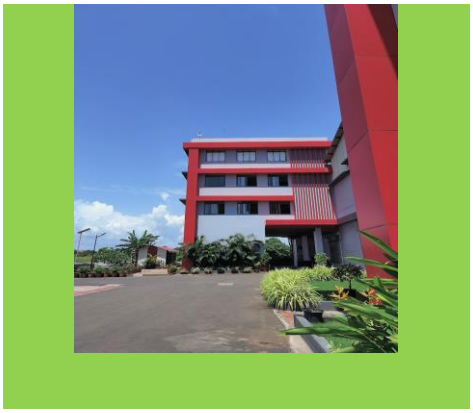

molbio

INTERNSHIP REPORT

"The capacity to learn is a gift; the ability to learn is a skill; the willingness to learn is a choice"
~Brian Herbert

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Molbio Diagnostics Pvt. Ltd. is a molecular diagnostics manufacturer that specialises in the low-cost diagnosis of tuberculosis and other diseases. During the Covid-19 pandemic, it revolutionised India's Covid-19 screening with an innovative portable and battery-operated real-time RT-PCR point of care (PoC) testing system. The Molbio Diagnostics RT-PCR PoC testing system helped in detecting infection in remote and rural villages.

The Truelab Real Time quantitative micro PCR system from Molbio Diagnostics brings PCR technology right to the point-of-care, at all laboratory and non-laboratory settings, primary centres, in the field, near patient, essentially at all levels of healthcare thereby decentralising and democratising access to molecular diagnostics.

Molecular Diagnostics is the state of the art in Diagnosis of infectious diseases. It works on the principle of DNA amplification, hence provides the ability to diagnose disease early in infection because of its excellent sensitivity and specificity. Some of the core values that this company stands for are collaborative innovation, being transparent and truthful, ensuring customer satisfaction, building relationships bases on trust, delivering excellence, etc.

Mission of Molbio Diagnostics Pvt. Ltd.

- To enable better medicine through precise, faster, cost effective diagnosis at the Point of Care (POC)
- To reduce patient suffering, fatalities, and resultant economic loss due to inadequate diagnosis
- To provide every patient access to best health care through cutting edge technologies
- To stay connected to the needs of the patient through constant engagement with all stakeholders

Vision of Molbio Diagnostics Pvt. Ltd.

- To transform healthcare practices by providing near-care diagnostic solutions using robust portable platforms
- To enable decentralisation and democratisation of diagnostics through high ended point of care technologies
- To be a leading global player in the point of care diagnostics segment
- To continue to innovate and bring new technologies for social betterment

The company Motto is "IC^{3}"-

- **I**ndependent Care
- **C**ontinuous Care
- **C**ost Effective Care
- **C**onnected Care

Molbio Diagnostics Pvt. Ltd. products fall under 5 sections-

- Truelab®
- Trueprep®
- Truenat®
- Truemix™
- STABILYSE®

Truelab® products

1.Truelab® Uno Dx Real Time Quantitative micro PCR Analyzer

Fully automatic Real time Quantitative micro PCR analyzer, three wavelength system, that performs 10-12 tests in 8 hours.

2. Truelab® Duo Real Time Quantitative micro PCR Analyzer

Fully automatic Real time Quantitative micro PCR analyzer, having a two channel-three wavelength system, which performs 20-24 tests in 8 hours.

3. Truelab® Quattro Real Time Quantitative micro PCR Analyzer

Fully automatic Real time Quantitative micro PCR analyzer, having four channel-three wavelength system, which performs 40-48 tests in 8 hours.

4.Truelab® micro PCR Printer

Bluetooth Printer, which prints wirelessly the results of the PCR tests performed by Truelab Uno Dx/Duo/Quattro Real Time Quantitative micro PCR Analyzer.

Trueprep® products

1. Trueprep® AUTO v2 Universal Cartridge Based Sample Prep Device

Fully automatic sample prep device works in tandem with Trueprep AUTO cartridge and Trueprep AUTO v2 Reagent Kits for extraction and purification of nucleic acids from clinical specimen.

2. Trueprep® AUTO Universal Cartridge Based Sample Prep Kit

Sample preparation kit for nucleic acid extraction on Trueprep AUTO/Auto v2.

