

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800

Open access books available

122,000

International authors and editors

135M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.

For more information visit [www.intechopen.com](http://www.intechopen.com)



# Good Clinical Laboratory Practice (GCLP) for Molecular Based Tests Used in Diagnostic Laboratories

Raquel V. Viana and Carole L. Wallis  
*Lancet Laboratories*  
*South Africa*

## 1. Introduction

Over the past decade there has been an expansion in molecular based technologies in the diagnostic environment. These molecular based technologies almost always involve *Polymerase Chain Reaction* of either DNA (PCR) or RNA (RT-PCR), but can also include isothermal amplification and/or sequencing. These molecular tests can be used for rapid qualitative or quantitative analysis for:

- Detection of infectious disease
- Viral load monitoring (HIV, HBV, HCV etc...)
- HIV diagnosis in paediatrics
- Translocations
- Mutations
- Gene rearrangements
- Forensic medicine

Several important steps need to be followed to ensure that a quality service is offered by a molecular laboratory. The quality of the test result is linked to a number of factors. It is reliant on activities that both directly and indirectly impact on the quality of the test ensuring that reliable and accurate results are obtained. There are several benefits to having a quality system in place, it allows for monitoring of the entire system, detects and limits errors, improves consistency among different testing sites and helps to contain costs.

Good Laboratory Practice (GLP) is defined in the Organisation for Economic Co-operation and Development (OECD) as “a quality system concerned with the organisational process and the conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, archived and reported”. The purpose of the Principles of Good Laboratory Practice is to promote the development of quality test data and provide a tool to ensure a sound approach to the management of laboratory studies, including conduct, reporting and archiving. Good Clinical Practice is an international ethical and scientific quality standard for designing, conducting, recording and reporting trials that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety and well-being of trial subjects are protected; consistent

with the principles that have their origin in the Declaration of Helsinki, and that the clinical trial data is credible. The conduct of the laboratory work involving diagnostic testing requires a hybrid of GLP and GCP requirements referred to as Good Clinical Laboratory Practice (GCLP). This would revolve around the application of those GLP principles that are relevant to the analyses of samples while ensuring the purpose and objectives of the GCP principles are maintained.

General GCLP principles, which also hold for Molecular GCLP, such as: *Organisation & Personnel Responsibilities*, ensure that there are quality policies and standards in place. Organisational charts and job descriptions should give an immediate idea of the way in which the laboratory functions and the relationships between the different departments and posts. Also by describing a defined list of responsibilities it ensures that there are sufficient resources established and clearly defined roles resulting in accountability for all steps in the laboratory. Furthermore, all involved in the process should be committed to a culture of quality. *Personnel* are an integral aspect of GCLP as this ensures that there are enough well qualified people to perform the assays. To aid this, systems need to be in place to plan for the number of staff required, employment and retention of existing staff using continual development programs and training of the staff. To ensure staff retention there should be active supervision and performance management of all the staff. *Data Management* is vital for a laboratory to work efficiently and therefore needs an information flow scheme established and a data collection and management system in place which also ensures patient privacy and confidentiality. A crucial part of data management is the adequate training of staff, so they can use it effectively.

Another important component of running a quality laboratory is the establishment of *Standard Operating Procedures (SOPs)*. This ensures that assay techniques and processes in the laboratory are standardised thereby contributing to reproducibility. Each SOP should detail one task in a clear and accurate fashion while also informing the operator of everything that needs to be known and how to do it. All SOPs and other documents in a laboratory need to be reviewed and approved by the laboratory manager on a regular basis to certify that all procedures used in the laboratory are up to date and accurate. To do this there needs to be a record of the number of copies (distribution list) of the SOPs and other documents in circulation within the laboratory. It therefore helps to number these documents in a consistent fashion so that there can be *Document Control* aiding in the location and removal of such documents from the laboratory when they are no longer in use. It is important that there is a *Stock Management* system in place. This allows for efficient management of reagents and consumables to ensure the continued ability to perform the assays the laboratory offers. To aid stock management there should be a procurement system in place, a mechanism of recording and managing the stock and adequate space to store the reagents and consumables correctly. There should also be appropriate *Facilities* to perform the assays (more details are described below), and to ensure quality results all the *Methods used should be Validated*, and appropriate *quality control* measures established and followed.

To ensure all of the above mentioned steps are followed it is important there be a *Management Review Process*, errors should be recorded (*Corrective Actions*), and all processes in the laboratory monitored through *Audits* (both Internal and External). This forms part of the Quality Assurance (QA) process. QA is defined as a team of persons charged with assuring management that GCLP compliance has been attained in the test facility as a whole

and in each individual study. QA must be independent of the operational conduct of the studies, and functions as a witness to the entire process. Moreover, the above mentioned criteria to run a quality service, there are additional specific requirements for performing molecular based assays and supplying accurate and reliable results. These requirements are a direct result of the basis of the molecular technologies which use the ability of PCR to make millions of amplicons of the desired gene of interest (Figure 1).

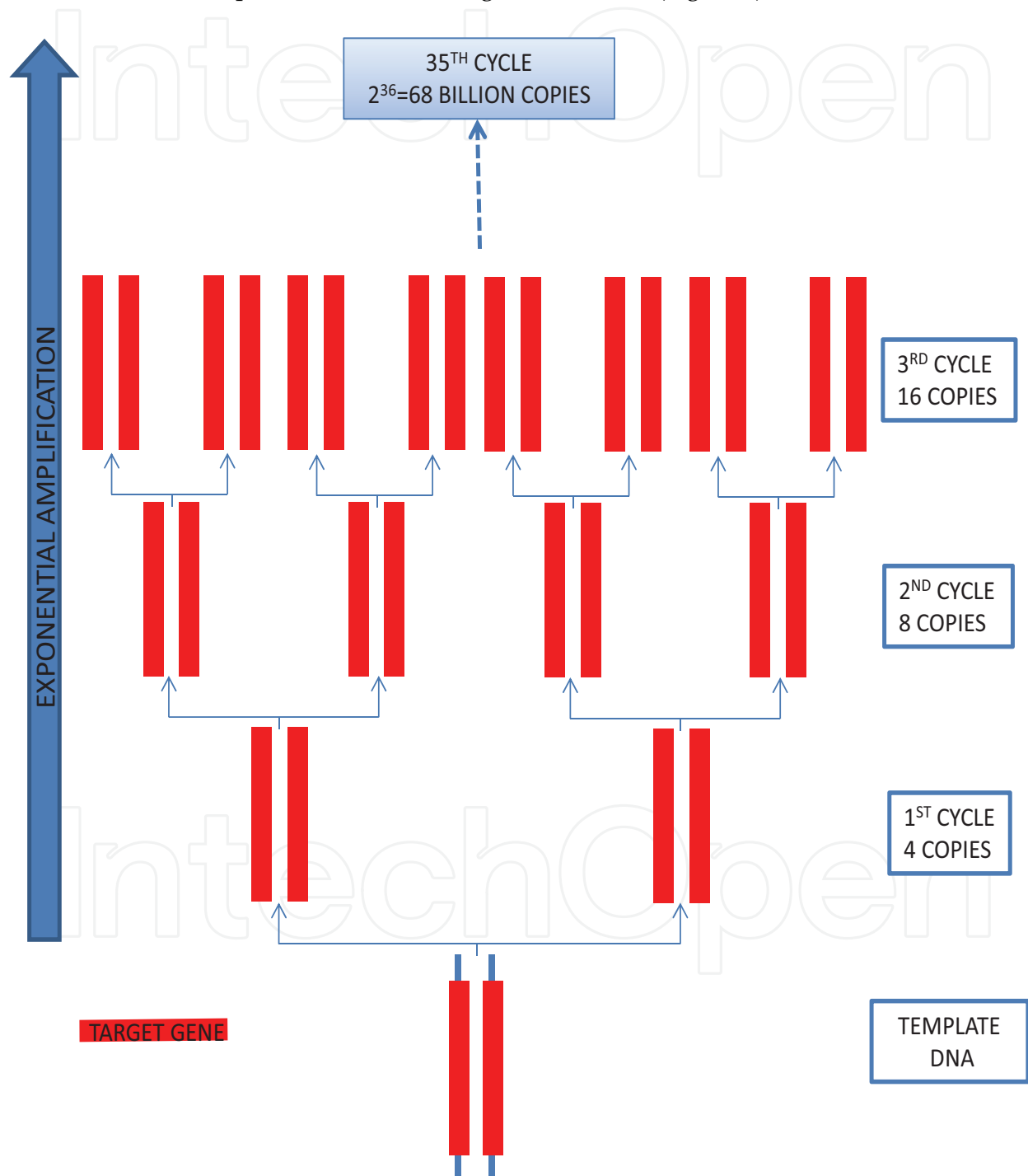


Fig. 1. The exponential amplification of a gene of interest during PCR  
(<http://users.ugent.be/~avierstr/principles/pcr.html>)

The major limiting factor for PCR based technologies is contamination, a direct result of either the highly sensitive nature of PCR amplification and/or the large amount of amplified target obtained. The aim of this chapter is therefore to provide useful information for the appropriate set-up of a molecular laboratory and the steps that need to be taken to ensure good quality results are produced.

