Public Health England

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UK Standards for Microbiology Investigations

Good Laboratory Practice when Performing Molecular Amplification Assays

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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).

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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.	9/16.12.13
Issue no. discarded.	4.3
Insert Issue no.	4.4
Section(s) involved	**Amendment**
Whole document.	Document transferred to an updated template.

Amendment No/Date.	8/04.11.13
Issue no. discarded.	4.2
Insert Issue no.	4.3
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. Scientific content remains unchanged.

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/webw/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.

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Scope of Document

This SMI describes key elements of how to organise facilities for polymerase chain reaction (PCR) testing including workflow, reagents, consumables and staff within a molecular diagnostic laboratory[1].

This SMI should be used in conjunction with other SMIs.

Introduction

The ability of PCR to produce large numbers of copies of a target sequence from minute quantities - sometimes single copies - of DNA has provided the exquisite sensitivity that makes PCR a powerful diagnostic tool. However, this ability also necessitates that extreme care be taken to avoid the generation of false positive results.

False positive results can result from sample-to-sample contamination and, perhaps more commonly, from the carry-over of DNA from a previous amplification of the same target. Other sources of contamination may include cloned DNA and virus cell cultures.

Careful consideration should be given to facility design and operation within clinical laboratories in which polymerase chain reactions are performed. This document describes procedures that will help to minimise the carry-over of amplified DNA.

Whilst the guidelines concern the majority of PCR applications, they are most relevant where 'in-house' assays are in use and may be less relevant when using commercial kits, and to other amplification procedures. These guidelines apply to many modifications of the basic PCR protocol eg nested PCR, although no specific provision is made within the guidelines. However, the greatest threat of contamination lies in laboratories that practise techniques that involve manipulation of amplified product or cloned DNA such as plasmids containing DNA target regions. Laboratories exclusively performing real-time PCR and discarding all amplified product without opening the tubes or sealed plates containing product are less liable to contamination. Further reassurance can be provided in many commercial or in-house systems by the enzymatic anti-contamination features described in section 5. Even in laboratories that avoid manipulation of product the good practice described in this document should be standard practice, especially in the clean room. Similar guidelines are available from other authorities[2].

1 General Considerations

1.1 Organisation of Work

Practise good housekeeping policy at all times. Do not keep tubes or reagents any longer than necessary. All reagents, reaction tubes etc. should be clearly labelled. Records of batch numbers of all reagent batches used in individual assays should be kept.

Avoid entering pre-amplification rooms immediately after working in rooms where products, cloned materials and virus cultures are handled. If working with these materials is unavoidable judicious use of clean laboratory coats, gloves and hand washing is necessary. Gloves should be changed frequently.

Ensure that all equipment, including paper, pens and lab coats are dedicated for use only in that particular laboratory eg a laboratory coat for each of the PCR rooms. Workbooks that have been in contaminated areas should not be taken into clean PCR areas.

PCR reagents should be aliquoted to avoid excessive freeze-thawing and to protect stock reagents from contamination.

Pulse centrifuge tubes before opening the reagents. Uncap and close tubes carefully to prevent aerosols.

Bench areas in PCR laboratories should be wiped daily with hypochlorite solution following use[3]. Validated chemical or a suitable alternative may be preferred. Containment areas can additionally be decontaminated using ultra-violet radiation if fitted.

All new members of staff, visitors and students must be trained in use of the PCR facilities. It is recommended that a formal induction process be established for these laboratory workers.

For reverse transcription (RT) PCRs, specific precautions are necessary to prevent contamination of equipment, consumables and reagents with RNAses, as these will lead to false negative results.

2 Specimen Processing

All accreditation schemes stipulate some requirements for the storage and retention of specimens and records.

Avoid molecular contamination problems of PCR through Good Laboratory Practice and following the unidirectional workflow[4] (see below).

2.1 Physical Separation of Pre-PCR and Post-PCR Assay Stages

To prevent carry-over of amplified DNA sequences[5], PCR reactions should be set up in a separate room or containment area ('PCR workstation' laminar flow cabinet) from that used for post-PCR manipulations.

A complete separate set of necessary laboratory equipment, consumables, and laboratory coats should be dedicated for the specific use of pre - or post-PCR manipulations according to the area designation. Care must be taken to ensure that

amplified DNA, virus cultures or DNA clones other than low copy number control material do not enter the 'Pre-PCR area'.

