MYLAB DISCOVERY SOLUTIONS

Internship report – July 2023

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Reflection

During my time at MyLab, I had the opportunity to observe and assist in the laboratory as well as read through various literature regarding safety, operating procedures, detection kits, and relevant scientific concepts such as PCR, which is a reliable process that is used in numerous MyLab products. Through my experience in the lab and reading, I was able to appreciate the meticulous nature of research as well as witness the application of previous theoretical knowledge in real-world healthcare solutions.

Furthermore, I was able to interact with many professionals in the field ranging from researchers and scientific officers to regulatory experts. The conversations with them provided invaluable insights into the dynamic realm of diagnostic testing as well as biomedical research and development. This internship equipped me with understanding on the science and technology behind disease detection and definitely fostered my curiosity about the healthcare field, which I will carry forward in my future endeavours.

Polymerase Chain Reaction (PCR)

Polymerase chain reaction, PCR, is used to amplify or generate multiple copies of a gene or segment of DNA. A precursor process (RT-PCR) can also be added if the target sample is RNA, where enzyme reverse transcriptase first converts the RNA to DNA, and then the PCR process follows with DNA amplification. Compared to traditional methods of DNA cloning and amplification, which can possibly take days, PCR requires only a few hours!

The Real-Time PCR uses a thermostable DNA polymerase called *Taq polymerase*, extracted from the thermophilic bacteria Thermus aquaticus. This enables it to work at high temperatures with high efficiency and amplification capacity. Furthermore, it utilizes two sets of primers (forward and reverse) complementary to both ends of the DNA template. *Primers* are short, single-stranded DNA fragments designed specifically for the target DNA region of interest, that provide the starting point for DNA polymerase. PCR can be performed on multiple target sequences at a time; these are called duplex or multiplex reactions. *Probes*, or fluorescently-labelled oligonucleotides, are also required to provide a detectable indicator signal as the amplification progresses; this helps visualise PCR in real time.

The steps of the polymerase chain reaction are:

1. **Denaturation:** The double-stranded DNA is separated or heat denatured. Single strands of DNA are formed.
2. **Annealing:** The reaction temperature is reduced to allow the complementary base pairing and annealing of the primer. (primers align to the single DNA strands)
3. **Extension:** The DNA polymerase adds nucleotides to the primer and extends the complementary strand in the 5’-3’ direction, resulting in two copies of the original DNA strand.

The denaturation, annealing, and extension process over a series of temperatures and times is known as one cycle of amplification. [figure 1]