## 2. Scope

This chapter is intended to serve as a guide for diagnostic companies planning on setting up a molecular laboratory, following acceptable quality control standards. The limiting factors of contamination and technique sensitivity have resulted in several specific recommendations for the use of these molecular based technologies in diagnostics. These recommendations will be described in this chapter and include:

### Section A:

Guidelines for working in a molecular diagnostic laboratory-this section will cover Sample Collection, Molecular Laboratory Layout, Staff Requirements and Competency, Quality Control around Equipment and Consumables, Laboratory Maintenance.

### Section B:

Molecular Assay Development and Quality Control-this section will cover appropriate technique selection, primer design, Appropriate Reagent and Enzyme Usage, Assay Validation and Measure of Uncertainty of Molecular Assays.

### Section C:

Controls to Monitor for Molecular Assay Performance-this section will ensure that contamination has not occurred and that the molecular technique is performing optimally. The following type of controls will be discussed: internal control, no template control, negative and positive control. Furthermore, corrective actions around the performance of the above mentioned controls will be discussed, including root cause analysis.

### Section D:

Data Tracking and Auditing of a Molecular Sample, this section will cover the three steps of processing a sample: Pre-analytical Phase (the recording of sample receiving), Analytical Phase (sample processing and assay analysis) and the Post-Analytical Phase (result recording and interpretation) and the quality control of the results.

## 3. Guidelines for working in a molecular diagnostic laboratory

### 3.1 Sample collection

The type of collection device used for collection of specimens that will be tested using molecular diagnostic techniques is very important. The reason for this is that some collection devices are coated with a substance that can result in inhibition of the molecular assay. For example, some coagulants such as heparin result in inhibition of the molecular assay and long and cumbersome methods are required to remove the heparin prior to starting any molecular assay. Therefore the preferred method of collection is in an EDTA coated tube.

Swabs and Dry blood spots (DBS) are also appropriate collection devices, however caution needs to be taken with swabs that are collected in a formalin based collection medium as this also inhibits PCR and must be removed prior to testing.

Depending on the nucleic basis of the test, RNA versus DNA, this will also impact on the time between specimen collection and sample storage. If the sample required is plasma to be used in an RNA based assay, whole blood should be spun down and plasma removed for storage at  $-70^{\circ}\text{C}$  until it can be tested. Some samples arrive in a storage medium, which allows for storage at room temperature for a certain amount of time prior to testing or long term storage. Whole blood and dried blood spots can be stored at  $4^{\circ}\text{C}$  for up to 24 hours for DNA based testing, but long term storage should be at  $-20^{\circ}\text{C}$ .

### 3.2 Molecular laboratory layout

It is vital that the correct workflow is followed in a molecular laboratory in order to minimise contamination and ensure good laboratory practises are followed. It is the responsibility of all laboratory staff to ensure that the workflow is followed. PCR is extremely sensitive and thus poses a HUGE risk of contamination. During each step of a molecular assay multiple copies accumulate and are compounded as one progresses through the different steps of the methodology. To minimize this and thereby reduce contamination the different areas in a molecular laboratory should be physically separated. Depending on the nature of the molecular assay the ideal number of separations differs. Firstly, there should be two major separations between the work done prior to amplification (PRE-PCR) generally known as the clean area and that performed after amplification (POST-PCR) generally known as the dirty area (Figure 2). Between these two areas the work flow should be uni-directional (Figures 2, 3, and 4) and the relative air pressure and direction should differ. The equipment, consumables and laboratory coats should be dedicated to each area. If possible it is helpful to colour code racks, pipettes and laboratory coats in the different areas to be able to easily monitor movement between the different areas. Furthermore, powder-free gloves should be used throughout the process in all the different areas as the power on powered gloves results in assay inhibition.

#### Clean area/room

The clean area is divided into two additional areas, namely, *specimen processing laboratory* and the *no template laboratory* (Figure 3). The air pressure should be positive and blow out of the rooms. The *specimen processing laboratory* is where specimens are received, stored, total nucleic acid is extracted and the generation of complimentary DNA (cDNA) is performed. The *no template lab* is where reagents are stored and mastermix preparation for cDNA and amplification are made. The clean areas **must** be kept free of amplicon at all times, to ensure this occurs there should be no movement back from the dirty area to the clean area. If under extreme circumstances a consumable or reagent needs to be moved backwards it must be thoroughly decontaminated with bleach and ethanol. Returning racks should be soaked in 1% bleach overnight before soaking in distilled water and placing in the clean area.

In the *sample processing laboratory* the following equipment would most likely be present:  $-80^{\circ}\text{C}$  and  $-20^{\circ}\text{C}$  freezers and a fridge for sample storage (depending on the specimens received in the laboratory), a biohazard hood for sample extraction (especially if infectious specimens are processed in the laboratory), a centrifuge (if required for specimen extraction), automated extraction platform, a PCR workstation (a contained area that contains a UV light with or without a timer), a thermocycler (for cDNA synthesis only), dedicated pipettes, dedicated vortex, a dedicated place to hang laboratory coats and the appropriate safety materials (eye wash, medical aid box, shower). If chemicals are stored in this area appropriate facilities and storage requirements should be in place.

In the *no template laboratory* the following equipment would most likely be present: -20°C freezers and fridge for reagent storage, dedicated pipettes, dedicated vortex, dedicated microfuge, a PCR workstation (a contained area that contains a UV light with or without a timer), a dedicated place to hang laboratory coats and the appropriate safety materials (eye wash, medical aid box, shower). If chemicals are stored in this area appropriate facilities and storage requirements should be in place.

To ensure that no specimen contamination in the *no template laboratory* occurs it is vital to discard ones powder-free gloves worn in the *specimen processing laboratory* and change ones laboratory coat. This **MUST** occur before you enter the *no template laboratory*. It is therefore useful to place a biohazard bin outside the *no template laboratory* where gloves can be discarded and a hook for the laboratory coat to be hung up prior to entering the *no template laboratory*. Furthermore, nothing may enter the *no template laboratory* from the *sample processing laboratory*; this includes racks, tubes and open reagents. If possible disposable lab coats are useful in these areas.

### **Dirty area/room**

Depending on the molecular methods performed in the laboratory the dirty area can be divided into one or two areas, namely, *post-amplification laboratory* and the *nested PCR laboratory* (Figure 3). The air pressure should be slightly positive for the *nested PCR laboratory* and neutral for the *post-amplification laboratory* and blow into both the rooms. The *post-amplification laboratory* is where the amplification reaction and detection of amplicon occurs. The detection of amplification can occur on a real-time PCR platform, gel electrophoresis, ELISA based detection and sequencing. **To note**, the *post-amplification laboratory* can be further divided into different rooms by each detection method, depending on the number of specimens and molecular assays run by a laboratory (Figure 4). In the *nested PCR laboratory* second-round amplification is set-up and a thermocycler is located there for this function. Nothing from these areas should move back into the clean area, without being completely decontaminated (as described above), under any circumstances. Gloves and laboratory lab coats must be removed when leaving this area.

In the *post-amplification laboratory* the following equipment would most likely be present: -20°C freezer and fridge for amplicon and reagent storage, a centrifuge (if required for the molecular assay performed), a PCR workstation (a contained area that contains a UV light with or without a timer), any equipment required for amplification, gel electrophoresis, sequencing or other amplicon detection methodology, dedicated pipettes, dedicated vortex, a dedicated place to hang laboratory coats and the appropriate safety materials (eye wash, medical aid box, shower). If chemicals are stored in this area appropriate facilities and storage requirements should be in place.

In the *nested PCR laboratory* the following equipment would most likely be present: -20°C freezer and fridge for reagent storage, dedicated pipettes, dedicated vortex, dedicated microfuge, a PCR workstation (a contained area that contains a UV light with or without a timer), a thermocycler, dedicated place to hang laboratory coats and the appropriate safety materials (eye wash, medical aid box, shower). If chemicals are stored in this area appropriate facilities and storage requirements should be in place.