Reagents and supplies should be taken directly from (clean) storage into the pre-PCR area and should never be taken or shared with areas in which post-PCR analyses are being performed. Similarly, equipment such as pipettes should never be taken into the containment area after use with amplified material.

2.2 The Unidirectional Workflow[6]

Where possible PCR facilities should be organised into four discrete areas/rooms as described below. Requirements may vary with the assay format and platform. For example, for real-time PCRs only 3 areas may be required as post-PCR analysis is not required. However, for nested PCR assays, the additional steps require that four rooms/areas are available.

Workflow between these rooms/areas must be unidirectional ie from clean areas to contaminated areas, but not from contaminated areas to clean labs. Dedicated laboratory coats shall be supplied for each area and gloves shall be changed between areas. Staff will have to leave product analysis areas and go back to the earlier rooms eventually. It is here that rigid adherence to good practise is most essential. Coats, gloves and any other personal protective equipment should be changed, and hands washed. No working materials can be brought back to earlier stages, not even notebooks or pens or memory sticks.

2.3 Reagent Preparation Clean Room

In this area, PCR reagents are stored. It is very important to keep this room/area free of any biological material (this includes DNA/RNA extracts, samples, cloned materials and PCR products).

Procedures carried out in this area include preparation and aliquoting of reagent stocks and preparation of reaction mixes prior to the addition of the clinical nucleic acid (which takes place in the nucleic acid extraction room or, in the second round of a nested PCR, in the PCR machine room). Aliquoting of primers and other reagents is recommended to minimise any consequence of contamination and reduce assay downtime.

2.4 The Nucleic Acid Extraction Room

Extraction of nucleic acid from clinical samples must be performed in areas where PCR products and stocks of cloned materials have not been handled. A second clean area is therefore required for this purpose. The second area is where the samples are processed, where the reverse transcriptase step of RT-PCRs is performed and where the extracted DNA or cloned DNA (cDNA) and positive control is added to the PCR reaction mixes (previously prepared in the reagent preparation room).

Specimens for PCR should come directly from the clean specimen receipt room into the extraction laboratory; the samples should never enter rooms where PCR products and cloned DNA are present.

2.5 The Amplification Room

The amplification room is the area in which the PCR machines are housed. It may also contain a containment area in which, for nested PCRs, the second round reaction

mixes are inoculated with the primary reaction product. Cloned DNAs should not be brought into this area.

Where PCR machines are shared, a clear booking system is recommended to provide a cohesive system for the assays. Individual users' PCR programs in the thermocyclers should not be edited by other users (even temporarily) without notification to the program owner.

2.6 The Product Analysis Rooms

This is the room in which post-PCR manipulations are performed eg agarose gel electrophoresis of products, PCR-ELISA detection systems. This is a contaminated area and therefore no reagents, equipment, laboratory coats etc. from this room should be used in any of the other PCR areas.

3 Handling of Mastermixes

- Mastermixes should be aliquoted in appropriate volumes for the usage requirement of an assay and to minimise the number of freeze/thaw cycles
- Mastermixes should be subjected to minimal thawing and put on ice as soon as possible
- Mastermixes containing either fluorophores, as in probe-based assays, or DNA-binding dyes, such as SYBR Green, should not be exposed to excessive light in order to prevent degradation by photo-bleaching.[8]

4 Selection of Controls

Assay controls are included according to the individual assay protocol. However, as general guidelines, the following are suggested:

- A positive amplification control: this should normally be an extract that amplifies weakly but consistently within an acceptable range. Use of a strong positive is an unnecessary risk as it can be a possible reservoir of contamination
- A positive amplification control derived from a plasmid should always be diluted to give a detection endpoint expected from a weak positive
- A negative or "no template" amplification control, eg nuclease free water, should always be included to control the reagent mastermix
- Extraction controls: known positive and negative specimens for an assay may be extracted and tested to act, respectively, as a control for successful nucleic acid extraction and a check on contamination during extraction
- Internal controls: these should be used to control for inhibitors of the PCR process and for failures of extraction or the PCR process including potential technical errors, for example failure to add the extracted sample to the PCR reaction. Internal controls should ideally be used to control for the whole process of sample extraction and PCR amplification. Demonstration of the internal control sequence by PCR in a duplexed reaction with the target sequence can therefore confirm potential to amplify the target sequence and validate a negative result. They should ideally represent the target organism as closely as possible. For RNA targets detected through reverse-transcriptase

