Technical Guide
PCR Analysis Guidelines

Second Edition July 2020

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Technical Guide

Chemical and Biological Testing Laboratories

PCR Analysis Guidelines

AS TG 6

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0 Background and Scope

The 2010s saw a marked increase in accredited microbiology laboratories adopting PCR technologies to complement and/or replace their traditional culture methodologies. Many of these laboratories were new to molecular biological testing techniques and disciplines and the development of expert guidance on the accreditation expectations of such testing was considered appropriate. This technical guideline is intended for those microbiology laboratories performing PCR analyses, either in dedicated zones fit for the purpose of PCR testing or in a shared environment. The guidance can equally be applied to applicant and accredited chemical testing laboratories expanding their capabilities into PCR analyses.

1 Staff training and experience

Staff approved to release results must be able to evaluate and interpret test results and have the ability to orally communicate the technical aspects and regulatory requirements of the analysis and reporting to a lay audience.

All staff members supervising and/or carrying out analyses shall have formal training in PCR theory and technique, commensurate with their qualifications and practical experience. This could include an external training courses and/or internal training courses, in either case records shall be maintained for this training.

Training shall be provided at a level appropriate to the staff member i.e. non-PCR staff members who work in the same area shall be provided with basic knowledge to ensure their activities do not inadvertently impact on PCR testing. Whilst PCR-technicians shall have at a minimum basic knowledge for the processes they are involved in. Key Technical Personnel or Signatories shall have sufficient training to troubleshoot any issues that arise i.e. a high level of competence and be able to assess issues in an ‘analyst independent’ manner.

2 Equipment

Use of specialised equipment, such as a thermocycler etc, should follow, at a minimum, the manufacturer’s instructions on use, calibration and maintenance. Where there are regulatory requirements, these requirements take precedence over the manufacturer’s instructions. Equipment such as thermocyclers sometimes have internal diagnostics controls; where this is the case the manufacturer’s instructions should also be followed.

Utensils and pipettes shall either be single use or decontaminated in-between use with an appropriate DNA decontamination solution. Plastic ware (tubes, pipettor tips, etc.) should be disposable and single use. Pipettes shall be left hanging on racks, and not left lying on the bench top. Balance pans shall be cleaned in-between use with an appropriate DNA decontamination solution. All waste shall be disposed of in biohazard bags.

Also see the IANZ Biological Specific Criteria for the recommended calibration frequencies.

3 Sample handling

Due to high sensitivity of these methods, samples shall be treated in a manner appropriate for PCR testing from sample receipt through all stages of testing to reporting and sample disposal. A higher than usual awareness of possible sources of DNA contamination is required during sampling, transport, receipt, storage, sample preparation and analysis of samples.

Accommodation considerations shall include separate sample bins, dedicated fridge storage areas (shelves) in sample receipt, transport to the test laboratory area, and incubators for sample incubation.

Fresh gloves shall be worn, or decontaminated with a DNA removal solution, when handling samples.

4 Quality control and proficiency testing

Under the requirements of accreditation, laboratories need to participate in some form of proficiency programme (see Biological Specific Criteria, Section 12). Where a test method is comprised of multiple individual test methods and target organisms, proficiency testing shall take this into account and cover all such testing.

In-process (broth) controls shall reflect the target organism, where more than one target organism is being analysed, the internal controls shall cover all possible variations. Controls shall include a positive (target)

control at a level just above the detection threshold (limit of sensitivity) of the assay and a reagent/broth blank.

If a control does not meet expected outcomes then the entire run of samples processed with those controls shall be repeated. This process must be clearly documented in laboratory procedures.

5 Sample enrichment

Samples for PCR testing shall be dedicated and not shared with other analyses. Sample enrichment, or extraction, shall take place within in a class 2 biohazard safety cabinet.

The presence of inhibitors is a major concern. Procedures shall be designed to minimise the risk of false negatives due to the presence of inhibitors of the PCR reaction.

Procedures shall be documented that include the use agents, or processes, to eliminate or reduce the effect of inhibitors.