To ensure minimal movement between areas during the running of molecular assays, it is optimal to have dedicated storage (freezer, fridge and room temperature) for each area. Furthermore, prior to starting the assay one must check that they have sufficient consumables and reagents to perform the test.

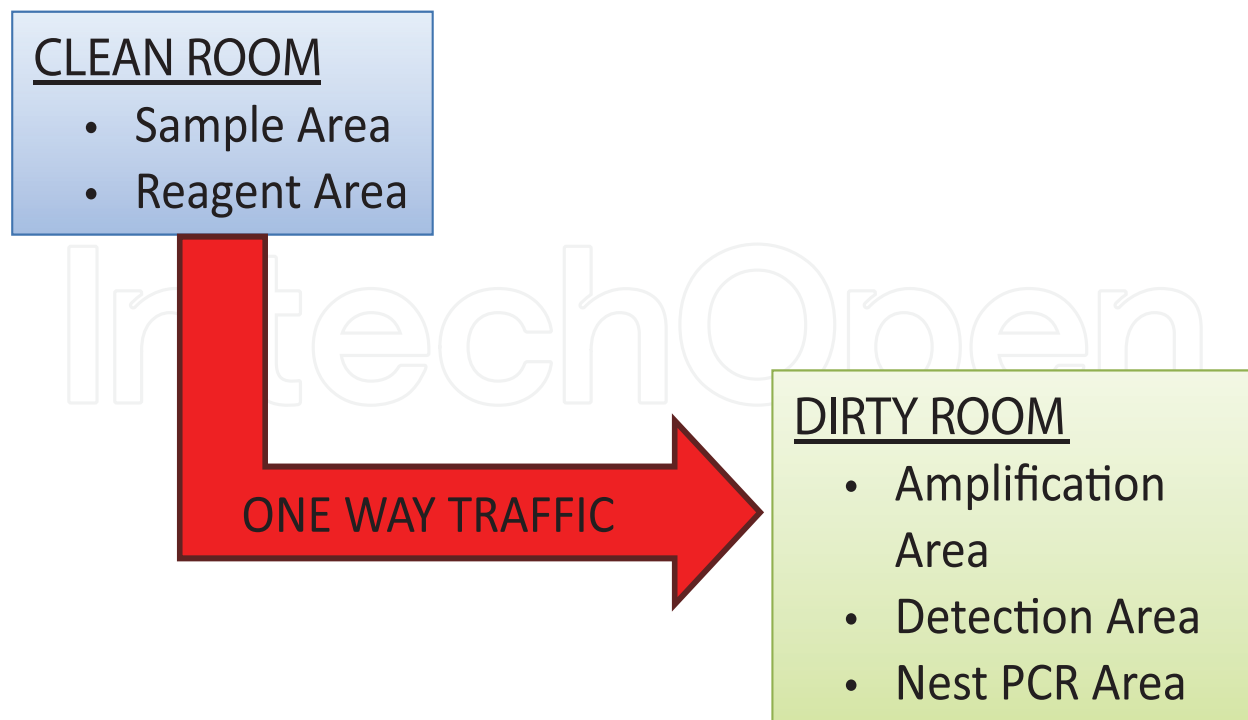


Fig. 2. Two room option for molecular lab layout. This is comprised of a clean area (for pre-analytical and sample preparation) and a dirty room (for analytical and post-analytical)

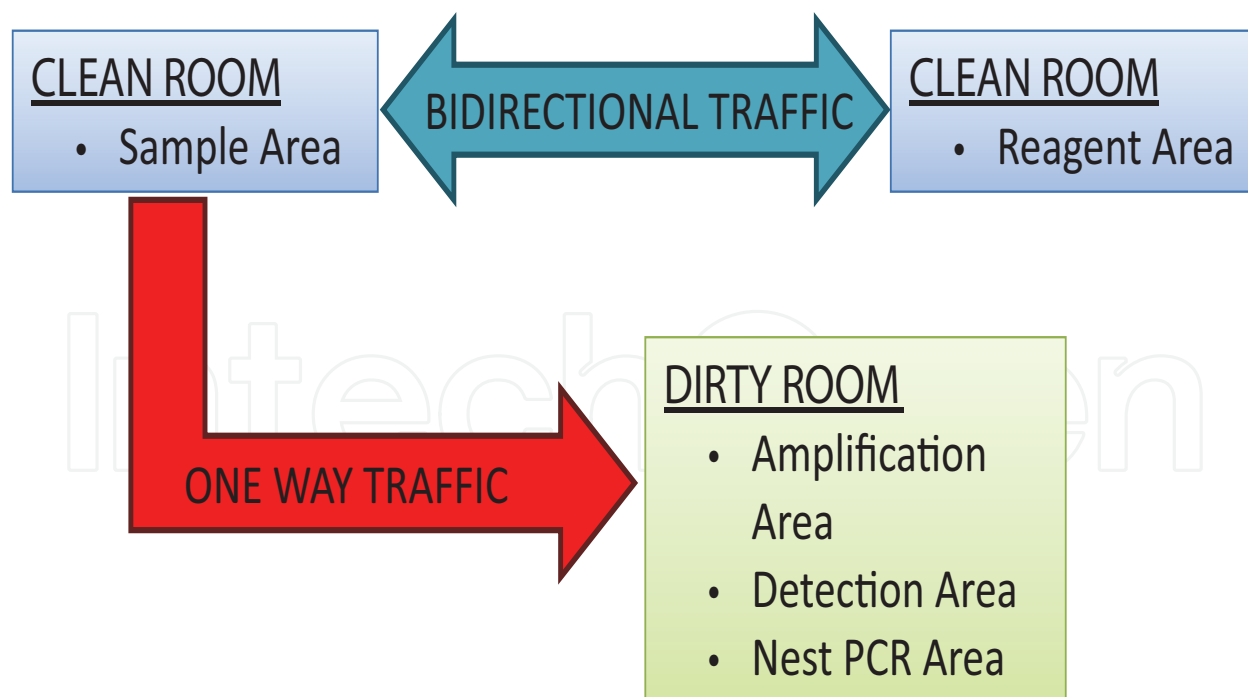


Fig. 3. Three room option for molecular lab layout. This is comprised of a clean area, which is divided into two rooms for 1) samples receiving and samples preparation and 2) room for preparation of reagents. As in the two room layout the dirty room (for analytical and post-analytical) remains the same



**NOTE:** To help in ensuring the above points are followed, it is important that each staff member organize their workflow as to ensure there is as little movement between clean and dirty areas during a shift and the laboratory policies should be incorporated and be well explained in a SOP that is easily accessible to all staff (including laboratory cleaners). Each work space should be kept tidy (minimal clutter) and each area should be closed to the other (with a door).

The above description of the different areas of a molecular laboratory describes the ideal laboratory layout. However, sometimes this is not always possible due to cost and space constraints, it is acceptable to divide the molecular area into just a clean and dirty area (Figure 2).