PCR, an RNA control should be used to control for this step. A range of approaches have been used including addition of bacteriophages such as MS2 or lambda phage or addition of DNA or RNA transcripts. Where human DNA or RNA is co-purified with the target organism detection of human gene targets especially 'house-keeping genes' such as β-globin have been used as internal controls. This has an added advantage of controlling for the adequacy of the sample, although assessments of partial inhibition are more difficult where expected levels of human DNA are not known

- Internal controls added at the PCR stage will control for inhibitors in the PCR but will not control for sample extraction

5 Other Non-Contamination Approaches

- A number of additional measures and procedures can be included in the assay protocol to minimise further the likelihood of contamination, the most common of which is the use of Uracil-DNA Glycosylase[9]. While use of such measures is recommended (as per assay protocol), it is to be noted that these are used in addition to the good laboratory practices outlined above, not as an alternative
- Decontamination is performed using UV-irradiation, sodium hypochlorite or 1M HCl[3,9]
- Regular environmental swabbing of areas where high through put PCRs are carried out is recommended
- Staff awareness of these issues and how they play an important role in the prevention of contamination

6 Quality Issues

It is important to demonstrate that assays are performing consistently and that results reported are reliable and accurate. Where available it is advisable to run external controls for commercial and in-house based assays. For many viral targets quantitated controls can be obtained from commercial and other sources (eg NIBSC). Keeping a regular record of these results will help to identify problems at an early stage.

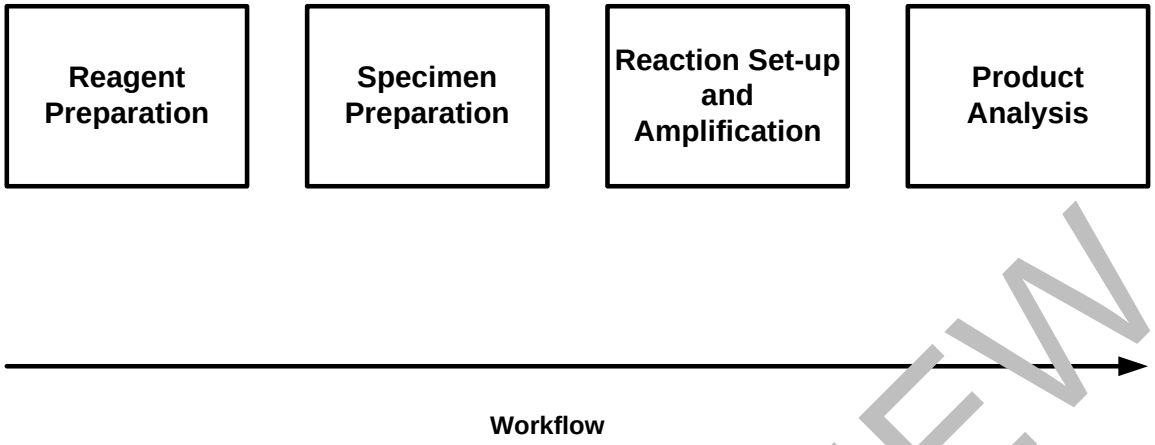
Assays should be appropriately validated before introduction into routine use (see Q 1 Commercial and In-House Diagnostic Tests: Evaluations and Validations). Note that the validation of an in house assay (as for a CE marked assay) is a validation of the total process. Any change in that process, be it in extraction procedure, reagents, cycling parameters, introduction of internal controls for inhibition, will necessitate a documented revalidation of the whole process.

New batches of reagents eg primers, probes PCR mix etc need to be assessed for performance against such well characterized control material and recorded as an auditable record.

Participation in quality assurance programmes is essential if a scheme exists eg National External Quality Assurance Scheme (NEQAS) and Quality Control for Molecular Diagnostics (QCMD). If no scheme exists interlaboratory collaboration for the exchange of samples is advisable to ensure that the test is performing correctly.

Contamination is a potential threat when using sensitive nucleic acid amplification techniques and regular environmental monitoring serves as a useful indicator of potential problems. Many commercial systems now recommend environmental monitoring as part of the housekeeping and maintenance procedures but this should also be carried out for in-house assays.

Appendix: Diagram Showing Workflow in a PCR Laboratory



Note: Although four rooms are ideal, many laboratories only have two rooms available.

Pre-PCR and extraction are therefore carried out within defined areas of a larger laboratory and amplification and product analysis are in a second laboratory.

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