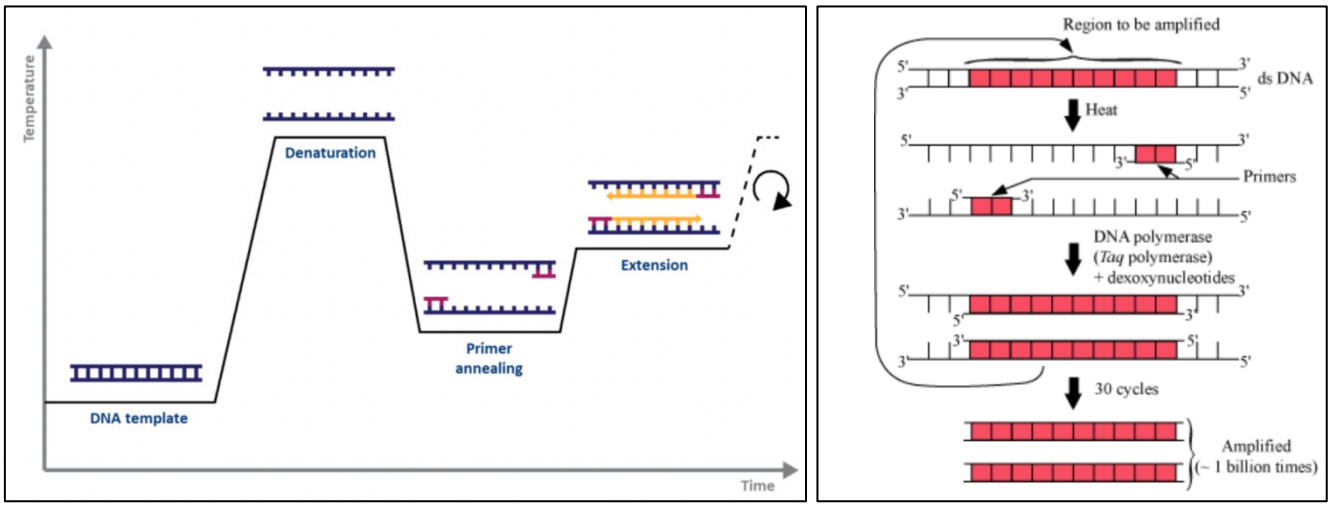


Figure 1. PCR amplification process

What is the difference between conventional PCR and qPCR?

PCR	qPCR (quantitative or real-time PCR)
Used to analyse a short stretch of DNA by amplification	Advanced PCR method used to amplify as well as quantify the amount of DNA
Primer is used for polymerisation	Primer, as well as fluorescent probes or dyes, are used
Results are investigated by gel electrophoresis	Fluorescence emitted by dye or probe is recorded during the PCR process
Data is recorded at the end of the process	Data is recorded during the amplification process at the exponential phase
Low-resolution technique	High-resolution technique
Distinct bands of various DNA fragments can be seen on agarose gel	Different peaks related to different DNA fragments are seen during qPCR
It is used to detect the presence or absence of DNA in the sample, detect gene mutations and amplify the template for DNA sequencing	It is used to quantify the DNA present in the sample. It is used to analyse gene expression, identification and detection of pathogens, quantification and identification of mutation

Lyophilization (freeze-drying)

Lyophilization is a water removal process used to preserve perishable materials and make materials more convenient for transport. It results in less weight, increased stability, and longer shelf life. The application of freeze drying in the pharmaceutical industry is beneficial since many enzymes or reagents tend to be heat-sensitive, making them susceptible to damage.

It works by freezing the material, then reducing the pressure and adding heat to allow the frozen water in the material to sublimate, and lastly removing ionically-bound water molecules in a process known as adsorption. [figure 2]

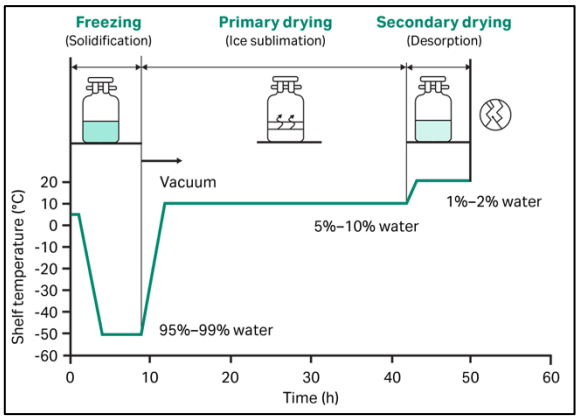


Figure 2. Lyophilization steps

As PCR is a highly sensitive method and small volumes are required for single reactions, the preparation of a master mix for several reactions is recommended. I assisted Kamini ma'am to make a *lyophilized* master mix for an assay to detect *human papilloma virus (HPV)*. It contained all reagents required for successful RT-PCR in one tube: the PCR mix (contains the enzyme), and specific detection mixes (here we used HPV 58 and 59). The detection mix is made of forward and reverse primers and a fluorescently-labelled probe. The master mix is split by the number of reactions, ensuring the same amount of reagents in each. As the reagents were stored in -20°C, the process started with thawing and vortexing the components, then diluting with nuclease-free water to make up the desired volume.