3. Trueprep® AUTO Universal Sample Pre-treatment Pack

Kit Contents

- Lysis buffer
- Disposable transfer pipette
- Packinsert

Used to pre-treat the samples such as whole blood/ serum/ plasma/ urine/stool/swab specimen before proceeding for extraction and purification of nucleic acids using Trueprep AUTO Universal Cartridge Based Sample Prep Device. Sample Pre-treatment decontaminates the specimen and makes it ready for storage/ transportation/ extraction.

4. Trueprep® AUTO Transport Medium for Swab Specimen Pack

Kit Contents

- Transport Medium for Swab specimen tubes (contains transport medium)
- Packinsert

To use with clinician-collected endocervical, vaginal, anorectal, nasal and throat swab specimens. The transport media is used as a medium for collection, decontamination, and transport of various types of swabs specimens before proceeding futher. The transport medium keeps the nucleic acids of the respective organism intact thus maintaining the integrity of the nucleic acids.

5. Trueprep® AUTO v2 Universal Cartridge Based Sample Prep Kit

Used for extraction and purification of nucleic acids from a wide range of biological specimens.

Kit Contents

A. The Reagent Pack contains the following reagents

1. Wash Buffer A

2. Wash Buffer B

3. Elution Buffer

4. Priming Waste

B. The Cartridge Pack contains the following

1. Cartridge

2. Elute collection tube

3. Elute collection tube label

4. Disposable transfer pipette

C. Disposable Transfer Pipettes (graduated) - 3 ml

D. Reagent Reset Card

E. Package Insert

Truenat® products

1. Truenat® MTB (Chip-based Real Time PCR test for Mycobacterium tuberculosis)

Kit Contents

Individually sealed pouches, each containing

1. Truenat MTB micro PCR chip
2. Microtube with freeze dried PCR reagents (master mix)
3. DNase & RNase free pipette tip
4. Desiccant pouch

Used for quantitative detection and diagnosis of *Mycobacterium tuberculosis* (MTB) in human pulmonary and EPTB specimens and aids in the diagnosis of infection with MTB.

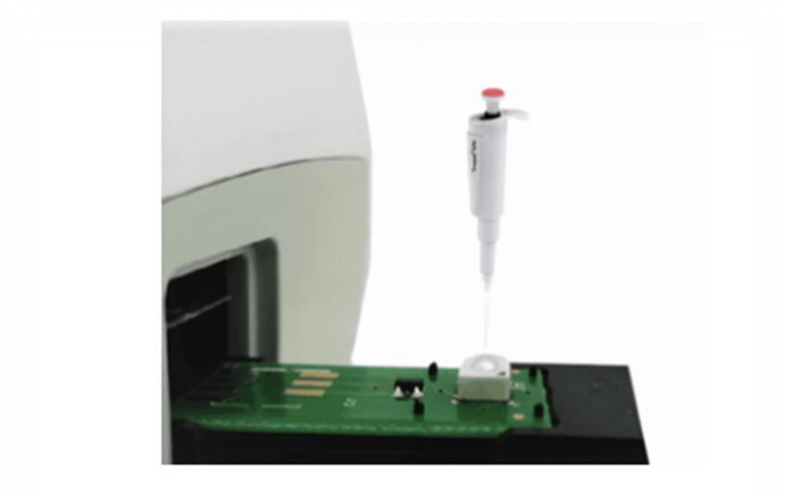
2. Truenat® HBV (Chip-based Real Time PCR Test for Hepatitis B Virus)

Kit Contents

Individually sealed pouches, each containing

1. Truenat HBV micro PCR chip
2. Microtube with freeze dried PCR reagents
3. DNase & RNase free pipette tip
4. Desiccant pouch

Used for quantitative estimation of the *Hepatitis B Virus* (HBV) in human blood/serum/plasma specimen and aids in the diagnosis of infection with Hepatitis B virus and in the estimation of viral load.



