INTERNSHIP REPORT

(2022)

MOLBIO DIAGNOSTICS PRIVATE LIMITED.

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Contents

Molbio diagnostic Pvt. Ltd, site V

Introduction

Molbio diagnostic is a molecular diagnostics manufacturer. Located at Verna Industrial Estate, Verna Goa. Molbio offers a platform that can perform molecular diagnosis for varies infectious diseases such as HIV, HAV, HBV, Covid-19, MTB etc at the point-of-care on the Truelab Real Time Quantitative micro–PCR System.

Its main aim is 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 and to provide every patient access to best health care through cutting edge technologies.

Molbio Diagnostics has a clear vision 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 and to continue to innovate and bring new technologies for social betterment.

Molbio Diagnostics also have their Research and Development Wing, located at Bigtec Labs in Bangalore India which has various tests and nucleic acid preparation devices to facilitate "sample to result" molecular diagnostics in resource limited settings. The micro-PCR system has since been launched in India through the parent company,

Molbio Diagnostics has manufacturing and marketing base in Verna Goa.

The internship at Molbio was conducted for a period of 30 days, which started from 1st December 2022. I along with three of my batch-mates were assigned to the Quality Control Department at site V. The Quality control department mainly aims to control the quality of the various manufacturing products produced or procured by following strictly the standard testing procedures and the standard operating procedures.

The main goal of undergoing an internship at Molbio Diagnostics was to get an insight on how it conducts various diagnostic and confirmatory tests, the equipment's used for conduction of these tests, the protocols and procedures followed and also to expand our knowledge on the various diseases prevalent in our society today and what are the kinds of tools used to diagnose these diseases.

Quality control

For the first few days we were told to understand the functioning of the various departments, how they all are integrated with one another and to understand the working and principle behind the various testing equipment's and procedures conducted at the company.

The Quality Control Department have different sections. They are as follows:

1) Incoming Material Room:

Induction was given by: Ms. Somini and Ms. Bhibijan

Incoming Raw Material: The incoming raw material section involves testing of all raw materials either procured from stores or from the production department. It is further categorised into 3 groups.

a) Incoming Packaging Material: This includes all the packaging components involved such as Cartons, Pouches, Buffer bottles, Labels, etc.

- All the raw materials that are procured from either vendors or the production department first arrive at this section.
- Every packing material product is tested by the QC officers before producing it in bulk.
- Every incoming material is tested for various parameters such as proper measurement, artwork, printing, overprinting, version, etc.
- A certain quantity of products in lots is sent by the production to the quality control department. After taking the sample size the QC further proceeds for testing.

- On receipt of sampling advice for incoming packaging material from stores department, verify the details of the received material.
- Verify the received packaging material details against the details given in the sampling advice such as: Name of the material, material code no., name of the manufacturer, name of the supplier, invoice no., date, GRN No., quantity received, total number of packages/containers, Batch No., Mfg date, Exp. Date, etc.
- The incoming material detail is entered in the respective logbooks.

Labelling:

For ease of identification and documentation. There are different labels assigned with their respective colour codes for the incoming materials that arrive in the Quality control for quality checks.

They fallow certain ISO standards for selecting the lot size for sampling and also fallow Standard operating procedures (SOP), and Standard testing procedure (STP) for testing.

- On initial arrival, the boxes are labelled with an 'UNDER TEST" label. At this stage the products lot number, batch number, manufactured date, expiry date is checked and matched to the official documents.
- On opening of the boxes, an 'OPENED FOR SAMPLING' label is affixed and an appropriate sample size is taken out for testing. The sample size is determined by number of units that arrive for testing. They check for proper measurements, prints, version, font, correct spellings etc, according to the SOPs and STPs, provided for each incoming material.
- If it complies with the specifications then affix 'APPROVED' label and send it back to the store.
- then an 'APPROVED' label is affixed and a release notice is passed.

- If the tested products are found faulty and fails the test, then a 'REJECTED' label is affixed and the whole lot that arrived for testing is rejected. If only a small amount of the products fails, then a retest date is fixed, and a new lot is to be sent for testing.

b) Incoming Raw Material: This includes non-chemical components used for the testing of samples such as Truenat chips, cartridges, sealed pouches, droppers, etc.

e) Incoming Raw Material (Chemical): This includes all the chemical components used in the testing procedures such as the lysis buffer, liquefaction buffer, Internal positive control etc.