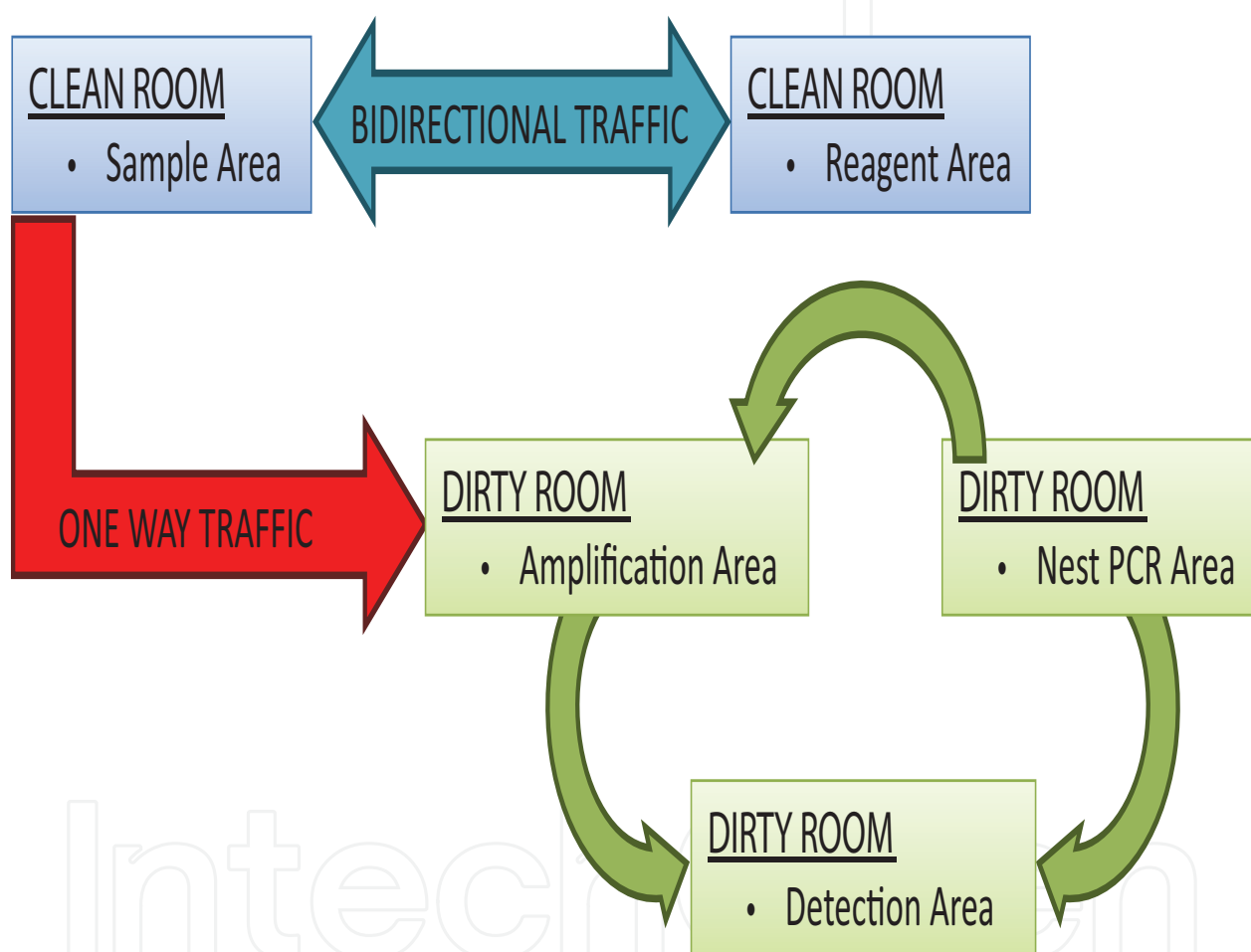


Fig. 4. Multiple room option for molecular lab layout. This is comprised of a clean area, which is divided into two rooms for 1) samples receiving and samples preparation and 2) room for preparation of reagents. The dirty area is divided into multiple rooms each with a specific function

### 3.3 Staff requirements and competency

Most molecular tests require highly skilled and well-trained staff. To achieve this all staff must be trained and then deemed competent prior to starting testing in the laboratory. Furthermore, it is advisable to assess the competency of the staff on an on-going basis using either external or internal quality control programs as described in Section 5. Once this has

been completed the laboratory manager should formally approve the staff member competent to conduct testing.

The procedure for staff training should include the following steps: a new staff member should be given an orientation of the facility. It is vital that all new staff also be trained in laboratory specific biosafety, biohazard waste management, personal protective equipment, and general laboratory safety including the procedures that need to be followed for all chemicals used in the laboratory. Once the new staff member has passed the above training they should be given an overview of all the tests performed in the laboratory. This will ensure the staff member has an understanding of the process (including PCR) in the laboratory and give them an understanding as to why certain measures should be followed. During this initiation orientation the staff member should also be advised of the correct work flow of a molecular laboratory. The staff member should also be given an overview of the maintenance required in the molecular laboratory (Section 3.4 and Section 3.5) and read, understand and sign all SOPs used in the molecular laboratory.

New staff members should then be trained on the methodologies they are required to perform. Firstly, the new staff member should observe the procedure whilst following the SOP, during this time the new staff member is able to ask questions and is given a brief explanation of each step and the importance of it. Secondly, the new staff member then performs the methodology under supervision of the trainer. Once they are able to successfully perform the assay under supervision the new staff member should perform the methodology independently on previously tested samples and the results compared for accuracy by the laboratory manager. This training should be done for all tests that the new staff member will be performing. The records for this training are then kept in the new staff members training file.

Once a staff member is trained the competence of the staff member needs to be performed. The criterion for competence needs to be determined prior to assessing it. Competency assessments should be done on all staff members on a continual basis, but it is recommended it be carried out at least once a year on each test the staff member is performing. Competency is assessed in one of the following ways:

- Completion of an external quality assurance panel.
- Comparison of results across staff members:
  - a. This can be performed in several ways, for example, staff can analyze the results of a molecular assay and these results are compared and similarity determined.
  - b. Parallel testing, this is where staff members perform the entire assays on the same samples. The results obtained from each staff member are compared and the similarity determined.
- The method used to determine competency is determined by the laboratory manager.

If the staff member is deemed to be incompetent they should be retrained on the appropriate methodologies and competency reassessed.

**NOTE:** The qualifications of laboratory staff and the training and experience are critical in ensuring a quality service is offered in a molecular laboratory, because the training and experience of staff can positively influence the rate of human errors in the laboratory.

### **3.4 Quality control around equipment and consumables**

Prior to setting-up a molecular assay in the laboratory it is important that one assess the equipment and reagents that are required. Each piece of equipment must meet the required specification of the laboratory and where the equipment can be sourced from. The

laboratory must ensure they have the correct space, electrical and plumbing facilities for the equipment. Consideration must be taken when determining who will supply the equipment. Are they reliable? Will they be able to support this piece of equipment and can they supply spare parts? All these factors will impact on the efficiency and reliability of the laboratory.

Once a piece of equipment is purchased, an SOP must be written defining how to use the machine, who is responsible and what the maintenance (daily, weekly, monthly and annual) procedure is. The maintenance must cover the routine checking that the machine is working correctly, if it is not, the appropriate troubleshooting is required and this must be recorded and regularly reviewed (see Table 1, an example of a maintenance chart). Furthermore, it must be determined if the piece of equipment requires a service or calibration by an external party and if so how frequently.

It is vital to train all staff on the machine (and when new ones are purchased) as correct operating of the equipment will lower the cost and regularity of repair, thereby preventing delays of tests and maintaining productivity.

Equipment	Calibrated	Maintenance			
		Daily	Weekly	Monthly	Annual
Pipettes					
Laminar Flow Hood					
Centrifuge					
Heating Block					
Waterbath					
Thermocycler					
Scale					
Plate Reader					
Sequencer					
Fridge/Freezers					

Table 1. Common equipment used in a molecular laboratory and the maintenance and calibration required

A similar process for supply of reagents needs to be followed with regard to assessing the need and establishing a reliable supplier. In the molecular laboratory one of the staff members should be put in charge of monitoring the stock levels, ordering (ensuring there is sufficient still left to perform the tests prior to running out) and ensuring reagents and consumables for each test are stored appropriately. The level of consumable and reagent wastage should also be recorded so that the efficiency of the tests and laboratory can be monitored.

### 3.5 Laboratory maintenance

All work surfaces should be cleaned prior to use with 1% bleach solution contained in an opaque vessel which inactivates pathogenic agents and destroys nucleic acids. Residual

bleach may affect stainless steel counter tops and the Perspex in hoods as well as contribute to inhibition of specific assays therefore it is advisable to then wipe down with distilled water to remove residual bleach that could form crystals. Finally 70% ethanol is used to further prevent transfer of pathogens. All cleaning solutions should be prepared daily. There are several commercially available products, such as DNA or RNA Away, that are specifically designed for removing nucleic acids or nucleases as well as pathogens, which can also be utilized for surface cleaning. Racks and trays should be soaked in the 1% bleach solution and then thoroughly rinsed with distilled water daily. Equipment such as thermocyclers and centrifuges should be cleaned with 1% bleach solution followed by 70% ethanol whenever contamination is suspected. Another means of decontaminating hoods, reagents, pipettes, tubes, and various other consumables, is exposure to UV light. Most biological safety cabinets are equipped with a UV light source. It is generally accepted that UV exposure at 254nm for a minimum of 5 minutes is sufficient for decontamination including the deactivation of nucleases and destruction of extraneous DNA on surfaces. Laboratory SOPs often include UV exposure steps as long as 30 minutes before and after use of hoods for PCR work. Wiping with bleach and/or detergents is still warranted as the penetrating power of UV light is minimal.