Loading of purified sample onto the micro PCR chip

Truemix™ products

1. Truemix™ COVID -19 (Real Time Duplex PCR Test for COVID -19)

Kit Contents

1. Lyophilized Truemix COVID-19 tubes
2. Reconstitution Buffer
3. Positive Control (Dried Down)
4. Negative Control
5. Strip of 8 PCR Flat Caps
6. Package Insert

It contains lyophilized, ready-to-use, open format, duplex Real Time Reverse Transcription Polymerase Chain Reaction (RT PCR) test for the qualitative detection of SARS CoV-2 RNA in human oropharyngeal and nasopharyngeal swabs and aids in the diagnosis of COVID-19. The test detects the E and Orf1a genes of the virus.

STABILYSE® product

Kit Contents

1. Collection and Lysis Medium Tubes
2. Packinsert

Sample collection and lysis medium for oropharyngeal and nasopharyngeal swab specimens (prep free Swab specimen).

Some of the Various Truenet and Trueprep sections we were given an introduction on during the tenure of our internship by the officers were:

Truenet Section

In Quality Control (QC) department

A. Incoming Raw material room

Testing of Incoming material

Upon receiving incoming raw material, Standard Testing Procedures (STPs) are used to test the material received. The results are recorded and an Analytical Test Report (AR) is prepared. If the material meets the specified criteria, it is approved and a release advice is issued to the store. If the material does not comply with the specifications, it is re-tested based on a different set of Standard Operating Procedures (SOPs). If it still does not comply, it is rejected.

Testing In-process material

Here, on testing the in-process material if desired results are obtained, the material is approved and a release advice is issued to the manufacturing department. The testing is done using a previously approved lot with positive and negative samples. If the desired results are not obtained, then an Out of specification is filed.

Testing Finished Product

On receiving finished product from the packaging department, the product is tested as per respective testing procedures in triplicate and the results are recorded. If the product complies, it is approved and a Certificate of Analysis (COA) is prepared.

Here, the approved finished product is also retained for a period of 180 days from the date of expiration of shelf life. The approved material details are also verified by QC and given for lot printing.

B. Instrumentation room

In this room, incoming raw materials such as primers, probes etc. are tested. Some of the instruments present in this room include UV visible spectrophotometer- for checking contamination, AlphaImager HP System- for taking photographs of the gel, Gel rocker- for staining of the gel with methylene blue, leak test apparatus- for checking leaks in pouches/bottles, pH meter, IR spectrophotometer, Capillary melting machine etc.

C. Stability room

In this room, the products are kept at a stable temperature. Studies on acceleration and shelf life, in-use stability, flex stability etc. are also done. Here, the products are also tested every 2 months during expiry term to see if they meet declared criteria and have not lost their effectiveness.

D. Rapid Testing Room

In this room, the cartridges are tested using a JIG machine. The cartridges are also given a 14-digit QR code which is read by the machine, along with checking for any defects in the cartridges. Any defect if present is shown on the machine screen and the defected ones get rejected and fall, while passed cartridges move forward. Apart from this, a certain number of cartridges also undergo leak tests where any error, if present, can be identified exactly. Physical fitting test is also done to check if the cartridge fits in Truelab device.

E. Documentation and Extraction room

In this room, the samples/cartridges are tested according to sampling plans. The nucleic acids are extracted in this room using Auto/Auto v2 devices and get collected in the elute chamber (150µl) of the cartridges.

F. PCR room

In this room, PCR reactions get carried out on loading the elutes in the Truelab®Real Time Quantitative micro PCR Analysers.

G. Sample Storage room

In this room, samples like blood, plasma, sputum, extracted DNA/RNA elute, serum, +HIV/+HBV/+HCV samples etc. are stored at appropriate temperatures.

H. Control Room

Here, approved samples are kept as a record till their expiry dates just in case any infield issue arises and needs to be addressed.

Master mix preparation room

In this room, the mix is prepared using standard formulas and then dispatched. The master mix contains all the requirements for the PCR reaction like primers, probe, enzymes, RNAase inhibitor, buffers, Double distilled water, reverse transcriptase etc.

Pre batch testing of primers, probes is done by QC and then once approved, it is used for making the entire batch.

Master mix filling room

In this room, a cold environment is maintained for the mix by using ice packs, the mix is loaded on the machine manually and after dispensing, the tubes are transferred to cold blocks for freezing the master mix at -46^0 C.