2) Instrumentation Room:

Induction was given by Ms. Saloni

Chemical component testing is carried out in this section instruments such as the spectrophotometer, alpha imager. making use of various PAGE equipment's etc.

3) Rapid Testing Room: Cosmetic defects and manual inspection of the raw material arrived is carried out in this section.

Cartridge samples received for QC testing are manually checked for any physical defects. "ZIG Machine" is also used to check the proper functioning of the cartridges.

4) Stability Room:

Induction was given by Mr. Adam.

In this section samples of all the tested products are stored at different storage conditions for stability study.

The purpose of stability testing is to provide evidence of how the quality of an Active Pharmaceutical Ingredient or Finished Product varies with time under the influence of a variety of environmental conditions such as temperature, humidity and light, Stability testing is important for determining factors such as a product's shelf life, optimal storage conditions, retest period, and assuring its overall quality for consumers.

During a stability test, the product is observed for any changes in the physical, chemical. biological, and microbiological makeup of the substance. All of these elements may impact the safety and efficacy of the product for the consumer, so it is vital to conduct extensive testing before putting a drug or cosmetic on the market.

5) Extraction Room:

Induction was given by Mr. Santosh

Extraction of Nucleic acids are carried out in this section. We were assigned practical work in the extraction room. We did validation of different concentration of IC (internal control) of the cartridges, ranging from 10ul, 15ul, 20ul, 25ul.

For each concentration, we did extraction for 40 cartridges, out of which, for 20 cartridges, we added only MSPT buffer, and for remaining 20 we added USPT and Negative HCV sample.

We performed leak test for cartridges, by extraction using Trueprep AUTO and Trueprep AUTO V2 devices. Lysis buffer and gelatine was used as sample for extraction process. The volume of elute collated was also noted from the elute chamber. Methylene blue dye was than introduced in the sample chamber for leak test.

6) PCR Room:

Induction was given by Mr. Rinoj and Mr. Arvind

Following extraction, the PCR process is carried out in this section.

Testing of Raw Material and Raw Material Chemical

• On receipts of sampling advice from stores department, verify the details of received material give in sampling advice and GRN and affix 'UNDER TEST' label on all received containers/vials.

• Check for printed details such as batch/lot no., date of manufacture

•Check for physical parameters such as appearance, colour, sealing of container, presence of any leakage. It should comply as per satisfaction. Recap the container and label it as 'OPENED FOR SAMPLING'.

For Primers and Probes

•For primer and probe they check the concentration by pedestal or cuvette method.

• Test in performed in triplicates, and the average is taken to calculate the concentration.

•The concentration should be above 70%. The obtained value for the primers is μM should be given to the manufacturing department to aliquot the primers and store below -20°C and use as and when required.

• The reconstituted primers/ probes are used for PAGE. The band should be crisp without smear formation.

• For probes, Probe check test is carried out on Truelab® Uno DX-Probe checking Device.

• If concentration, PAGE result and probe signals are as per specification, further performance test is carried out.

• Performance test can be done using Applied Biosystems 7500 Real time PCR.

Products manufactured at Molbio Diagonostics

Trueprep®

A) Trueprep AUTO and Trueprep® AUTO v2 Universal Cartridge Based Sample Prep Device

Trueparep is divided in two types; Trueprep AUTO and Trueprep® AUTO v2.

Trueprep AUTO takes 18 minutes and Trueprep® AUTO v2 takes 20 minutes respectively for extraction.

The products included are cartridges, reagent packs (buffers), devices and transport medium for specimens.

Trueprep AUTO and Trueprep AUTO V2

This is a fully automatic sample prep device which works in tandem with Trueprep AUTO cartridge and Trueprep AUTO v2 Reagent Kits for extraction and purification of nucleic acids from clinical specimen. The PCR process necessitates the extraction and purification of nucleic acids from clinical specimens to free it from potential PCR inhibitors. The Trueprep AUTO v2 Universal ® Cartridge Based Sample Prep Device together with Trueprep AUTO v2 Universal Cartridge Based Sample Prep Kit provides an easy method of nucleic acid extraction and purification.

Principle of working

The Trueprep AUTO v2 is an electromechanical system pre- programmed to sequentially heat, mix and add reagents to the contents of the cartridge placed in the cartridge holder and has a 2-line LCD screen that displays the status. Specimen pre-treated with lysis reagent is added to the sample chamber of the cartridge which is then placed in the cartridge holder of the device. Sample processing is initiated upon pressing the start button on the device, through an automatic pre-programmed process wherein nucleic acids released by chemical and thermal lysis of cells bind to the proprietary matrix in the matrix chamber. In subsequent steps, the captured nucleic acids are washed with buffers to remove the PCR inhibitors and finally eluted from the matrix using the elution buffer. At the end the cartridge is automatically ejected and the elute containing purified nucleic acids is then collected from the elute chamber for further analysis.