All equipment should be properly calibrated and maintained to ensure reliable and accurate performance. Records of repairs and routine maintenance as well as non-routine maintenance should be kept. Routine maintenance records should be documented in such a way that users of equipment can be assured that it is reliable and not outside its service interval. A good way of ensuring this is by attaching a service label to the equipment and by making provision for a clear service plan. Early warning that equipment is malfunctioning is important therefore the checking interval should be assigned to assure this. Alarms are very useful, especially if a problem occurs at a time when staff are not present. Back up for vital equipment should be available whenever possible as well as back up (generator) in the event of service failures such as power cuts. Records of equipment calibration, checking and maintenance demonstrate that the respective SOPs have been followed and that the equipment used was adequate for the task and operating within its specifications. The records should also demonstrate that the required action was taken if the equipment failed these checks and that staff were aware of this and took appropriate remedial action.

## 4. Molecular assay development and quality control

### 4.1 Appropriate technique selection

PCR has been adapted to fit several applications, including detection of target DNA, sequencing stretches of target DNA, and amplification and detection of mRNAs, ribosomal RNAs, and viral RNAs after using reverse transcriptase to make complimentary DNA. There are currently five common types of PCR used:

#### 4.1.1 Conventional PCR

This type of PCR uses a thermostable DNA polymerase to make multiple copies of a target region of DNA defined at each end (3' and 5') by a specific primer.

PCR typically consists of three basic steps:

**Step 1. Denaturation**, requires that the sample DNA become a single-stranded template. To achieve this, the sample DNA is typically heated between 94°C and 97°C for 15 to 60 seconds, to separate or denature the two strands of the DNA.

**Step 2. Annealing** step, in which the reaction temperature is lowered typically between 47°C and 60°C for 30 to 60 seconds, to allow the oligonucleotide primers to bind to the single-stranded template.

**Step 3. Elongation**, during which the temperature is raised, typically to 72°C, allowing the polymerase enzyme to make a complimentary copy of the template. The length of the elongation step (30 seconds to three minutes) is determined by the speed of the enzyme, its ability to continue moving down the template DNA referred to as *processivity*, and the length of the DNA segment to be amplified.

One repetition or thermal cycle of these three abovementioned steps theoretically doubles the amount of DNA present in the reaction. The number of repetitions needed for a PCR application is determined by the amount of DNA present at the start of the reaction and the number of amplicon copies desired for post-PCR applications. Typically 25 to 40 cycles are performed.

#### 4.1.2 Real-time PCR

Real-time PCR detects and measures the amplification of target nucleic acids as they are produced. Real-time PCR requires the use of primers similar to those used in conventional PCR, but in addition also requires an oligonucleotide probe labelled with fluorescent detection chemistry, and a thermocycler able to measure the fluorescence. Typically, the binding of a dye-labelled probe to the template sequence causes fluorescence to increase in direct proportion to the concentration of the PCR product being formed. A real-time machine monitors the fluorescence increase and calculates a cycle threshold (CT) value. This value, which represents the first cycle in which there is a detectable increase in fluorescence above the background level, is used to measure relative or absolute target quantities. In the absence of an absolute standard, the starting copy numbers of nucleic acid targets from different samples can be determined in a relative sense (e.g., sample one has 20 times more target than sample two). If an absolute standard, which contains known quantities of the target nucleic acid, is run to generate a standard curve, the starting copy number in the test samples can be estimated. Real-time PCR also differs from conventional PCR in that the target selection for real-time PCR is more restricted due to requirements of a smaller target fragment and the need to select probes with a higher melting temperature than the primers to ensure that the probe is fully hybridized during primer extension. In addition, the annealing and elongation temperatures are usually combined in a two-step PCR process that is performed at an intermediate temperature (e.g., 60°C) for one to two minutes.

There are several different fluorescent detection chemistries used for real-time PCR, including the following:

- *SYBR® Green I*, a fluorescent dye, is frequently used in real-time detection chemistry. This dye intercalates into double-stranded DNA, including PCR products and fluoresces. Therefore when used to detect amplification the level of fluorescence increases with each amplification cycle. This detection chemistry is not target sequence specific and is therefore more versatile than probe-based detection, but is susceptible to false positives due to the formation of non-specific PCR products or primer-dimers. Melting curve analyses are often used as an additional confirmation of product size for procedures using SYBR® Green.
- *Dual-labeled fluorogenic oligonucleotide probes* are most frequently used. These probes (e.g., TaqMan® probes) have a reporter fluorescent dye at the 5' end and a quencher dye at the 3' end. The probes are added to the PCR master mix along with the PCR

primers. During the PCR, if the target sequence is present, the probe anneals downstream from a primer site and is cleaved by the 5' nuclease activity of *Taq* DNA polymerase during polymerization. This cleavage releases the reporter dye from the probe and away from the quencher dye, resulting in fluorescence that is detected by the instrument. These probes can be modified with a minor groove binding (MGB) protein, allowing for shorter probes to be designed, which increases specificity in assays detecting a single nucleotide change.

- *Fluorescent resonance energy transfer (FRET) probes* involve the hybridization of two probes to adjacent sequences within the amplified product. The upstream probe has a fluorescent dye at the 3' end and the adjacent probe has a fluorescent dye at the 5' end. Correct hybridization of these probes brings the two dyes into close proximity. The laser excites the first fluorescent dye, which emits light at a different wavelength. This light then excites the second fluorescent dye by FRET between the adjacent probes. The real-time PCR machine detects the wavelength of light emitted by the second fluorescent dye.
- *Molecular beacon probes* use a variation of this same process, wherein reporter and quencher dyes are held together by a hairpin structure in the probes but become sufficiently separated by linearization of the probe after annealing with the template to allow the reporter dye fluorescence to be detected.

#### 4.1.3 Multiplex PCR

Multiplex PCR involves the amplification of two or more different PCR products within the same reaction. This type of PCR is a modification of a conventional or real-time PCR with the use of multiple sets of primers in each reaction. Multiplex PCR requires less time and effort in amplifying multiple target templates or regions than individual reactions and may be utilised as an effective screening assay. While multiplex PCR provides potential time saving by allowing simultaneous detection of multiple targets, significant optimization is required to obtain all of the products with equal efficiency and sensitivity. Extra precaution must be taken to the design and concentration of the primers so that they do not interact or compete with each other.

#### 4.1.4 Reverse transcription (RT)-PCR

RT-PCR is used to amplify RNA target sequences, such as messenger RNA and viral RNA genomes. This type of PCR involves an initial incubation of the sample RNA with a reverse transcriptase enzyme and a DNA primer. DNA primers that are used commonly include oligos dT (an oligo consisting of only thymidine residues), random hexamers (primers made of six random nucleotides), or a sequence specific primer. Oligos dT will hybridize to the poly-A tail of messenger and certain viral RNAs and prime DNA from the 3'-end of the RNA molecule as a consequence of this amplification of RNA near the 5'-end of the molecule may not occur. Random hexamers work with any RNA, but require an extra initial incubation at 25°C. Specific primers can be either the PCR primer that hybridizes to the RNA at the 3' side of the amplification region or a primer that hybridizes further downstream from the PCR primers. RNase inhibitors should be added to RT reactions to prevent the degradation of the RNA target sequence by RNase present in the sample or introduced as contamination. The reverse transcription and the PCR amplification can be performed in a one- or two-step process. In general, the two-step process is more sensitive, while the single-step reactions are less likely to be contaminated, because the tube is not

opened after reverse transcription. The determination of which process should be used depends on the level of sensitivity required and the likelihood of contamination. There are many types of reverse transcriptases available for RT-PCR. The characteristics of the enzymes make some better suited for a one- or two-step reaction and other downstream applications. Some enzyme characteristics that impact the type of reverse transcriptase used for RT-PCR include: the presence or absence of RNase H activity that degrades RNA in an RNA:cDNA hybrid, processivity of the enzyme, divalent ion requirements, specificity and sensitivity, ability to incorporate dUTP for UNG carryover contamination, and optimum temperature for function.

#### 4.1.5 Nested PCR

Nested PCR is a conventional PCR with a second round of amplification using a different set of primers annealing within the first round amplicon which helps increase the specificity and sensitivity of the target amplicon. The use of a second amplification step with the "nested" primer set results in a reduced background from products amplified during the initial PCR due to the nested primers' additional specificity to the region. The amount of amplicon produced is increased as a result of the second round of amplification. Used correctly, the multiple rounds of nested PCR should increase both the sensitivity and specificity of the PCR. However, this technique also increases the chance of carryover or cross-contamination because of the additional interaction with the first amplicon. The following precautions need to be followed to limit the chance of sample contamination and false-positives:

- Never opening more than one tube at a time.
- Adding an additional negative control for the second-round of amplification.
- Including first-round negative controls in the second-round of amplification to check for false-positives.
- Designating a fourth room or separate area for sample preparation after the first amplification (see Figure 3).