Lyophilization room

This room has 28 freeze dry machines. Before receiving the master mix batch, the machines are pre-cooled for 2 hours at -46° C and the master mix room is informed to begin preparing and dispensing. The master mix tubes are then transferred to cold trays and capped with bungs. Probes are inserted in the blocks, trays, and products to know the precise temperature of each. The product is then freeze dried for 42 hours to form lyophilization cakes, followed by vacuuming and tightening of bungs. All details and every step of the freeze dry process can

be observed on the display screen and needs to be continuously monitored. In this entire procedure 2 dryings take place i.e.; primary (2hrs) and secondary (2hrs) and 38 hours of vacuuming in between the two. If any component stops working the full procedure gets halted as they are interconnected to each other. Once this is done the product is maintained at environmental temperature and humidity, which is critical for the 2 years warranty period provided. Once the lyophilization is complete the product is given for testing. The capacity of each lyophilizer is 26,880.

Trueprep Section

1. Chip Assembly/ Washing/ Drying/ UV room

In this room, the Truenet chips obtained from vendors like Danlaw, Cyient undergo various steps like washing in a PBC washing machine using EDM (electron deionized) water and Ultra-pure water, followed by drying in hot air ovens (80^0C) for 20 mins, treatment in UV chambers, assembling and dispensing. These chips are made of a LTCC (low temperature ceramic core) and can be arranged manually on trays that fit 240 chips or with the help of a 'pick and place' machine. These trays are transferred from one department to another using mobile cabinets. The role of QC here is to check received chips (diameter, resistance, conductivity, quantity etc.) and give approval if all criteria are met.

2. MG prep room

In this room, the coat mix having 5 components like MgCl, glycerol, trehalose, BSA and Tween20 is prepared. There is also a vacuum dryer in which trays are placed to avoid exposure to heat and the coat is dispensed (At every step QC approval and QA clearance is taken).

3. Polymer dispensing room

In this room, the master chips which contains the sample loading well, are coated with wax to prevent moisture from entering the sample and to prevent the sample from evaporation. This is done by a polymer dispensing machine that deposits wax within the range of 5.8mg-6.2mg on the chip through syringes in the machine jets for melting and dispensing. The moisture is removed and kept below 5%. Finally, a dryer is applied to retain concave shape of the wells and remove any bubbles present.

4. **Chip sorting and holding room**

In this room, the chips that have undergone coating are manually check using overhead lamps as a precaution. The chips are segregated into high wax and low wax chips. Those that are defective are discarded.

5. **Flash Writing room**

In this room, the chips are given an identity (i.e., batch number etc.). On a memory card, details are fed through flash writing (disease details, expiry date, lot number, manufacturing date, CT value etc.). After flash writing, the chips are loaded at the loading station and then sent to the testing station where POGO pins connect to gold bars on the chip and feed in the information. Manual verification is then done at the unloading station.

6. **Assembly Room- 1**

Here, on receiving the chips from the flash writing room, the Quality Assurance (QA) department verifies the number received. The chips are then placed in true net pouches. Each pouch contains 1 disease specific chip, 1 master mix tube, 1 tip and 1 silica. The pouches also contain all information like batch number, expiry date, usage number, manufacturing date etc. Those that are going to be exported have the CE mark. A finished pouch is sent to QC for testing and approval.

7. **Packing Room**

In this room, printers for pouch printing are present. These printers help print the lot number, chip number, manufacturing date, expiry date, storage temperature etc. Here, cartons are also printed (i.e., 50 Test, 25 Test, 20 Test, 5 Test) and packed. A party label is also present on the carton as the products are exported, allowing the buyers to get in contact with the company, if any issue arises. The cartons are packed with 4 main products: USPT/MSPT/ATM/STABILIZE. Each

carton contains 1 fitment, pipette packet (depending on test size i.e., if 50 test than 50 pipettes), package insert, buffer (number of bottles as per kit test size) and additional liquification buffer for MTB Test.

Some Finished kits are given to QC for testing and approval.

8. **Printing room**

In this room, the lot number, manufacturing date, expiry date, address etc. that is mentioned on the packaging is printed using a printing machine. If any mistakes occur during the printing process, they are easily rectified using acetone as an eraser.