B) Trueprep® AUTO/AUTO v2 Universal Cartridge Based Sample Prep Kit

Trueprep® AUTO Universal Cartridge Based Sample Prep Kit

Trueprep® AUTO v2 Universal Cartridge Based Sample Prep Kit

Universal Cartridge Based Sample Prep Kit uses a Trueprep proprietary matrix enclosed in a cartridge to purify nucleic acids from clinical samples. The cartridge also contains pre-loaded Internal Positive Control (IPC). The IPC is a full process control that undergoes all the processes the specimen undergoes, from extraction to amplification, thereby validating the analysis from sample to result.

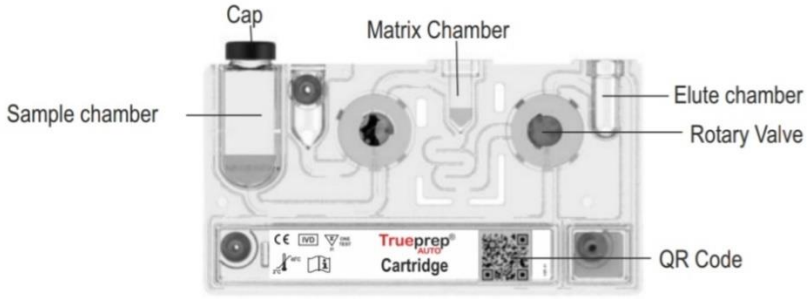


Figure 3: Disposable **Trueprep® AUTO v2** cartridge for sample processing

There are two types of cartridges, Ultrasonic (black in colour) and Laser (transparent) channels are welded with laser.

Contents in the reagent pack:

1. Wash Buffer A -To wash inhibitors from the sample-GREEN CAP
2. Wash Buffer B-To wash inhibitors from the sample-BLUE CAP
3. Elution Buffer - To elute nucleic acids-WHITE CAP
4. Priming Waste- To purge residual liquid from tubing-RED CAP
5. Reagent Reset Card-1 No. (Applicable for Trueprep AUTO v2 Users only)

Contents in the Cartridge Pack:

1. Cartridges containing immobilized internal positive control (IPC) for extraction.
2. Elute collection tube- Capped tubes for collection and storage of (ECT)extracted nucleic acids.

3. Label - To label Elute collection tube (ECT) Label

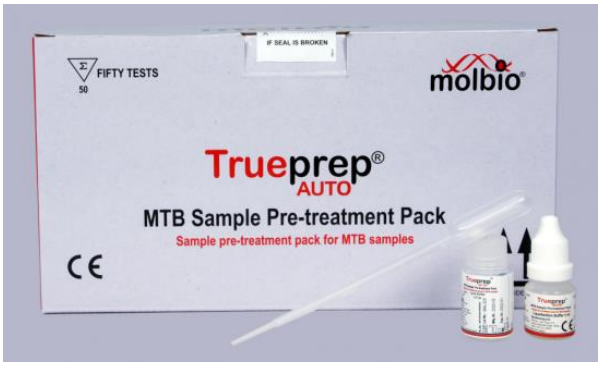
4. Disposable Transfer Pipette-To pierce the seal of elute chamber and to transfer Pipette extracted nucleic acids from elute chamber of cartridge into the Elute collection tube (ECT)

5. Package Insert

C) Trueprep AUTO Universal Sample Pre-treatment Pack (USPT)

The Trueprep AUTO Universal Sample pre-treatment pack employs a reagent to digest complex specimen to release the bacteria/virus, concentrate to get better yields and also discard potentially inhibitory substances and to enable further extraction and purification of the target DNA/RNA using Trueprep® AUTO/AUTO v2 Universal Cartridge Based Sample Prep Device together with Trueprep® AUTO/AUTO v2 Universal Cartridge Based Sample Prep Kit. Sample Pre-treatment decontaminates the specimen and makes it ready for storage/ transportation/ extraction. The samples used for analysis include whole blood, plasma, serum, urine, 25 Disposable transfer pipette (graduated).

D) Trueprep® AUTO MTB Sample Pre-treatment Pack



It is similar to the Trueprep® AUTO Universal Sample Pre-treatment Pack with an additional liquefaction buffer for digesting the sputum samples extracting nucleic acid in MTB diagnosis.