RNA extraction and wipe test

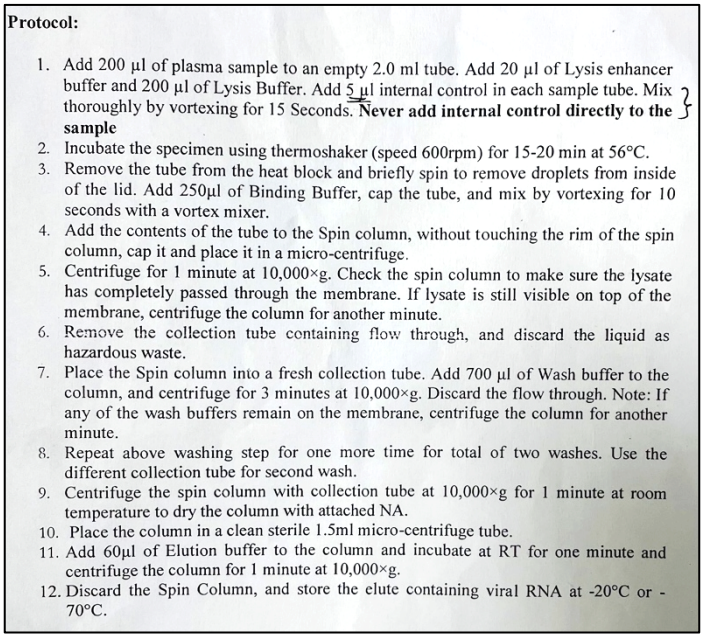
A wipe test is used to determine the presence of contamination on a surface in the lab. Frequent decontamination of work areas in the lab ensures safety and credibility of the experiments. A cotton swab or filter paper is used to wipe the surface of the target area to collect the sample and RNA extraction is performed. I assisted Pooja ma'am in the process of RNA extraction using a series of buffers; the various buffers and their functions are outlined below.

Lysis buffer: disruption of the exterior environment of a cell in a way that causes it to break open and release its contents.

Lysis enhancer (Proteinase K): destruction of proteins in cell lysates (tissue, cell culture cells) and release of nucleic acids, by very effectively inactivating DNases and RNases.

Binding buffer: binding of RNA to the columns for subsequent RNA purification and concentration.

Wash buffer: removal of all traces of protein and salt by washing all excess and unbound components from the reaction surface. As the



Protocol:

1. Add 200 µl of plasma sample to an empty 2.0 ml tube. Add 20 µl of Lysis enhancer buffer and 200 µl of Lysis Buffer. Add 5 µl internal control in each sample tube. Mix thoroughly by vortexing for 15 Seconds. **Never add internal control directly to the sample**
2. Incubate the specimen using thermoshaker (speed 600rpm) for 15-20 min at 56°C.
3. Remove the tube from the heat block and briefly spin to remove droplets from inside of the lid. Add 250µl of Binding Buffer, cap the tube, and mix by vortexing for 10 seconds with a vortex mixer.
4. Add the contents of the tube to the Spin column, without touching the rim of the spin column, cap it and place it in a micro-centrifuge.
5. Centrifuge for 1 minute at 10,000×g. Check the spin column to make sure the lysate has completely passed through the membrane. If lysate is still visible on top of the membrane, centrifuge the column for another minute.
6. Remove the collection tube containing flow through, and discard the liquid as hazardous waste.
7. Place the Spin column into a fresh collection tube. Add 700 µl of Wash buffer to the column, and centrifuge for 3 minutes at 10,000×g. Discard the flow through. Note: If any of the wash buffers remain on the membrane, centrifuge the column for another minute.
8. Repeat above washing step for one more time for total of two washes. Use the different collection tube for second wash.
9. Centrifuge the spin column with collection tube at 10,000×g for 1 minute at room temperature to dry the column with attached NA.
10. Place the column in a clean sterile 1.5ml micro-centrifuge tube.
11. Add 60µl of Elution buffer to the column and incubate at RT for one minute and centrifuge the column for 1 minute at 10,000×g.
12. Discard the Spin Column, and store the elute containing viral RNA at -20°C or -70°C.

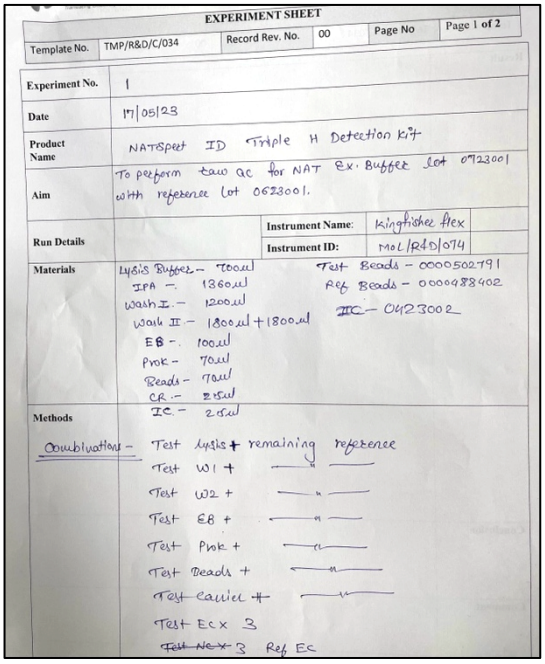
wash buffer flows through the membrane, it reduces the interference and ensures that all contaminants are removed.