The different packages available are 50 test pouches, 25 test pouches, and 5 test pouches. Each pouch has an information pamphlet within it. Each pouch has a standard weight and before it is dispatched the pouches are weighed to check if anything is missing. The pouches contain 1cartridge, 1 elute label, 1 collection tube, and 1 disposable pipette.

9. **Reagent preparation room**

In this room, pre-treatment packs are made. There were three reagents prepared A, B, and C in the stock preparation area. The buffers were prepared using overhead stirrers. All SOPs are followed for preparing the buffers. Since line clearance is needed all equipments are placed for Quality Assurance checks and the buffers are filled under a lamina air flow.

For example, in the 50 test Reagent bag the reagents (wash A, wash B etc.) are filled using a machine and the entire process takes around 150s - (Vacuum process (20s), priming process (20s), pouch filling (90s), air filling (10s)).

10. **Filling Room**

In this room, reagents are filled in bottles according to requirement.

Eg. MSPT- 2.5 ml per bottle

There is a 3-piece machine for filling Universal Sample Lysis buffer which is semi-automatic.

Bead placement in MSPT bottles and bung placements are done manually here by workers.

Reagent pack filling

Here, the reagent is filled using a 5-station reagent filling machine. Before starting the filling procedure, the pH of the reagent is checked every morning to ensure that there is no change. The machine is cleaned and volume for dispensing is set. The filling procedure involves 4 steps which takes a total of 2.24 mins.

1. Vacuum process (30s)
2. Priming process (15s)
3. Pouch filling (90s)
4. Air filling (30s)).

A seal for connector housing to seal connector of pouches is done. The priming bottle is also provided with a pin hole for trapped air to escape. The final dispensed pouches/bottles are weighed to check if dispensing was done correctly by comparing the weight to given weight SOPs. (On a daily basis 4 kits are sent to QC for lot approval).

11. **Labelling room**

In here, labels are printed and attached to the bottles by an automatic set adhesive vertical sticker labelling machine that contains 5000 labels in a roll.

12. **Cartridge Selection room**

Here, the cartridges present are universal cartridges that can be used for testing any disease. There are 2 types of cartridges Laser cartridges and Ultrasonic

cartridges. These cartridges consist of polycarbonyl and are manually filled with an Internal Positive Control (IC) to validate the data. The IC is manually dispensed according to tests done for approved IC volume. Once IC dispensing is done the cartridges are placed on trays for drying. The cartridges contain a matrix chamber that contains cellulose which helps trap nucleic acids. The cartridges have a 2 years warranty. Some cartridges are also sent for QC testing and approval.

Cartridge pouching/sorting/labelling room

The cartridges are then labelled (QR Code, lot number., manufacturing date, expiry date, running number etc.) and then placed into pouches. Each pouch contains 1 cartridge, 1 elute dropper, 1 elute collection bottle and 1 label.

Quality Control (QC) Department

During the one-month tenure of my internship at Molbio, I was placed in the Quality Control (QC) department.

This Department plays a crucial role in testing and verifying the quality of products with regard to predefined standards.

The QC department functions for assuring the quality of all the batches manufactured at every stage of production. This is achieved by sampling, inspection, and testing as per specifications of Raw material/packaging material/in-process products etc.

https://solutionpharmacy.in/wp-content/uploads/2021/08/Responsibilities-of-Quality-Control-QC.jpeg

The Quality Control Department also handles sampling, specifications, testing, documentation, release procedures, validation etc. which ensure that the necessary and relevant tests are carried out and that the materials are not released for use, nor are products released for sale or supply until their quality has been deemed satisfactory.