Contents

Lysis buffer, Liquefaction buffer, Disposable transfer pipette (graduated), Pack inserts.

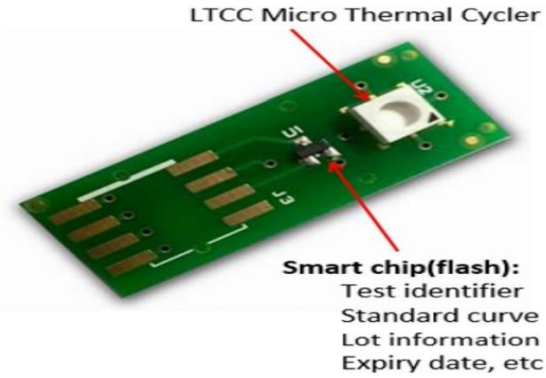
E) Trueprep® AUTO Transport Medium for Swab Specimen Pack;

It is intended for 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 for pre-treatment using Lysis buffer, extraction and purification of nucleic acids.

Contents

Transport Medium for Swab Specimen Tubes (contains transport medium).
Pack inserts.

Truenat®



Induction was given by Mr. Arvind

Truenat is a chip-based, point-of-care, rapid molecular test for diagnosis of various infectious diseases such as Covid-19, Malaria, Dengue, Chikungunya, Salmonella, HINI, HBV, Rabies, Influenza A/B, HAV, HEV, HBV etc. The technology is based on the TaqMan RTPCR chemistry which can be performed on the portable, battery operated Truelab real time Micro-PCR platform.

Contents of a Truenat Kit:

Individually scaled pouches, each containing a

1. Disease specific micro-PCR chip.

2. Microtube with freeze dried RT PCR reagents.
3. DNase & RNase free pipette tip.
4. Desiccant pouch.

Truenat COVID-19

The Truenat chip for Covid-19 has been widely used for the detection of COVID-19 cases in the state as well as in the country. Truenat COVID-19 is a chip-based Real Time duplex Reverse Transcription Polymerase Chain Reaction (RT PCR) test for the semi quantitative detection of SARS CoV-2 RNA in human oropharyngeal and nasopharyngeal swab specimen and aids in detection and confirmation of SARS COV-2 infection and diagnosis of COVID-19. It is a disposable, room temperature stable, test with dried MgCl in reaction well and freeze-dried RT-PCR reagents in microtube for performing Real Time RT-PCR test for the viral infection. The test detects the E and Orfla genes of the virus. Truenat COVID-19 runs on Real Time quantitative Micro-PCR analyzers.

We tested our self for COVID-19 by performing the extraction and amplification process by following the standard procedure, which is as fallow:

Procedure

→ We took our oral swab sample which was then transferred in the transport medium bottle. From that Bottle 0.5ml the solution was removed and was transferred in the lysis buffer bottle having 2.5ml lysis buffer (USPT). After that the entire solution(3ml), was transferred in the sample loading chamber of the cartridge. With the help of Trueprep AUTO/AUTO v2 device, the RNA from the sample was extracted. (Covid-19 test can also be performed

without the step of purification or extraction of nucleic acids by using **STABILYSE® Prep Free pack**).

→ The COVID-19 chip was placed on the Truenat tray of the Real Time micro–PCR Analyzer. 6 µL of the purified RNA was then dispensed using the provided micropipette and tip into the microtube containing freeze dried PCR reagents, including reverse transcriptase (RT) and allowed to stand for 30-60 seconds to get a clear solution.

→ 6 µL of this clear solution is then pipetted out using the same pipette and tip and dispensed into the reaction well of the Truenat COVID-19 chip and the test was inserted in the Real Time Quantitative micro Truelab PCR Analyzer.

Principle

Truenat Polymerase Chain Reaction (RT-PCR) is based on Taqman chemistry. When loaded in PCR analyzer, the RNA is first converted into complementary DNA (cDNA) by the RT enzyme and further thermal cycling takes place. A positive amplification causes the dual labeled fluorescent probe in the COVID-19 chip-based Truenat Real Time PCR test to release the fluorophores in an exponential manner which is then captured by the built-in opto- electronic sensor and displayed as amplification curve on the analyzer screen, on a real time basis during the test run. The Cycle threshold (Ct) is defined as the number of amplification cycles required for the fluorescent signal to cross the threshold (i.e., exceed the background signal). Ct levels are inversely proportional to the amount of target nucleic acid in the sample (i.e., the lower the Ct level the greater is the amount of target nucleic acid in the sample).