Elution buffer: disruption of the bonds between the column and the substrate; this extracts the bound RNA/DNA nucleic acid from the matrix into the solution for storage and further use.

Raw QC for the Natspert ID triple H detection kit

The NATSpert detection Kit by MyLab is an all-in-one qualitative multiplex real time PCR test for simultaneous detection of HIV, HBV and HCV in a single tube format.

Quality control testing for raw materials ensures the purity, identity and quality of reagents. I assisted Aditi ma'am to perform a raw QC test of the buffers involved in the triple H detection kit, such as the lysis buffer, wash buffers 1 and 2, and elution buffer. Each buffer was tested against a QC-passed reference buffer by making multiple combinations, some using Proteinase K and magnetic beads as well.


EXPERIMENT SHEET

Template No. TMP/R&D/C/034 | Record Rev. No. 00 | Page No | Page 1 of 2

Experiment No. 1

Date 17/05/23

Product Name NATSpert ID Triple H Detection Kit

Aim To perform raw QC for NAT Ex. Buffer lot 0723001 with reference lot 0623001.

Run Details — Instrument Name: Kingfisher flex; Instrument ID: MoL/R&D/074

Materials
Lysis Buffer – 700ul
IPA – 1360ul
Wash I – 1200ul
Wash II – 1800ul + 1800ul
EB – 100ul
Prok – 70ul
Beads – 70ul
CR – 25ul
IC – 25ul
Test Beads – 0000502791
Ref Beads – 0000488402
IC – 0423002

Methods
Combinations – Test lysis + remaining reference
Test W1 +
Test W2 +
Test EB +
Test Prok +
Test Beads +
Test carrier +
Test Ecx 3
~~Test Ncx~~ 3 Ref Ec

Standard Operating Procedures (SOPs)

An SOP is a tested, verified, and documented way of executing operations that form the pharmaceutical industry's basis. It provides step-by-step guidance for the personnel to perform a specific process. [figure 3] I had the opportunity to read through multiple SOPs regarding product validation, kit development, and stability testing as well as brochures on various PCR and detection kits.