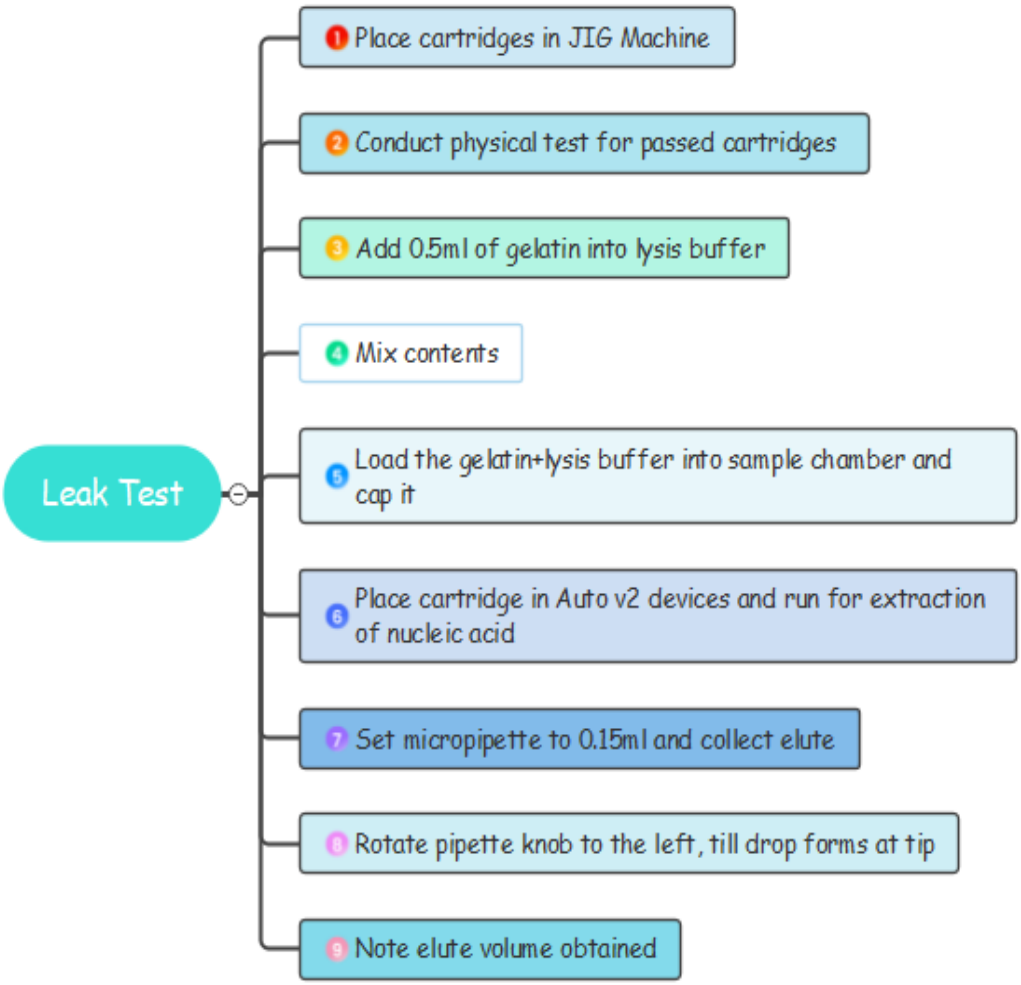
Work carried out

1. CARTRIDGE LEAK TEST

Purpose

- To check if the cartridges obtained are proper.
- To check if the amount of elute volume formed is proper.
- To check for any capillary leaks due to improper welding.
- To check exact error of a cartridge rejected during the JIG test.