In the case of negative samples, amplification does not occur and a horizontal amplification curve is displayed on the screen during the test run. At the end of the test run, E/Orfla gene "DETECTED" or "NOT DETECTED" result is displayed and in positive cases, semi quantitative result is also displayed on the screen. Based on the detection of the internal positive control (IPC), the validity of the test run is also displayed.

The IPC is a full process control that undergoes all the processes the specimen undergoes - from extraction to amplification thereby validating the test run from sample to result. Absence of or shift of IPC Ct beyond a pre-set range in case of negative samples invalidates the test run. While IPC will co-amplify in most positive cases also, in some specimen having a high target load, the IPC may not amplify, however the test run is still considered valid.

Three amplification curves are displayed on the Truelab Real Time micro PCR Analyzer screen to indicate the progress of the test.

Both the target and the internal positive control (IPC) curves will take a steep, exponential path when the fluorescence crosses the threshold value in case of positive samples. The time taken (Ct) of the specimen will depend on the number of virus copies of the sample.

The curve will remain horizontal throughout the test duration and the IPC curve will take an exponential path in case of negative samples. In case the IPC curve remains horizontal in a negative sample, the test is considered as valid. While IPC will co-amplify in most positive cases also, in some specimen having a high target load, the IPC may not amplify, however the test run is still considered valid.

At the end of the test run, the results screen will display "DETECTED" for Positive result or "NOT DETECTED" for Negative result.

The results are printed using the Truelab micro-PCR printer. This principle is applicable for all the various tests for which the disease specific Truenat chip is available.

Quality control of Truenat® products:

Truenat Chips

Manual Inspection of the chips are initially carried out, when they arrive from production department, for quality testing. Here the chips are inspected for any physical damage, presence of any particulate material in the chip and the presence of the amount of wax in the sample well. In the instrumentation room, physical parameters like length and breadth of the chips are cheeked with the help of Vernier Calliper. Resistance of chip is also cheeked using Multimeter. LTCC concentration is also cheeked. Performance of the chip is also tested using Truelab® Uno Real Time Quantitative micro–PCR Analyzer. 5 chips are tested, i.e., with 1 pre-known negative and 4 positive samples. The CT values of these samples are pre-determined. Therefore, if the chip is functionally accurate it will display the Ct value of the samples, to be in range with which was previously known.

In the Positive samples, the expected Ct should be detected in both; the Test and the IC (Internal Control) In the Negative sample, the expected Ct should be detected only in the IC.

Master-mix tube:

In the master mix tubes there are freeze dried PCR reagents. These include the DNA primers. probes, free nucleotides called ddNTPs, and DNA polymerase.

Primers: Primers are short, single-stranded DNA sequence used to hybridize with the sample DNA and define the region of the DNA that will be amplified. The purpose of PCR primers is to provide a "free" 3'-OH group to which the DNA polymerase can add dNTPs.

Probes: Probes are fluorescently labelled DNA oligonucleotides. They are designed to bind downstream of one of the primers during the PCR reaction and to give a fluorescent signal during the reaction.

The concentration of these primers and probes has to be in the acceptable range, only when which the PCR process would run smoothly with no errors. Therefore, a quality and concentration check serve to be very essential for further Quality testing of the primers and probes are done in the instrumentation room of the department.

Truenat Pouch Testing

The sealed Truenat pouches which contain the microchip, the mastermix tube and the pipette tip, are tested for the scale integrity on the **Dye Leak Apparatus**. Procedure:

→ The device is plugged in and switched on

→ 0.2% methylene blue solution is then added in the lower half of the device, below the tray

→ The pouches to be tested are then placed in this methylene blue solution. The device and the air valve are then closed.

→ The vacuum valve is then tightened and the pressure is set to -15 hg → It is then run for a total time of 20 minutes.

After the run is complete, the air valve is opened and the pressure is slowly released, the pouches are then taken out from the solution, wiped dry with clean tissue paper so as to remove all the solution from the surface of the pouches.

The pouches are then torn open, and the insides are checked for any leakage of the dye into the pouches, which would be very evident by blue staining, if any leakage had to occur.