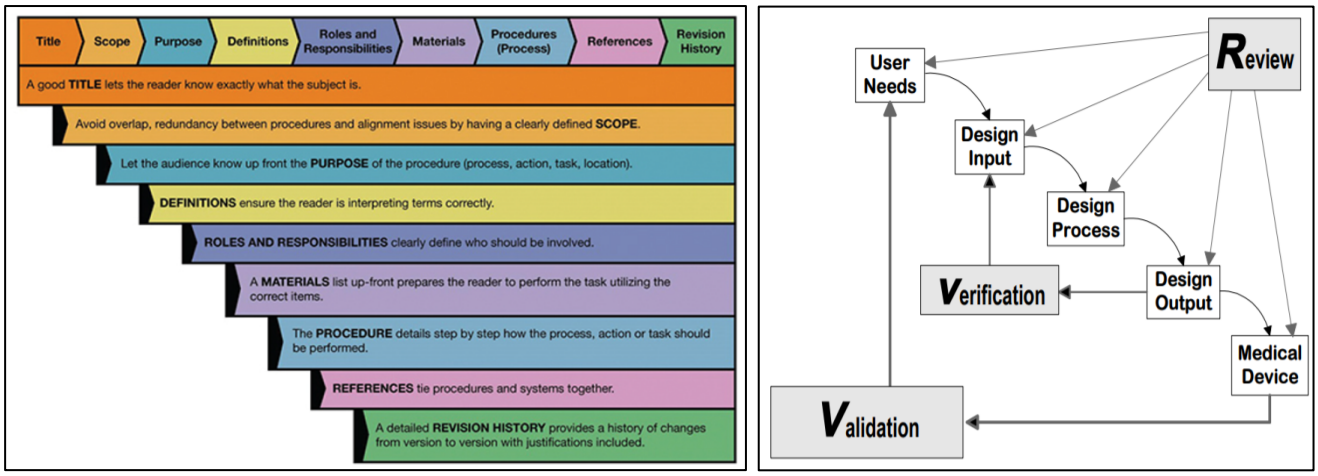


Figure 3. Components of an SOP

Validation refers to documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its pre-determined specifications and quality attributes. Interference studies involve adding a potential interferent to a sample and determining any bias of the test parameter relative to the control. This can be tested in terms of accuracy and stability!

Accuracy (trueness and precision): Trueness is the closeness of agreement between the arithmetic mean of a large number of test results and the accepted reference value; precision refers to the closeness of agreement between each of the test results.

1. **Analytical sensitivity:** ability of a method to detect the analyte of interest with accuracy and specificity, in the presence of other components in a specimen matrix under stated conditions.
   - *Limit of Blank* and *Limit of Detection* describe the smallest concentration that can be reliably measured. *Limit of Quantitation* is the lowest concentration at which the analyte can not only be reliably detected but at which some predefined goals for bias and imprecision are met.
2. **Specificity:** ability of a measurement procedure to detect or measure only the analyte to be detected in presence of other substances in the sample.

Stability: testing how the quality of a product varies with time under the influence of environmental factors such as temperature, humidity, and light to establish a re-test period for the drug substance or its shelf life.

- **Acceleration aging factor (AAF):** factor by which the rate of spoilage increases when the temp is increased by 10°C. It is a prediction of a product's shelf life under real-life conditions.

$$AAF = Q10^{\{(TAA-TRT)/10\}}$$

where TAA is the accelerated aging temperature and TRT is the expected storage temperature.

TaqPath MTB RIF & INH resistance detection kit – Instructions for use (IFU)

Tuberculosis is a chronic airborne disease caused by the Mycobacterium tuberculosis (MB) complex. Multidrug-resistant tuberculosis (MDR-TB) is a global problem due to its resistance to treatment with powerful first-line anti-TB medications, Rifampicin (RIF) and Isoniazid (INH). Rapid and accurate assays are necessary to identify resistance-associated mutations against the first-line anti-TB drugs and are crucial for effective therapy. The failure to appropriately detect the disease can lead to rising mortality, secondary resistance, and transmission of drug resistant-TB.

TaqPath™ MTB RIF & INH Resistance Detection Kit is a multiplex real time PCR test intended for the simultaneous *in-vitro* qualitative detection of Mycobacterium tuberculosis complex (MTB) and its resistance to Rifampicin and Isoniazid antibiotics. The kit contains oligonucleotide primers and dual-labeled hydrolysis probes that target the genes for specific detection of the disease and its mutations.

Coviswift covid 19 S plus rapid PCR kit – Device master file

The kit is based on real time PCR for amplification of specific regions of the target genome using hydrolysis probe chemistry. It uses reverse transcriptase PCR to generate the amplified product from clinical specimens. The amount of target sequence that is present at each amplification cycle is measured through the use of fluorescent-labelled oligonucleotide probes, which do not generate signals unless they are specifically bound to the amplified product. Components of the kit are outlined below:

PCR component	Component	Source	Role
Sample tube	Pre-filled with molecular transport medium for sample collection	Transport medium	Sample collection
Lysis tube	Pre-filled buffer tube for nucleic acid lysate	Chemicals	Lysate preparation
COVID-19 Reaction tube	Lyophilized reaction mix containing reverse transcriptase enzyme, Taq polymerase and specific primers and probes for N gene, ORF lab, S gene, and RNaseP targets.	Recombinant molecular compound, synthetic compound, and enzymes	PCR amplification
Reconstitution buffer	Buffer components for reconstitution of lyophilized reaction mix	Salts, dNTPs	Reconstitution of lyophilized reaction mix
COVID-19 positive control	In vitro transcripts for SARS-CoV-2 specific N gene, ORF lab, S gene, and RNaseP targets	Recombinant molecular compound or synthetic molecule resp.	Quality check
No template control	DNase/RNase free water for no template control	Nuclease free water	Quality check