#### 4.2 Primer design

Well-designed primers are essential for ensuring accurate and efficient detection of the desired gene of interest in a molecular assay. Primers are essential in PCR analysis and are short segments of chemically synthesized DNA (which are called oligonucleotides or, more commonly, "oligos"). A length of 18-27 base pairs, ensures adequate specificity and are short enough to ensure easy binding to the template during annealing. Primer sets are oligos with nucleotide sequences that are designed specifically to prime the amplification of a portion of a targeted nucleic acid. Hybridization probes are oligos with specific nucleotide sequences that are internal to the sequences of the primers and which are used to confirm the amplification of the target or quantitate it. Design and selection of the specific primer and probe set to be used for an experiment is based on the application, the type of PCR and hybridization that will be performed, and the segment of the target nucleic acid sequence that is known. Primers should be designed to amplify only the DNA or RNA of interest and be specific for that region. Primer melting temperature ( $T_m$ ) is by definition "the temperature that the one half of the DNA duplex dissociates and becomes single stranded, thereby indicating the duplex stability". The optimal  $T_m$  range is 52-58°C, primers with melting temperatures above this (65°C) are prone to secondary binding. The  $T_m$  is directly linked to the GC content of the primer.

As a general rule, well designed primers are characterized by the following:

- Length of 18 to 27 base pairs
- No homology within or between primers, especially at the 3' end to avoid primer-dimer formation.
- No guanine-cytosine (GC) stretches greater than four base pairs
- *GC content*: (the numbers of C's and G's in the primer as a percentage of all the primer nucleotides) of 40% to 70%.
- *GC Clamp*: to promote specific binding there should be a G or C nucleotide present within five bases of the 3' end of the primer.
- $T_m$  of the two primers should be as close as possible, however, a  $T_m$  of between 52-58°C tends to give the best result.
- Secondary primer structures:
  - A *hairpin* is formed by intramolecular interaction within the primer and reduces binding to the target, therefore, no hairpin loops with a Gibbs Free Energy of -2 kcal/mol or less.
  - *Self-Dimer*: this is formed when two primers in the same direction bind as a result of intermolecular interactions. To reduce self-dimers a primer should have a 3' end dimer of less than a Gibbs Free Energy of -5 kcal/mol or less and an internal self-dimer with a Gibbs Free Energy of -6 kcal/mol or less.
  - *Cross-Dimer*: this is formed when two primers of two different directions bind as a result of intermolecular interactions. To reduce cross-dimers a primer should have a 3' end dimer of less than a Gibbs Free Energy of -5 kcal/mol or less and an internal self-dimer with a Gibbs Free Energy of -6 kcal/mol or less. Nucleotide repeats should be avoided.

A variety of computer programs are available to aid in the creation of the best possible primers and probes, such as Primer Premier and PrimerPlex. These programs can help determine the optimum annealing temperature for newly created oligos and check for the formation of intra- and intermolecular dimers and hairpin loops. Laboratories should consider repeating the design process with more than one computer program, because these programs represent a simulated environment that may not include all the variables that affect oligo design.

For laboratories that are performing real-time PCR, the software provided with the real-time PCR instrument may be used for primer design. New primers and probes should always be tested experimentally for sensitivity and specificity before use in any method. The specificity of a chosen sequence should be evaluated using BLAST (Basic Local Alignment Search Tool) or its equivalent. Versions of BLAST are available on the WEB at a number of sites, including [www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov). BLAST compares the designed oligo sequences to known nucleic acid databases such as GenBank and EMBL. The search determines the potential of hybridization of the chosen oligo with sequences from other organisms. The results of this search should be used to define any relevant, closely matched sequences for specificity testing. The primer concentrations used in each newly developed PCR assay should be optimized to obtain maximum amplification efficiency. Optimization of primer concentrations is especially important when performing multiplex PCR.

#### 4.3 Appropriate reagent and enzyme usage

*Taq* DNA polymerase, which is isolated from the thermophilic bacterium *Thermus aquaticus*, is the primary enzyme used in the amplification of DNA in nearly all procedures.



Modifications of this enzyme or other DNA polymerases with specific functions and unique properties, including different extension rates, processivity, greater proofreading ability, and different temperature tolerances, generally expressed as a half-life at the denaturing temperature, may be more appropriate for some PCR applications. Hot-start DNA polymerases are enzymes that are inactive until a specified temperature is reached. Use of hot-start enzymes reduces the production of non-specific products by preventing the elongation of primers that have non-specifically annealed to the template at lower annealing temperatures (which may happen during master mix preparation or in the first step of PCR cycling). When selecting an enzyme type for a method or study, the analyst should evaluate the different strengths and weaknesses of the DNA polymerases available to determine which individual polymerase, or combination of polymerases, will work with their template nucleic acid. Records of lot numbers of all reagents should be captured and stored. Reagents should be aliquoted to avoid excessive freeze-thawing and to protect stock reagents from contamination. These reagent tubes should be clearly labelled. All reagents containing fluorescent probes should not be exposed to excessive light to prevent degradation by photo-bleaching.

#### 4.4 Assay validation

Before an assay can be implemented in the laboratory, several performance factors should be considered such as intended use of the test (target gene, sequences and mutations); the population the test will target; the test methodology and the type of sample that will be used. To establish the performance factors of an assay relevant information from scientific studies should be obtained, the correct testing population should be defined, the correct test method to obtain the desired result chosen, the quality control parameters decided and that the results obtained from the test can be interpreted.

Once the parameters have been chosen and prior to implementation of the molecular assay it is important to perform a validation on the assay to ensure quality results are obtained. To do this certain parameters should be accessed depending on the assay this includes both samples and analytical performance specification.

Samples used in the validation are very important. Specimens chosen for the validation should be chosen to test a specific parameter and samples used should be well characterised prior to starting the validation. The samples should represent the type of samples expected to be tested routinely. Furthermore, there should be a variety of samples to ensure that the test results can be interpreted for specific patient conditions. The results from this will allow for limitations of the methodology and results to be known. When selecting the samples for a validation the following guidelines should be followed:

- Irrespective of the prevalence of the disease/mutations/variation being tested the sample number of samples should be chosen and consistent across tests.
- All sample types expected for the assay should be included.
- Samples that include all the possible results should be included.
- Controls and calibration materials should be obtained and included.

The analytical performance parameters that can be included are: precision, accuracy, reproducibility, analytical specificity, analytical sensitivity (amplification sensitivity and sensitivity of variants), linearity, reportable range of test results, reference range and lot specific testing. Once the acceptable level for each criteria is set it cannot be modified once the validation has begun. Furthermore, the validation should be performed using the same conditions that will be used for the processing of routine specimens. This includes: the

facility, equipment, reagents and personnel. The same holds true for when external quality assurance panels are performed (refer to Section 5).

**Precision** is the ability to obtain the same result from multiple replicates (5-10) of a sample. The closeness of each result for the same sample is then assessed. Depending how close the replicate results are will determine the precision of the assay. **NOTE:** the replicate results **might not be close** to that obtained from a different method.

**Accuracy** is performed to determine how close the results from two different tests are and is used for both a quantitative and qualitative assay. To assess accuracy it is best to use a very well characterised sample. This parameter is easy to measure; however, it is often difficult to determine the criteria prior to performing the validation of a new assay.

**Reproducibility** is the ability to produce the same result even though there are changes to the conditions the assay are performed under. For example, if a different staff member performs the assay, different lot numbers or different thermocyclers are used. This assessment, unlike precision and accuracy requires a larger number of samples with fewer replicates and often over a longer time period.

**Analytical Specificity** is the ability of the assay to determine only the target analyte that is being detected and that there are no interfering substances. Interfering substances can be associated with specimen type and patient associated factors such as clinical condition, medication or disease stage.

**Analytical sensitivity** (*to note these two types of sensitivity criteria are not mutually exclusive*).

- **Amplification sensitivity** is determined by what is the minimum starting material (lower limit of detection) required to generate an accurate result. Importance should be placed on the different types of samples that can be obtained for the test.
- **Sensitivity of variants** is determined by what percentage of a specific target must be present to be detected. For example, the amount of tumour versus non-tumour cells to detect the presence of a mutation.

