standards 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.

7 Reporting Procedure

7.1 Reports

Negative specimens should be reported as

“Virus not isolated”.

Positive specimens should be reported as

“Enterovirus (type xx) isolated” or “Enterovirus isolated. Typing results to follow”.

8 Notification to PHE^{27,28} or Equivalent in the Devolved Administrations²⁹⁻³²

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

Other arrangements exist in Scotland^{29,30}, Wales³¹ and Northern Ireland³².

References

1. Pringle CR. Virus taxonomy at the XIth International Congress of Virology, Sydney, Australia, 1999. Arch Virol 1999;144:2065-70.
2. Corless CE, Guiver M, Borrow R, Edwards-Jones V, Fox AJ, Kaczmarek EB, et al. Development and evaluation of a 'real-time' RT-PCR for the detection of enterovirus and parechovirus RNA in CSF and throat swab samples. J Med Virol 2002;67:555-62.
3. Santos AP, Costa EV, Oliveira SS, Souza MC, Da Silva EE. RT-PCR based analysis of cell culture negative stools samples from poliomyelitis suspected cases. J Clin Virol 2002;23:149-52.
4. European Parliament. UK Standards for Microbiology Investigations (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".
5. Official Journal of the European Communities. Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on *in vitro* diagnostic medical devices. 7-12-1998. p. 1-37.
6. Health and Safety Executive. Safe use of pneumatic air tube transport systems for pathology specimens. 9/99.
7. Department for transport. Transport of Infectious Substances, 2011 Revision 5. 2011.
8. World Health Organization. Guidance on regulations for the Transport of Infectious Substances 2013-2014. 2012.
9. Home Office. Anti-terrorism, Crime and Security Act. 2001 (as amended).
10. Advisory Committee on Dangerous Pathogens. The Approved List of Biological Agents. Health and Safety Executive. 2013. p. 1-32
11. Advisory Committee on Dangerous Pathogens. Infections at work: Controlling the risks. Her Majesty's Stationery Office. 2003.
12. Advisory Committee on Dangerous Pathogens. Biological agents: Managing the risks in laboratories and healthcare premises. Health and Safety Executive. 2005.
13. Advisory Committee on Dangerous Pathogens. Biological Agents: Managing the Risks in Laboratories and Healthcare Premises. Appendix 1.2 Transport of Infectious Substances - Revision. Health and Safety Executive. 2008.
14. Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. MMWR Surveill Summ 2012;61:1-102.
15. Health and Safety Executive. Control of Substances Hazardous to Health Regulations. The Control of Substances Hazardous to Health Regulations 2002. 5th ed. HSE Books; 2002.
16. Health and Safety Executive. Five Steps to Risk Assessment: A Step by Step Guide to a Safer and Healthier Workplace. HSE Books. 2002.

17. Health and Safety Executive. A Guide to Risk Assessment Requirements: Common Provisions in Health and Safety Law. HSE Books. 2002.
18. Health Services Advisory Committee. Safe Working and the Prevention of Infection in Clinical Laboratories and Similar Facilities. HSE Books. 2003.
19. British Standards Institution (BSI). BS EN12469 - Biotechnology - performance criteria for microbiological safety cabinets. 2000.
20. British Standards Institution (BSI). BS 5726:2005 - Microbiological safety cabinets. Information to be supplied by the purchaser and to the vendor and to the installer, and siting and use of cabinets. Recommendations and guidance. 24-3-2005. p. 1-14
21. Standard biosafety level 2 requirements. WHO Laboratory Biosafety Manual. 3rd ed. World Health Organisation; 2004. p. 9-19.
22. Iturriza-Gomara M, Megson B, Gray J. Molecular detection and characterization of human enteroviruses directly from clinical samples using RT-PCR and DNA sequencing. *J Med Virol* 2006;78:243-53.
23. Chonmaitree T, Ford C, Sanders C, Lucia HL. Comparison of cell cultures for rapid isolation of enteroviruses. *J Clin Microbiol* 1988;26:2576-80.
24. Schmidt NJ, Ho HH, Lennette EH. Propagation and isolation of group A coxsackieviruses in RD cells. *J Clin Microbiol* 1975;2:183-5.
25. Lim KA, Benyesh-Melnick M. Typing of viruses by combinations of antiserum pools. Application to typing of enteroviruses (Coxsackie and ECHO). *J Immunol* 1960;84:309-17.
26. Clinical Pathology Accreditation (UK) Ltd. Standards for the Medical Laboratory. Sheffield. 2004. p. 1-56
27. Public Health England. Laboratory Reporting to Public Health England: A Guide for Diagnostic Laboratories. 2013. p. 1-37
28. Department of Health. Health Protection Legislation (England) Guidance. 2010. p. 1-112.
29. Scottish Government. Public Health (Scotland) Act. 2008 (as amended).
30. Scottish Government. Public Health etc. (Scotland) Act 2008. Implementation of Part 2: Notifiable Diseases, Organisms and Health Risk States. 2009.
31. The Welsh Assembly Government. Health Protection Legislation (Wales) Guidance. 2010.
32. Home Office. Public Health Act (Northern Ireland) 1967 Chapter 36. 1967 (as amended).