Procedure

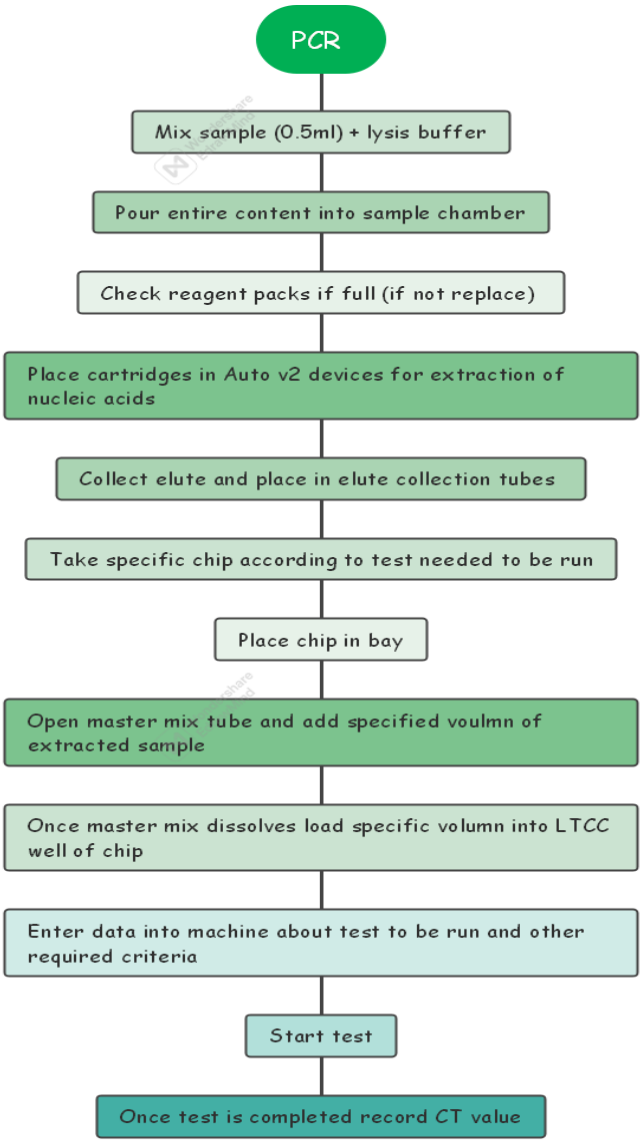


2. PCR TESTING

Purpose

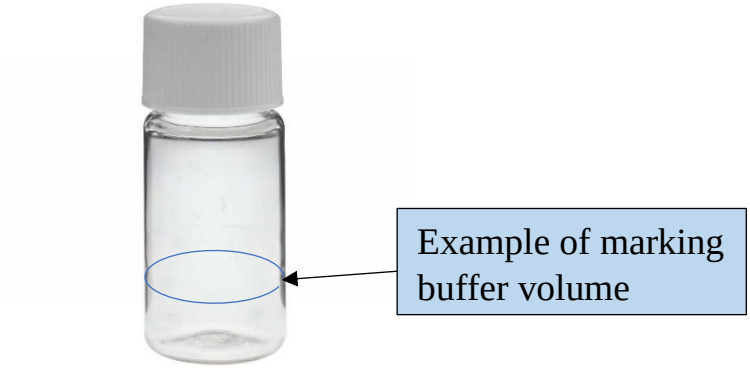
- To test if the samples are positive or negative.
- To obtain viral load.
- To find out the CT value.

Procedure



3. LEAK TEST FOR BOTTLES

Buffer bottles are tested for any leak using the leak test apparatus. For this the level of the buffer was marked so that if the bottle is leaking it can be noticed by checking the level of the buffer solution post the leak test. The bottles were also labelled.



4. STABILITY STUDY OF LYSIS BUFFER

The lysis buffers were checked for their stability at different temperatures- Room temperature (RT), 40℃ and 45℃. The bottles were checked for any precipitation around the rims or change in the buffer transparency. 10 bottles from each carton were randomly opened and checked, if precipitation was observed in any bottle in the lot, every bottle in that lot was checked and the number of bottles showing precipitation was noted.

5. DATA ENTRY

The data of the tests run on the Truelab® Real Time Quantitative micro PCR Analyzer can be printed using the Truelab® micro PCR printer. These thermal prints obtained were checked and stuck. The data was also entered on the computer and previously entered data was also cross-checked.

Additionally, we were also given a theoretical explanation on how PAGE is carried out, a demonstration on using Quant Studio 5 PCR software and a demonstration on using the leak test apparatus for bottle leak test and pouch dye leak test.

CONCLUSION

"*The only source of knowledge is experience*."

~Albert Einstein

This month-long internship at Molbio Diagnostics Pvt. Ltd. helped broaden my horizons and increase the range of my knowledge and understanding. It also provided me with a rich and rewarding hands-on practical experience. Thereby, helping me to appreciate my field of study more, understand first-hand how things work in an industrial set-up, and practically apply topics that I studied theoretically. I also learned so much through the expertise shared with us by the officers. Throughout this internship, I was provided with ample opportunities to grow in a participatory environment and thereby, grow my strengths. Overall, this internship was an enriching and stimulating academic experience that will help me grow in awareness, realisation, and motivation.