**NOTE:** A validation is also required if during the routine running of a methodology there is a change to the procedure. The change will dictate the level of validation that is required. If there is a minor change to the method - equivalency testing can be performed. For example, if the primer sequences are modified the results obtained from the previous and new primers should be compared. However, if there is a major change - a complete re-evaluation should be performed. This could include a new extraction or detection methods or chemistries.

#### 4.5 Measure of uncertainty of molecular assays

For all assays introduced into a molecular laboratory the limitations of the assay need to be determined. For example, is there a limit of detection of the assay? Does it require a certain amount of sample for the assay to be accurately detected? And what steps have been taken to overcome these limits.

Limitations to molecular assays can include the following:

- a. Collection devices and sample storage
  - The assays are normally validated on a certain collection device, such as in an EDTA tubes.
  - Specimens collected in Heparin are not suitable.
  - Once plasma has been isolated from the sample, it must be stored at -70°C.
  - Accurate and reliable results are dependent on proper sample collection and storage prior to testing.

- Samples are only processed if they are collected in the correct collection device.
- b. Contamination
  - The PCR based protocol has the limitation of contamination.
  - To reduce contamination the following steps have been taken:
    - Three separate areas for reagent and sample preparation and post-PCR analysis and sequencing.
    - Powder-free and filter tips are essential when performing PCR.
    - Cross-contamination is further reduced by adding AmpErase UNG
- c. RNA degradation
  - For genotyping success it is critical that you prevent RNA degradation by RNases.
    - Sources of RNases contamination are: skin, hair, general laboratory glassware and contaminated solutions.
      - To reduce RNA degradation powder-free gloves should be worn at all times.
      - RNases Inhibitor is added to the Reverse Transcriptase step.
- d. Internal controls:
  - Internal controls (positive and negative) should be included in every run:
    - No template Control (blank).
    - Positive control.
    - Negative Control.
- e. Sequencing and interpretation of results
  - Accurate and reliable results are dependent on good sequence quality. High background and noisy data can interfere with precise base calling.
  - Population based sequencing only detects a population that is present in greater than about 25%.
  - Some assays the interpretation system is based on a proprietary algorithm and these may need to be updated regularly and the software may at times be outdated.
- f. Staff
  - The level of skill and training required for staff members.
- g. Limit of detection
  - The assay can be dependent on the amount of initial sample material required or the amount of amplicon added to the detection step.
- h. Analytical specificity
  - How accurate is the method you are using? Does it detect other substances or viruses present in the assay and can this impact on the results.

## 5. Controls to monitor for molecular assay performance

There are several ways to monitor a molecular assay and these range from procedures put in place in the laboratory (internal) or external panels performed.

**External Quality Assurance (EQA)** panels assess the results of samples supplied to the laboratory to monitor the performance of the assay. The panels should be treated the same as routine samples in the laboratory, and should be rotated through all staff performing the assay. This type of proficiency testing monitors and enables a platform for improving the quality of a laboratory. EQA panels normally occur twice a year. Within ones molecular laboratory, you can run similar panels across staff members and include several controls as described below to assess the procedures in the laboratory on a more routine basis.

Internal Quality Controls need to be in place to ensure that the laboratory can control the procedures of the molecular assay. This includes the number, type and frequency of controls used for the assay. This enables the laboratory to detect errors caused by the test, environment or operator. The exact controls that can be used are described in Section 5.1.

## **5.1 Contamination control**

### **5.1.1 Internal control**

This is a control that is run in the same tube as the sample. Its level of amplification ensures there is nothing in the PCR that is resulting in inhibition.

### **5.1.2 No template control**

The reaction is set-up without the presence of the starting material and DNase and RNase water is used in its place. With this sample you do not expect any amplification to occur. If there is amplification it indicates that one of the reagents or consumables are contaminated with a sample containing the desired target. In this case, you cannot use any results obtained in this run. Therefore, immediate action must be taken by the staff member by contacting the laboratory manager and all areas should be cleaned with 1% bleach and 70% ethanol and the contaminated reagents discarded. The test must then be repeated with only the controls prior to running samples to confirm that the contamination has been eliminated.

### **5.1.3 Negative control**

The negative control is when starting material is added that does not have the target gene present. With this sample you do not expect any result if a result does appear then this indicates that either there is contamination from another sample or reagent in the run or the assay is not specific for the gene target (this should have been determined in the assay validation described Section 4.4). If this occurs immediate action must be taken by the staff member contacting the laboratory manager and the entire run must be repeated. If there is still a contamination the entire Pre-PCR area must be decontaminated using a 1% bleach solution, followed by 70% ethanol and distilled water. The PCR must then be repeated with only the positive and negative controls. If the contamination is still present the kit must be discarded and a new kit opened.

### **5.1.4 Positive control**

The positive control is a sample that contains the target of interest and is known to work. If the positive control does not work, but the samples amplify, one can continue with the assay if it is a qualitative assay-but the reason for the control not amplifying should be investigated to ensure it does not happen regularly. However, if the positive control and the samples do not amplify further investigation is required. The assay should be repeated with only the positive and negative controls, if there is still no amplification the storage of the reagents must be checked as well as the expiry dates. It should be determined if there is anything that could be inhibiting the PCR. If everything appears in order the laboratory head will contact the appropriate reagent rep.

For qualitative assays it is **very important** that the positive control works as it identifies the amplification efficiency of the assay.

## 5.2 Corrective actions around the performance

The objective of a corrective action for either an internal or external quality control (QC) failure is vital as quality control is an important measure of the analytical and interpretive performance of the laboratory. Any failures are therefore an indicator of potential problems in the system and should be dealt with as a priority. QC also serves as an educational process, identifying areas of deficiency in knowledge and facilitating correction thereof through supervised feedback sessions. All corrective actions must be documented and filed in a designated place (such as a corrective action file) once it has been reviewed and signed off by the laboratory manager or head.

NOTE:

- For all corrective actions the following should be checked:
  - Are all reagents stored in the correct place and temperature?
  - Is the person performing the assay trained to perform it?
  - Check the expiry date of all reagents used in the assay.
  - Have all instruments been serviced and correct maintenance followed?

## 5.3 Root cause analysis

A root cause analysis is important to monitor problems that occur regularly in the laboratory and make appropriate changes to prevent them from reoccurring in the future. This is normally performed monthly by the laboratory manager. Corrective actions linked to Samples, Procedural/Technical Issues, Laboratory Equipment, Assuring Quality and Laboratory Safety should be monitored for the number of events and duplicate events. This allows for re-occurring events to be identified. Once a re-occurring problem has been identified it should be investigated and a resolution determined and actioned.

## 6. Data tracking and auditing of a molecular sample

It is important to have a system in place that can be used to document and record the movement of samples within the laboratory, from the pre-analytical, to analytical phase and finally to post-analytical phase. During these phases the samples processed need to have all reagent lots recorded, storage and staff member handling the samples recorded. Furthermore, all these procedures need to be written in a document controlled SOP that has been read and signed by all staff and that is accessible to staff in the laboratory on a daily basis.

### 6.1 Pre-analytic phase

The pre-analytical phase includes: test selection, ordering, specimen collection, processing handling and delivery to the testing site and sample receiving. Studies have shown that this step (in conjunction with the post-analytic phase) has a high level of errors and therefore needs to be closely monitored in the laboratory to ensure quality results are reported. One major contributor to this high error rate is the inaccurate selection of the molecular test to be performed. To reduce the number of incorrectly ordered molecular tests it is vital that the laboratory inform the health care professionals using this service of the tests that are offered and the diagnostic purpose (including assay limitations) of each assay. It is also vital to provide the correct collection, specimen handling, transport and submission of specimen information. Furthermore, if any patient specific information, such as treatment history, is required to interpret the test results this should be provided.

Another contributor to the error rate of the pre-analytic phase is specimen handling errors. When a sample is received in a laboratory it is given a unique number. This unique number allows for the correct test to be assigned to the sample and allows the movement of the sample through the assay steps in the laboratory to be monitored. This unique number should also be used for short or long term storage once the sample is received and/or processing is complete. During the entering of specimen information of this unique number, data entry errors can occur. Furthermore, specimens can be stored incorrectly prior to sample testing which could impact on the test. To ensure this does not occur and thereby reduce the error rate, it is important that all staff are adequately trained on sample receiving, and defined SOPs are in place to aid staff. The laboratory should have a data checking system in place to help reduce data entry errors.

During sample receipt in the laboratory the person receiving the specimen should check that the correct sample was received for the test, the correct collection device was used and there is adequate sample to perform the test. These parameters of sample acceptance or rejection should be well defined by the testing laboratory in a SOP available and understood by all staff.