Where the use of such agents are mandated by a regulator, then regulatory procedures shall be followed and not left to individual staff members to decide whether or not to add such agents.

Where commercial kits are used and include inhibition controls as an optional component it is recommended that these be used.

6 Accommodation guidelines and environmental monitoring

In microbiology laboratories where cultured samples are present with micro-organisms in high concentrations there is greater chance for aerosol contamination. To reduce false positives by cross contamination, or carry-over contamination of samples and reagents by other samples or amplified material, four physically separate and contained areas within the laboratory are required.

These areas are a sample enrichment area, reagent preparation area, sample preparation (DNA extraction) area and an amplification and detection area. It is preferred that these areas are self-contained where no other work is occurring. If this is not possible then restricted access to these areas is required where, at a minimum, other staff not performing PCR are not allowed to enter.

The laboratory shall provide gloves and gowns in each designated area that are not worn in other areas of the laboratory. Gloves shall be discarded after use and hands shall be washed before leaving that area.

Cleaning of large areas shall be with a 10% bleach solution (or equivalent) with a minimum of ten minutes contact time. Cleaning the area with an ethanol after the bleach cleansing is recommended to protect surface areas. For smaller areas where a quick decontamination is required, specialised proprietary cleaning agents for removing DNA other than bleach should be used. It should be noted that the level of decontamination is critical and must be of a high standard.

Swabbing of the laboratory environment for potential sources of DNA contamination should be performed on a regular basis alongside other pathogen environmental testing after the method has been validated to ensure that the swab matrix can be confirmed as appropriate.

Where the workspace has a shared environment with other organisms, then the laboratory shall endeavour to analyse these other organisms by the PCR method employed to check that there is no amplification that could result in a false positive result.

Once samples have been cultured or extracted, samples shall be transported within the laboratory area inside sealed containers. Any containers or racks used shall be washed in a 10% bleach solution (or equivalent) either by overnight soaking, or for a minimum of ten minutes contact time. Any trolleys used by the laboratory to transport large volumes shall be cleaned before and after use with a DNA removing solution that has an immediate effect.

7 Reagent preparation, sample preparation and DNA amplification/detection layout

Workflow shall be arranged so that staff do not take samples into a previous area i.e. movement shall be unidirectional. Workflow shall go from sample enrichment, to reagent preparation, to sample preparation to the amplification areas. All work areas shall contain their own designated supply of consumables and equipment (e.g. pipettes etc).

Post-PCR (amplification) areas should be in a separate area to other pre-amplification samples/areas to avoid DNA cross-contamination. Where this is not possible, the amplification area shall be positioned to avoid the possibility of contamination of the pre-amplification samples/areas.

The reagent preparation area shall be dedicated, separate, clean and in a contained area. This area should be physically separate from other areas of the laboratory. Where it is not possible for a separate designated room, this area shall be as far away from other processes in the laboratory as possible and use either a class 2 biohazard cabinet or Laminar flow set-up cabinet.

The sample preparation/DNA extraction area shall be dedicated, separate, clean and in a contained area. This area should be physically separate from other areas of the laboratory. Where it is not possible for a separate designated room, this area shall be as far away from other processes in the laboratory and should use a class 2 biohazard cabinet.

Sub-sampling of large sample containers into smaller containers shall be performed inside the biohazard cabinet. Any reusable auto-pipettes used shall be used in a manner so that the main portion of the auto-pipette does not enter into the larger sample container. Single use disposable transfer pipettes are preferred. The layout of equipment inside the cabinet shall be arranged in such a way as to not carry used items that may carry DNA contamination over fresh samples or consumables.

The amplification and detection area shall be dedicated, separate, clean and in a contained area. This area should be physically separate from other areas of the laboratory. As this stage of the testing generates high concentrations of target DNA (amplicon), this area shall be located as far away from high traffic areas as possible to avoid the possibility of contamination of the pre-amplification samples/areas.

Amplification tubes shall be discarded in such a manner that there is no risk of contaminating laboratory areas e.g. upturning the Rotogene carousel and disposing of the amplification tubes into a contained biohazard bag.