Truelab®

Micro-PCR Analyzer

There are 3 versions of Micro-PCR analyzer which are manufactured by molbio diagnostic, they are:

Truelab® Uno Real Time Quantitative micro–PCR Analyzer

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

Truelab® Duo Real Time Quantitative micro–PCR Analyzer

↓

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

Truelab® Quattro Real Time Quantitative micro–PCR Analyzer

↓

It is a fully automatic Real time Quantitative micro analyzer having a four channel-three wavelength system and performs 40-48 tests in 8 hours.

Production

We were further given induction sessions on how the production departments functions, and how its work is also linked to the Quality department. A brief description on how the production department is as follows.

Truenat section

1) Truenat chips preparation

Procedure- There are several processes and each process have been validated for their accuracy and reproducibility. The desired amount of Truenat" blank chips are issued from stores.

1. **Chip sorting**

Chip sorting is done manually. Ceramic well of chips are checked, also QR code is checked. If any faults are found, then they removed as rejected chips.

Washing, Drying and UV treatment of chips

Washing is done using PCB Air Dry System conveyer. Chips are washed with UP water and the used water then drains back into tank, they are also washed with EDI water followed by air flow for drying. Chips are arranged on to the chip tray either manually or with the help of pick and placed machine. It needs to be arranged in one order only, so that further processing of it can be done smoothly. The chips well lie above the hole of the chip tray. Chip is perfectly aligned in its position and the chip is not lifted or placed holding ceramic portion of the well but should be held using the PCB. Later, the chips are dry with air pressure which is fixed to the machine.

Drying- Hot air oven is used for drying the Truenat" blank chips. It is dried at 80° for 20 minutes.

After washing and drying, the chip tray must be labelled with identification label. After the chips are dried, they have to be virtually observed for the presence of dirt, metallic particles or any damage and same need to be segregated.

UV treatment

UV treatment is given after chip drying and segregation is done. Chips are transferred to the UV chamber where they are treated for 20 minutes.

Dispensing

As per the requirements 10 µL of BSA was dispensed in each chip via the biodot reagent dispensing machine. After dispensing the BSA coat, chip tray was kept in lyophilizer and used the vacuum dryer for draying the chips for 1 minute. Similar steps were followed in dispensing the hydrophilic coat After drying the chips containing BSA coat in vacuum dryer, 6 µL of hydrophilic coat dispensed in the chip well. Later, it is lyophilized and dried in vacuum dryer.

Later, 5.8- 6.2 mg wax is dispensed in each chip via the biodot wax dispensing machine. Here, dryer is used to melt the wax to get the concave shape.

Master mix preparation room

The liquidated master mix is created in this room. This master mix contains of primers, probes, buffers all in the right proportions. Trehalose is also added to the master mix for stability during freeze drying. At every step of preparation, a sample is sent to QC department for quality check. Preparation of the master mix is done in cold conditions as the enzymes and buffers used are heat specific.

Flashwriting of the Truenat® Chips

The chip contains various components: Printed Circuit Board (PCB), Flash Memory IC, Low temperature confine ceramic well (LTCC). Process of flashwriting is carried out to add memory and data to the chip and this memory is added to the flash

memory IC. Data such as Disease ID, slope value, Intercept value etc are added to the memory.

Pouching Assembly

After flashwriting of the chips, the chips are assembled into the pouches along with the silica gel, mastermix tube (to the right), pipette tip (to the left) and the chip in the center.

Silica gel is activated at 110°C for 1 hour and the pouches are sealed at 260°C. The finished pouch is sent to the QC for testing.

Lyophilisation room

Induction was given by: Ms. Shruti.

In the Lyophilisation room the liquid master mix is subjected to lyophilisation/ freeze drying. Lyophilisation is the process in which water is removed from a product after it is frozen and placed under a vacuum, allowing the ice to change directly from solid to vapor without passing through a liquid phase. The entire process takes 42 hours.

Conclusion

In the course of the 30 days internship in Molbio Diagnostics, I was able to gain a lot of insightful information on the diagnostic methods and devices used to diagnose many diseases such as Covid-19, HAV, HBV, HEV, MTB etc. I got to learn the functioning of a diagnostic company, how it works on an industrial scale, how it manufactures its diagnostics products from the raw materials and all the sectors associated with it, also got to know how different departments like Production, Manufacturing, Validation, Quality Assurance connects and communicate with each other, along with hands-on practical and training experience on how to operate various laboratory-oriented devices; documentation and perform diagnostic tests. It provided me an insight on professional practise.

The internship was a very useful exposure as it enabled me to develop and master new skills through training and overall gave an industrial exposure. Overall, it was a pleasant learning and experience to remember.