## 6.2 Analytical phase

The analytical phase includes the sample processing and testing. Once a sample has been received, a staff member can begin processing the sample. To ensure there are no errors during the processing of samples it is important to have defined SOPs for the method being performed and that these procedures are correctly followed. Controls for the assay must be included in each run. Reagents must be prepared correctly and the appropriate safety precautions followed throughout the test.

The following should be recorded for each sample processed in the molecular lab (Figure 5):

- Test to be processed.
- Operator.
- Date for each step (if the assay occurs over multiple days).
- Lot numbers of the reagents used (each reagent used should be recorded).
- Controls used in the run (any information about the control that is important in the test).
- Specific equipment used during the assay that could impact on the test outcome.
- List of samples processed together.
- Area for review by a manager.

These sheets are commonly known as **record sheets** and can be made to suit the molecular assay being performed in the laboratory and can be test specific or generic depending on the assay requirements.

## 6.3 Post-analytic phase

The post-analytical phase includes assay analysis, result recording and reporting. During *assay analysis* it is important to ensure that all staff members processing samples analyse and interpret the results in a standardised manner. To control for this a detailed document controlled analysis SOP should be in place for each assay performed in a molecular laboratory. The use of a defined analysis procedure minimises the individual variances that could occur during the result analysis, thereby ensuring reproducible and accurate results are obtained and released.

Assay: _____				
Operator: _____				
	Extraction	cDNA synthesis	Amplification	Detection
Date				
Lot number of reagents				
1)				
2)				
3)				
4)				
5)				
Controls				
1)				
2)				
3)				
4)				
Samples				
1)				
2)				
3)				
4)				
5)				
6)				
7)				
8)				
9)				
10)				
Reviewed: _____				
Date: _____				

Depending on the number of steps the assay has this can be modified.

Depending on the number of reagents involved this can be modified.

Fig. 5. Example of record sheet

*Result recording:* Once the molecular assay has been completed on the samples and the results analysed. The results need to be reviewed. This should be done in the following manner:

- a. The results from the controls of the run are checked to determine they are correct or in range. For a quantitative test the controls should indicate that there has been successful amplification and detection of the target region. For qualitative tests the controls need to be within the appropriate ranges.
- b. Each sample identifier is checked and confirmed to ensure no data entry or clerical errors occurred during the assay.
- c. The results then need to be reviewed (normally by the laboratory manager or laboratory head).
- d. The specimen results should also be checked for any outliers or unusual results that do not fit the clinical picture and/or previous results obtained.

A study may have to be reconstructed many years after it has ended therefore storage of records must enable their safekeeping for long periods of time without loss or deterioration and preferably in a way which allows for quick retrieval. Access to the archive data should be restricted to a limited number of personnel. Records of the people entering and leaving the archives as well as the documents logged in and out should be kept.

#### **6.4 Interpretation and the quality control of the results**

To ensure accurate results of tests performed in a molecular laboratory are reported, additional analysis is required. For example, with sequencing to minimise the chances of sample contamination or mix-up one can align the sequences in a program such as Clustalw2 program (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>) that is freely available on the internet. This program aligns the sequences and draws either a phenogram or cladogram which can be used for a crude analysis. *Parameters to look for are* if there are multiple sequences from the same sample do they cluster together? If you are using a positive control does it cluster with previous positive controls? (if the same sample is used as a positive control). Do samples from the same region cluster together (normally the case for infectious diseases)? Are any sequences very closely related or identical as these should be investigated further.

Once the results have been checked, the testing report should also include additional information that differs for each test but provides an accurate understanding and interpretation of the test results. All reports should contain the following information (according to CLIA guidelines):

- Patient name, Unique Laboratory Number used throughout the test and patient date of birth.
- Name and Address of the testing laboratory.
- Test performed and the date it was performed.
- Specimen information.
- Patient management recommendations (for genetic testing for heritable conditions).
- Name of referring doctor.
- Test methodology.
- Test limitations.
- Test result and interpretation of the result.

### **7. Conclusion**

The recommendations described in this chapter should be considered in conjunction with Good Laboratory Practice and other regulatory guidelines in country. When deciding to set-up a molecular laboratory or to introduce a new test it is important to consider the requirements such as infrastructure, staff, equipment, supplier support, what are the current molecular tests that are available and will these tests complement and/or improve those that are currently in use. The clinical validity of the assay also needs to be assessed during implementation and then through the running of the assay.

The quality management approach described in this chapter allows for the monitoring and continual assessment of the assays through a defined quality control process. Furthermore, the information provided in this chapter can be used to set-up a new molecular laboratory or enhance an existing molecular laboratory. The guidelines described can be adapted for use in different settings and depending on the assay requirements.



*To summarize:*

- a. It is important to ensure health care workers referring specimens understand the use of molecular tests.
- b. To achieve Molecular GCLP the attitude of those in charge is vital.
- c. To get staff to comply to the above mentioned criteria one must write brief and clear SOPs and ensure all staff read, acknowledge and observe the SOPs.
- d. Be meticulous with sample labeling.
- e. Ensure all quality control parameters are implemented and followed.
- f. Ensure all maintenance in the laboratory is routinely performed.
- g. Ensure the housekeeping guidelines are followed.
- h. Everything needs to be documented (if it is not written down...it did not happen).
- i. Assay design, choice and implementation must be considered carefully as this directly impacts on quality of the tests performed.

## 8. References

- Centre for Disease Control and Prevention. Good laboratory Practices for Molecular Genetic Testing for Heritable Disease and Conditions. Morbidity and Mortality Weekly Report, June 2009, p.1-37 Vol. 58, No. RR-6 [www.cdc.gov/mmwr](http://www.cdc.gov/mmwr).
- PCR Primer Design Guidelines.  
[http://www.premierbiosoft.com/tech\\_notes/PCR\\_primer\\_Design.html](http://www.premierbiosoft.com/tech_notes/PCR_primer_Design.html).
- PPD and DAIDS. Global Solutions for HIV. DAIDS Guidelines for Good Clinical Laboratory Practice Standards. 2008.  
<http://www3.niaid.nih.gov/research/resources/DAIDSClinRsrch/Labs/>
- Burd, EM. Validation of Laboratory-Developed Molecular Assays for Infectious Diseases. CLINICAL MICROBIOLOGY REVIEWS, July 2010, p. 550-576 Vol. 23, No. 3.
- Principles and guidance reports for Good Laboratory Practice. Organisation for Economic Co-operation and Development (OECD). <http://www.oecd.org/ehs/>
- GLP Handbook (2<sup>nd</sup> Edition). World Health Organisation.  
<http://apps.who.int/tdr/publications/training-guideline-publications/good-laboratory-practice-handbook/pdf/glp-handbook.pdf>.
- Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples, EPA doc number 815-B-04-001, October 2004.  
[http://www.epa.gov/ogwdw/ucmr/ucmr1/pdfs/guidance\\_ucmr1\\_qa-qc.pdf](http://www.epa.gov/ogwdw/ucmr/ucmr1/pdfs/guidance_ucmr1_qa-qc.pdf).



## **Wide Spectra of Quality Control**

Edited by Dr. Isin Akyar

ISBN 978-953-307-683-6

Hard cover, 532 pages

**Publisher** InTech

**Published online** 07, July, 2011

**Published in print edition** July, 2011

Quality control is a standard which certainly has become a style of living. With the improvement of technology every day, we meet new and complicated devices and methods in different fields. Quality control explains the directed use of testing to measure the achievement of a specific standard. It is the process, procedures and authority used to accept or reject all components, drug product containers, closures, in-process materials, packaging material, labeling and drug products, and the authority to review production records to assure that no errors have occurred. The quality which is supposed to be achieved is not a concept which can be controlled by easy, numerical or other means, but it is the control over the intrinsic quality of a test facility and its studies. The aim of this book is to share useful and practical knowledge about quality control in several fields with the people who want to improve their knowledge.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Raquel V. Viana and Carole L. Wallis (2011). Good Clinical Laboratory Practice (GCLP) for Molecular Based Tests Used in Diagnostic Laboratories, Wide Spectra of Quality Control, Dr. Isin Akyar (Ed.), ISBN: 978-953-307-683-6, InTech, Available from: <http://www.intechopen.com/books/wide-spectra-of-quality-control/good-clinical-laboratory-practice-gclp-for-molecular-based-tests-used-in-diagnostic-laboratories>

**INTECH**  
open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen