INTERNSHIP REPORT

Jochebed M. Fernandes

Roll No:21P044009

MSc. Part II

School of Biological Science and Biotechnology

Department of Zoology

Goa university

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INTRODUCTION

Moll bio is a 'global first' platform that can perform Molecular Diagnostics for infectious diseases at the point of care. 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. The institution comprises three main departments: Trueprep, Truelab, and Truelab. Moll bio Diagnostic Centre provides IVD (In-vivo diagnostics) kits. Moll bio aims to provide high quality affordable Diagnostic kits across the world, they also enable high-quality equipment for such communities which have very fewer healthcare facilities. In addition, provide Diagnostic kits for high-resource limited settings for communities that cannot afford good health care facilities. It aids in preventing diseases and has also impacted society's well-being. the main aim of mall bio–Diagnostic Centre is to provide leading Global players leading Diagnostic kids with innovative and new technology for the social betterment of the people. Today Moll bio has a staff strength of 700 people and it's already the largest IVD company in India, with over 4,500 installations in the public and private sectors globally and an installed base of around 1500 systems, Truenat is spread across 35 countries all over the world. The platform is infrastructure independent and provides a complete end-to-end solution for disease diagnosis. With a proven ability to work even at Primary Health Centres and with wireless data transfer capability, this game-changing technology brings a paradigm shift to the global fight for control and management of devastating infectious diseases. The system works on disease-specific Truenat microchips for conducting a real-time PCR. The sample preparation (extraction and purification) is done on a fully automated, cartridge-based Trueprep AUTO sample prep device. Moll bio not only produces kits for COVID -19, but it also produces kits for different types of diseases /viruses such as rotavirus, mucormycosis, Scrub typhus, leishmania and RSV viruses, HIV, HAV, HBV, and mainly for tuberculosis (TB). They also have sophisticated instruments such as FTIR which is used to study the chemical bonding of any compound. The mission of Moll bio diagnostics 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, and to stay connected to the needs of the patient through constant engagement with all stakeholders.

As a part of the validation team, we were given hands-on training in validating different equipment at Moll Bio diagnostic center. Validation is a process of initiating shreds of evidence that will provide proof or Assurance that a particular system is working within the specified

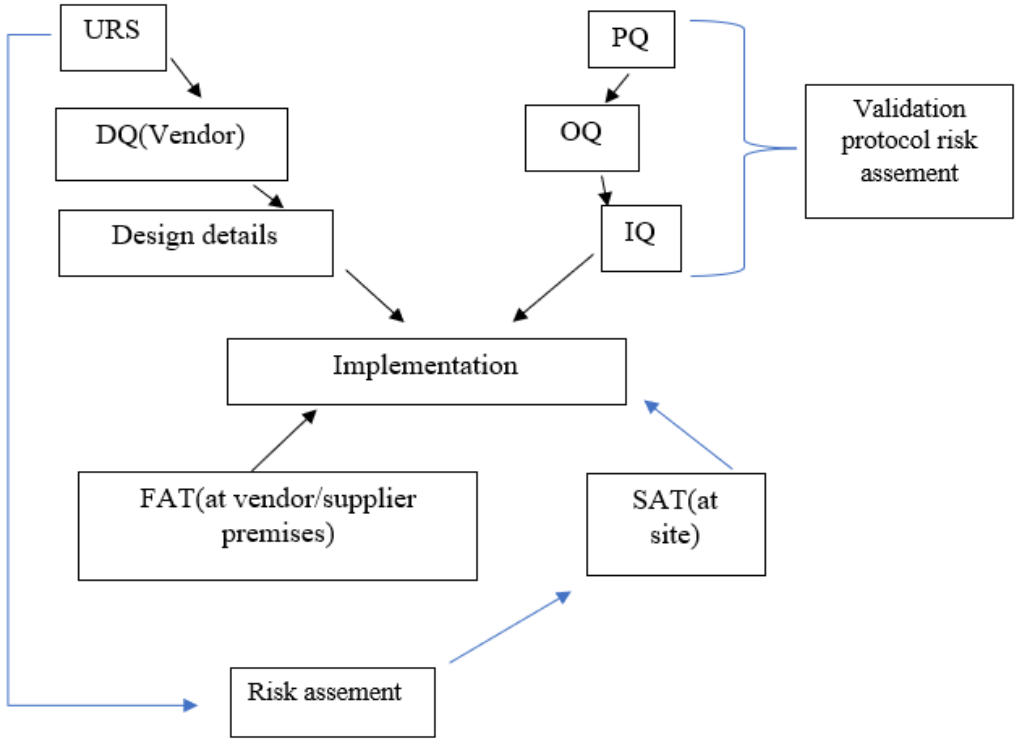
range and we produce good quality products or services complying with the predetermined specification. This system can be designed as per the needs of the company with different software processes, products, and equipment facilities based upon the need of the company. Basically, validation is done to check whether the equipment is working properly or whether any other chemicals such as the buffers used in the company will have a particular validity for a particular period of time and will provide good-quality products.

Prospective validation, is a study done at the developmental stage of the product. Validation is a process done for overall products that are produced at Mall bio diagnostic centers such as the cartridges, chips, cartridge coating machine, Lyophilized, Silicon bungs, Reagent kits (ABS frames and fitment testing), embossing Machines and the different reagents that are prepared, and many more.

Qualification is basically a part of validation where the person performing different installations of the Machines or designing the machine should be qualified to perform all of the work given to him and the person should be able to properly install the equipment and check whether it is working correctly and it is compiling with specific requirements given by the company. Qualification needed for validation includes **DQ** (Design Qualification) **IQ** (Installation qualification), **OQ** (Operation Qualification) **PQ** (Performance Qualification), prospective validation, concurrent validation, and revalidation.

The validation protocol produces acceptance criteria for the validation report. Types of validation include (1) process validation, (2) facility purity validation, and (3) equipment qualification. Equipment qualification includes design qualification, installation qualification, operation qualification, and performance qualification. (4) software system validation, (5) Test method validation, (6) cleaning validation, (7) HVAC validation, (8) User validation, and (9) shipping validation.

Validation process:



FAT-Factory acceptance test

SAT-Site acceptance test

Fig: 1

❖ Truenat® Department-Products manufactured.

Disease-specific Real Time micro–PCR Tests

The micro–PCR Chips are pre-loaded, ready-to-use, and disposable. They are disease-specific and can be run on the Truelab® Uno Dx/Duo/Quattro to get quantitative real-time PCR results.

The introduction was given by Mr. Goraksh.

Truenat is a cheap based point of 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 artificial chemistry which can be performed on a portable battery operated through the real-time micro-PCR platform

- **CONTENTS OF A TRUENAT KIT.**

A. Individually sealed pouches, each containing

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

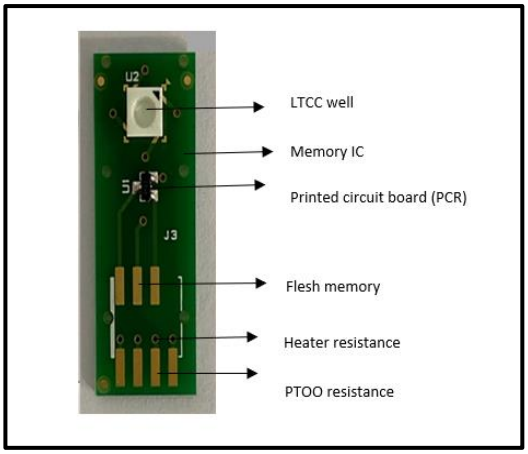


Fig .1- loading of the sample

Fig. 2- Chip

- **Products of Trurenat.**

A. Truenat® MTB

Chip-based Real-Time PCR test for Mycobacterium tuberculosis.

Fig.4-Truenat kit

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

Principle of the test:

The Truenat™ MTB chip is placed on the chip tray of the Truelab™ Real Time micro–PCR Analyzer 6 µ of the purified DNA is then dispensed using the provided micropipette and tip into the microtube containing freeze-dried PCR reagents and allowed to stand for 30-60 seconds to get a clear solution. Six µL of this clear solution is then pipetted out and dispensed into the reaction well of the Truenat™ MTB chip and the test is started. A positive amplification causes the dual-labeled fluorescent probe to release the fluorophore in an exponential manner which is then captured by the built-in optoelectronic sensor and displayed as an 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. 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, an MTB "DETECTED" or "NOT DETECTED" result is displayed and in positive cases, quantitative value is also displayed on the screen. Based on the Ct 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. The target sequence for this kit is part of the ribonucleoside-diphosphate reductase gene, the product of which provides the precursor for DNA synthesis. The region selected is specific to the MTB complex. The used Truenat™ MTB micro-PCR chip in submerged in freshly prepared 0.5% sodium hypochlorite solution for 30 minutes before disposal as per the standard medical waste disposal guidelines. The samples which were positive for MTB were loaded into another chip designed for detection of rifampicin resistance (Truenat™ MTB/RIF micro-PCR chip) and loaded into Truelab™ Real Time micro PCR Analyzer.

B. Truenat® HIV-1

Chip-based Real-Time PCR Test for HIV-1 Virus. The assay is based on Taqman chemistry, High primer sensitivity and specificity, Microchip-based real-time PCR assay, Minimal sample requirement @ 6µL, Smart chip with lot specific data for quantitation of results, Chip re-use lock, Reaction port with contamination/evaporation resistant design. The kit contains individually sealed pouches each containing a Truenat HIV-1 micro-PCR chip, a microtube with freeze-dried RT-PCR Reagent, DNAs and RNase is free pipette tip, and a desiccant pouch. The Test Duration lasts for 35 minutes and this kit must be stored at 2-30°C for two years.

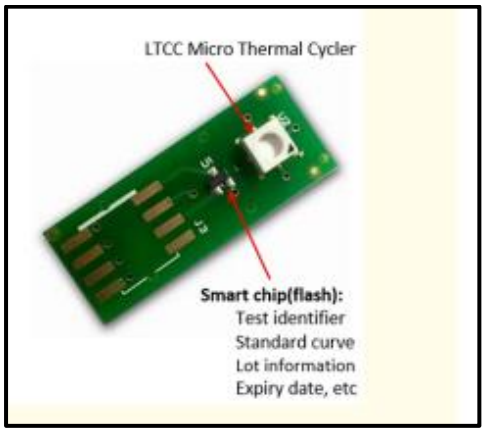


Fig.5 **Fig.6**

The chip is made of three important layers: the 10 µl BSA the first layer covered with the 6 µl hydrophilic layer and a 5.8-6.2 mg of wax.Wax-filling coating that prevents the evaporation of the sample.

C. Truenat® COVID-19

Chip-based Real Time Duplex PCR Test for COVID-1

Fig.7

Semi-quantitative detection of SARS CoV-2 RNA in human oropharyngeal and nasopharyngeal swab specimens aids in the detection and confirmation of SARS CoV-2 infection and diagnosis of COVID-19. The test detects the E and Orf1a genes of the virus. COVID-19 Assay based on Taqman chemistry, High primer sensitivity and specificity, Micro chip-based real-time PCR assay, Minimal sample requirement @ 6µL, Smart chip with pre-set data for quantitation of results, and Chip re-use lock, and Reaction port with contamination/evaporation resistant design.

❖Truenat departments

The Truenat department consists of different rooms such as the chip washing room, Mg preparation room, polymer or wax filling room, master mix preparation room and flashes writing room, lyophilization room, and pouching and packaging room. We were given detailed information on how each department functions for example in the chip washing room.

1) **Chip washing room:** the chip are purchased from the vendor and they are washed with different two different types of water the UPI and the EDI water air pressure is used alternatively after washing the chips are than chips are sent for incubation and drying in the oven and later transported into the UV Chambers.

Later on, Mr. Goaraksh introduced us to the MG preparation wherein the LTCC wall of the chip is filled with the BSc, Wax and, and hydrophilic court. 10 ml of BSA is first added into the walls to fill up the pores and make the surface smooth the hydrophilic coat is then kept for drying

2) **The Mg preparation room**: In the ceramic walls of the Trurenat chip, two types of courts are dispensed, the BSA coat and the hydrophilic coat. 10µ of BSA is first added into the walls to fill up any pose and make the surface smooth than the hydrophilic coat is then added which contains 10% of twee 20 and 40% of trehalose 50% of glycerol and 50 mMCl2 is used in the coating mix which lines the walls of the well. In PCR MgCl2 is an essential factor that enhances the activity of Taq DNA polymerase which increases the amplification rate. The coat dispensing is done by the bio-dot reagent dispensing machine.
3) **Polymer/wax filling room:** Wax is first melted on a hot magnetic plate and then a filter to remove any contamination wax is added to the LTCC well to prevent the evaporation of the sample. The biodot will dispense 5.8 to 6.2 mg of wax in the wall there are 12 Jets present in the bio dot machine which are filled with the wax it will depend on the specified amount of wax when melted. The wax is that allowed to dry and to check if there is any remaining in the Well a device called a coulometer is used.
4) **Master mix preparation room:** The liquefied master mix is created in this room the master mix contains the primers probes purpose all in the right proportions. Trehalose

is added to the master mix for stability during freeze-drying at every step of preparing of the aster mix sample is sent to the QC department for quality checking. Preparation of the master mix is done in cold conditions because the buffers designed are heat specific.

5) **Flash writing room:** After dispensing of the coat t and the wax into the well the chips are sent to be flash written. The chip consists of a flash memory of 16 bytes which contains its lot number, ID, and three slope values which essential for reading by the micro-PCR analyzer.

6) **Lyophilisation room:** The liquid master mix is subjected to lyophilization freeze drying. Lyophilization is the process in which water is removed from the product after it is frozen and placed under a vacuum allowing the ice to change directly from solid to Vapour without passing through a liquid phase. The entire process takes 42 hours.

7) Pouching and packaging:

Induction was given by Ms. Shabana

the final live final lyophilized of mastermixs tubes, coated ships, and pipette tips are then pouched in the sleeves which are then sealed by the embossing machine into pouches. These pouches also contain the Silica Gel packet to absorb for these is packaged according to the requirements of the customer.

❖Products produced in Trueprep.

Fig. 8- Products of Trueprep

Fig.1 Trueprep AUTO Universal Cartridge Based Sample Prep kit

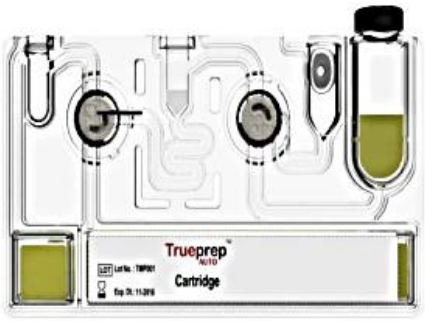


Fig.2 Trueprep AUTO Universal Cartridge Based Sample Prep device

Fig.3 Truelab™ Real Time micro PCR Analyzer

Fig.4 Truenat™ MTB chip

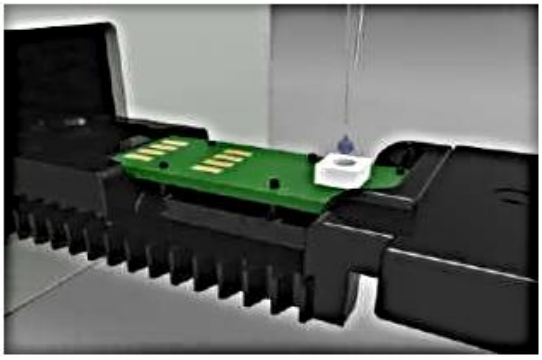


Fig.5 Loading of the sample

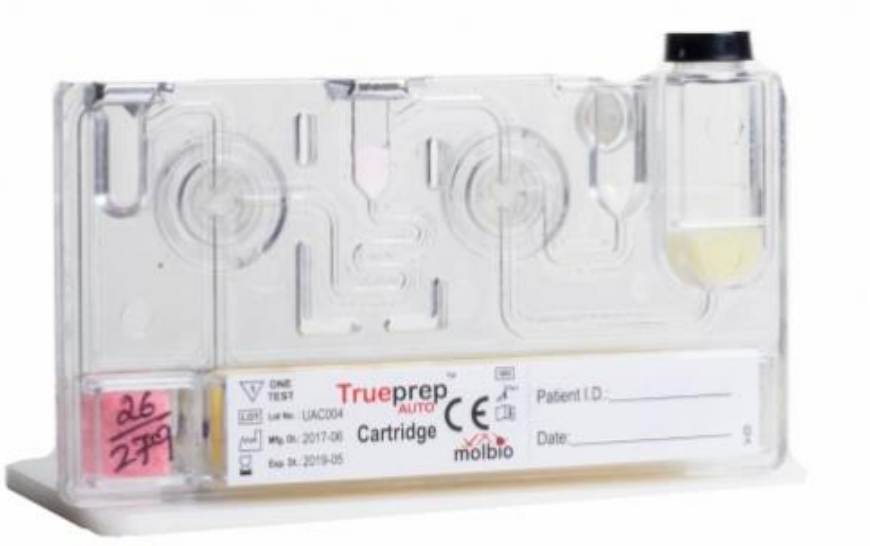


Fig.6 Universal cartridge

There are two types of cartridges produced, laser welding and plastic cartridge. The laser welding cartridge does not have DCM (**Methylene Chloride or Dichloromethane)** coating and is faster to produce. The plastic cartridge is made of polycarbonate. By using ultrasonic welding, ultrasonic waves are used to vibrate the plates which cause friction and heating in this way it causes the plastic to melt and sticks the two plates together the energy directors or the grooves of the two alone together the cartridge is ready for further inspection. Cartridge with pre-loaded Internal Positive Control (IPC) that validating the analysis from sample to result.The cartridage has a matrix chamber which helps in trapping the nuclic acids.In sample chamber the 0.5µl of sample is added,than 2.3µl of lysis buffer is added.In total 3ml of lysis buffer and sample is present.

- **<u>Working of the cartridge.</u>**

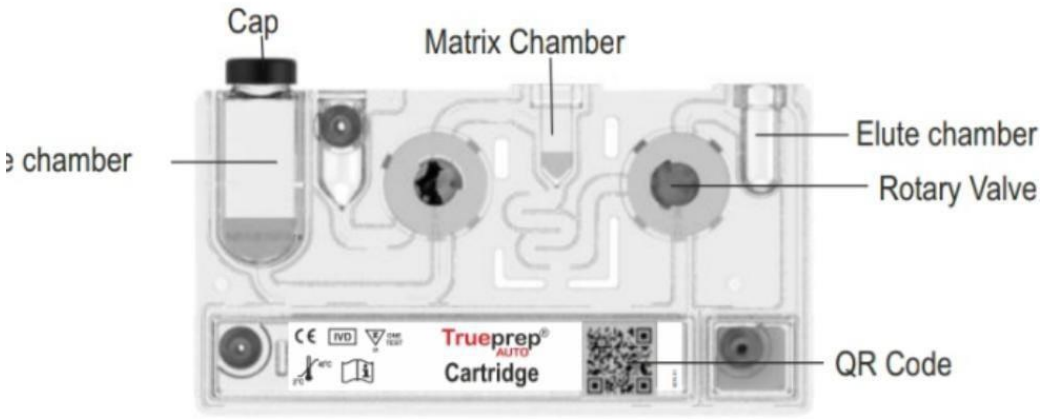


Fig.9-Ultrasonic cartridge

0.5µl of sample + 2.55µl of lysis buffer is added in the sample chamber

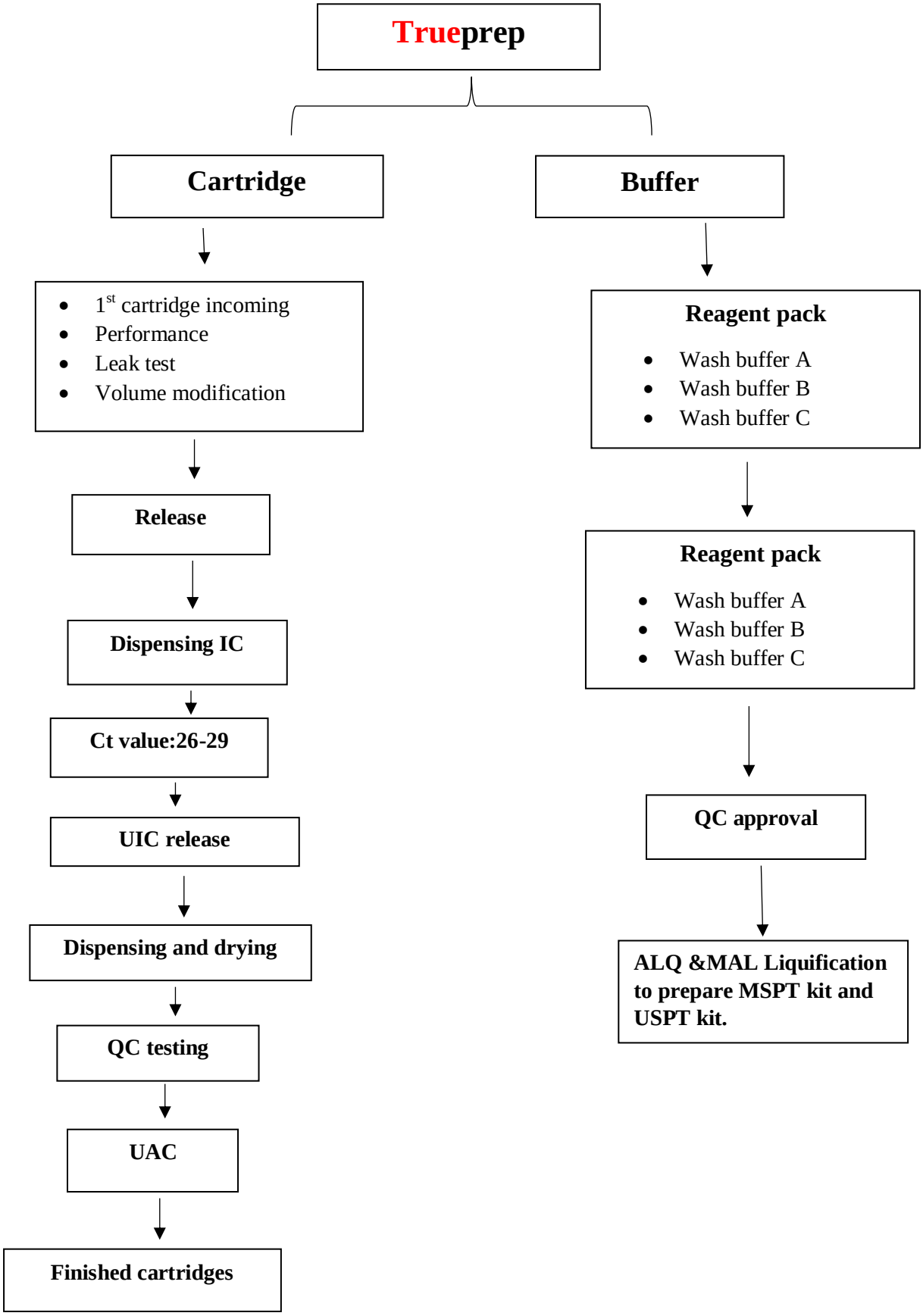
↓

The cartridge is inserted into the Auto V2 machine

↓

Than the sample travels through the capillaries and the movement of the rotary valve moves the sample from the matrix chamber to the elute chamber. Total sample present in the elute chamber is 1.5ml.

❖ Trueprep® Department



General flowchart for cartridge testing parameters

PHYSICAL PARAMETERS

- physical defects
- cosmetic effect
- presence of all the parts

FITTING TEST

using ultrasonic cartridges should fit inside the tray and cartridge chamber cap fitting test

QR code details

automated cartridges jig test ultrasonic cartridge testing shall be performed on automated cartridges testing zinc machine for checking vacuum test and QR code reading will be displayed on the jig machine display.

REJECTION CARTRIDGES

QR code will be displayed on the jig machine display

THE LEAKY TEST

will be performed for the age in the cartridge

- Elute quantity in microliter
- Capillary movement in the cartridge
- Capillary movement in cartridges

FINALLY, THE PERFORMANCE TEST

on the artificial system will be performed.

Fig.10

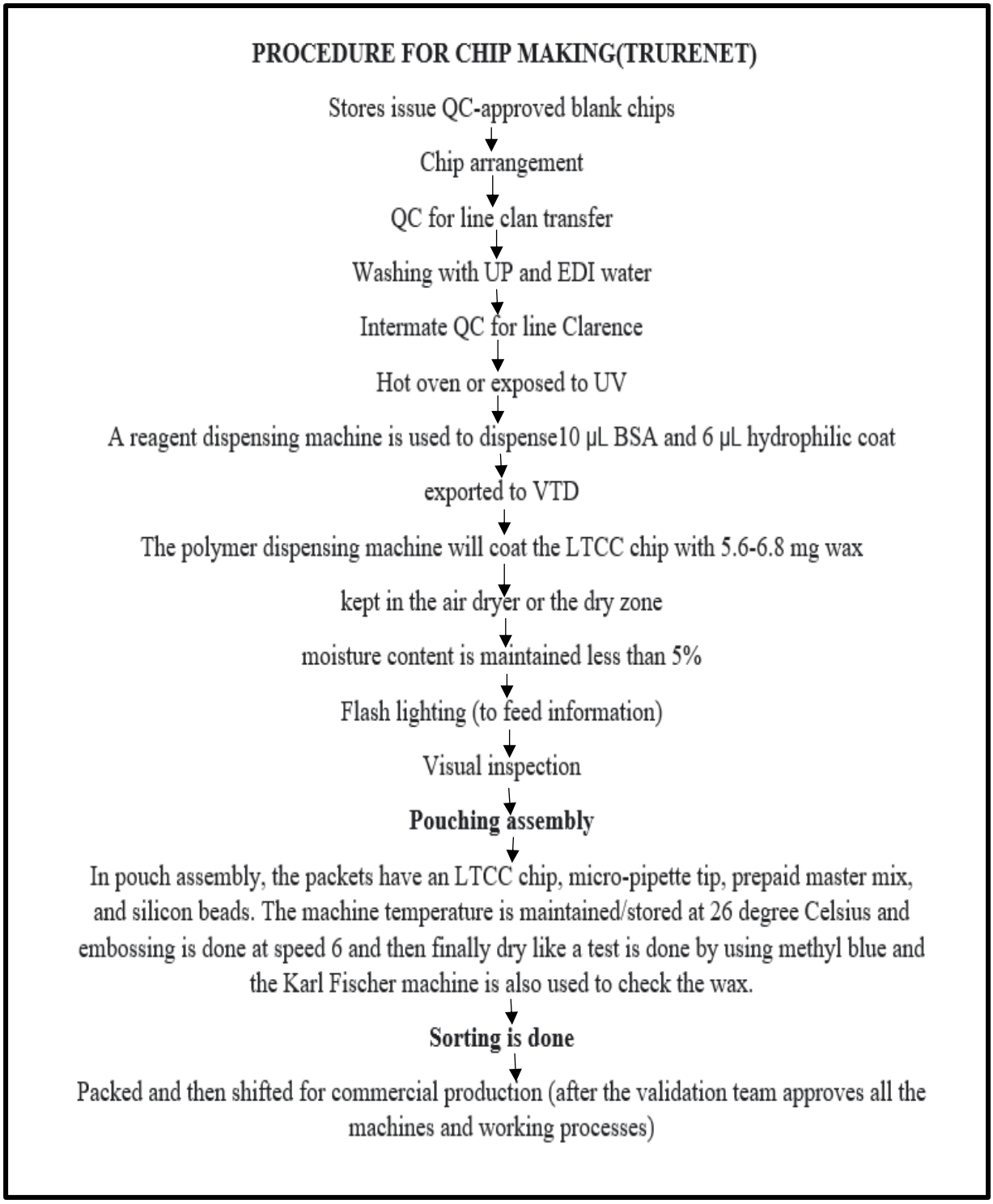

PROCEDURE FOR CHIP MAKING(TRURENET)
Stores issue QC-approved blank chips
Chip arrangement
QC for line clan transfer
Washing with UP and EDI water
Intermate QC for line Clarence
Hot oven or exposed to UV
A reagent dispensing machine is used to dispense10 µL BSA and 6 µL hydrophilic coat
exported to VTD
The polymer dispensing machine will coat the LTCC chip with 5.6-6.8 mg wax
kept in the air dryer or the dry zone
moisture content is maintained less than 5%
Flash lighting (to feed information)
Visual inspection
Pouching assembly
In pouch assembly, the packets have an LTCC chip, micro-pipette tip, prepaid master mix, and silicon beads. The machine temperature is maintained/stored at 26 degree Celsius and embossing is done at speed 6 and then finally dry like a test is done by using methyl blue and the Karl Fischer machine is also used to check the wax.
Sorting is done
Packed and then shifted for commercial production (after the validation team approves all the machines and working processes)

Fig.11

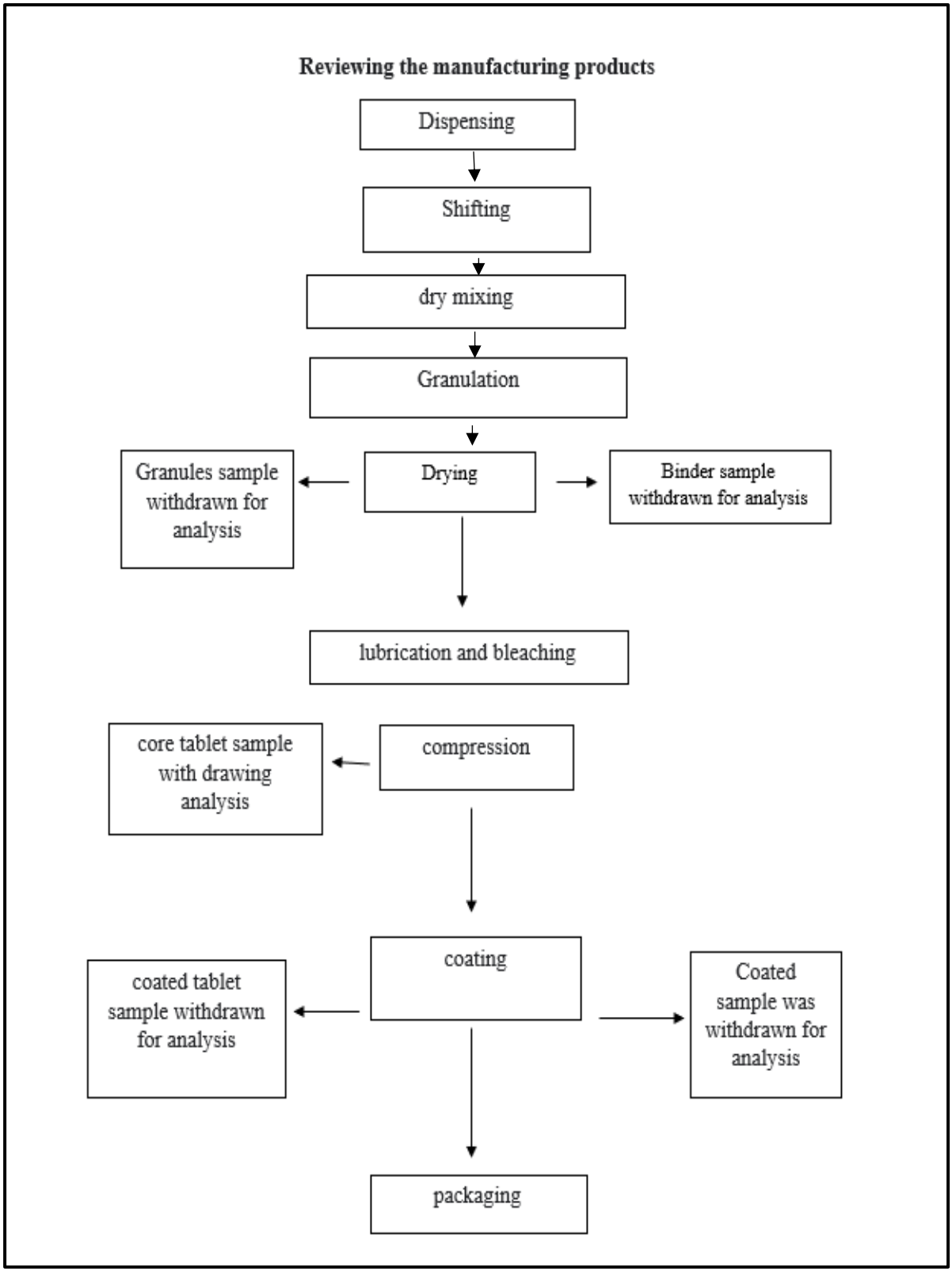

Reviewing the manufacturing products
Dispensing
Shifting
dry mixing
Granulation
Drying
Granules sample withdrawn for analysis
Binder sample withdrawn for analysis
lubrication and bleaching
compression
core tablet sample with drawing analysis
coating
coated tablet sample withdrawn for analysis
Coated sample was withdrawn for analysis
packaging

Fig.12

Each sample was processed by Trueprep Auto MTB sample pretreatment pack which employs a combination of reagents. These reagents homogenize the sample to release the bacilli, concentrate to get better yields of bacilli, and also discard potentially inhibitory substances. The pre-treated sample is loaded in Trueprep AUTO Universal Cartridge Based Sample Prep kit and Device to enable further extraction and purification of the bacterial DNA so that it is free from PCR inhibitors. It is a lightweight, portable device designed to operate at room temperature. It operates on mains and/or rechargeable batteries and is fully automatic with minimal hands-on time. The cartridge-based extraction process is quick, reliable, and efficient and does not require highly skilled personnel to carry out the extraction process. All the waste from the processing of the sample is contained within the cartridge dump area thus posing no risk from potentially biohazardous material. At the end of processing the bound DNA is eluted and collected in an elution chamber. The entire process takes 20 min. The elute is transferred to the Elute Collection tube (ECT).

❖ Truelab Department

1) Truelab® Uno Dx Real Time Quantitative micro–PCR Analyzer

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

Components	Quantity
Truelab Uno Dx Real Time Quantitative micro PCR Analyzer	01
AC Adapter to power the Truelab Uno Dx	01
Truelab Antenna	01
Truelab Microtube Stand	01
Truepet SPA fixed volume precision micropipette-6 µl	01
Truelab Uno Dx Real Time Quantitative micro PCR Analyzer User Manual	01

2) 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

Components	Quantity
Truelab Duo Real Time Quantitative micro PCR Analyzer	01
AC Adapter to power the Truelab Duo	01
Truelab Antenna (Black) for cellular connectivity	01
Truelab Antenna (Green) for WiFi connectivity	01
Truelab Microtube Stand	01
Truepet SPA fixed volume precision micropipette 6 µl	01
Truelab Duo Real Time Quantitative micro PCR Analyzer User Manual	01

3) Truelab® Quattro Real Time Quantitative micro PCR Analyzer

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

Components	Quantity
Truelab Quattro Real Time Quantitative micro PCR Analyzer	01
AC Adapter to power the Truelab Quattro	01
Truelab Antenna (Black) for cellular connectivity	01
Truelab Antenna (Green) for WiFi connectivity	01
Truelab Microtube Stand	01
Truepet SPA fixed volume precision micropipette - 6 µl	01
Truelab Quattro Real Time Quantitative micro PCR Analyzer User Manual	01

4) Truelab® micro PCR Printer

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

The standard Truelab micro PCR Printer package contains the following components are Trurelab micro PCR printer and an AC adapter(SMPS).

❖ WORK DONE AT MOLL BIO DIAGNOSTICS.

The internship started on the 1st of December 2022 at the Mollbio diagnostics center. Firstly, in the morning we were welcomed by Dr. Ruchira Malik (HOD) of the Validation department. She enlightened us about the process of validation and what they do in the department and a brief introduction about the Moll bio diagnostic center, Real-time PCR techniques, and the difference between RT-PCR, PCR, and qPCR were elucidated. Secondly, we were taken to the validation room and our work was allotted to us.

validation is a process that does risk analysis, intended uses, vendor, diligence, training, installation testing, intended use validation of the automated test system, maintenance revalidation of the automated test system, maintenance revalidation, and configuration management. The validation room functions to install, adjust the machines, and check the working of the cartridge. Thirdly, a talk on safety aid was delivered to us by Mrs.Sherya at the library. Furthermore, on day one Mr. Prakesh and Mr. Saurav demonstrated the working of the cartridge coasting machine. The cartridge coasting machine is used to add load desired amount of chemical or IC into the cartridge. The machine has two parts: the inlet connected to the reservoir that adds the IC, and the outlet connected to the positive dispensable pump which pumps 30µL of the IC or as desired by the designer. It works on the principle of IPC (Internal positive control). The cartridge coating machine also has PLC (Programable logical control) The PLC helps in controlling the volume, by moving the knob clockwise we can decrease the volume and by moving the clock anticlockwise we can increase the volume. The machine also has a homing sensor belt which indicated the stop signal. The IPC is made of plasmids which are of two types internal IPC and External IPC.

As a part of the validation team, we were given hands-on training in validating different equipment at Moll Bio diagnostic center. Validation is a process of initiating shreds of evidence that will provide proof or Assurance that a particular system is working within the specified range and we produce good quality products or services complying with the predetermined specification. This system can be designed as per the needs of the company with different software processes, products, and equipment facilities based upon the need of the company. Basically, validation is done to check whether the equipment is working properly or whether any other chemicals such as the buffers used in the company will have a particular validity for a particular period of time and will provide good-quality products.

In the first week of December, we were given a tour of the Truenat department. The Truenat is a real-time quantitative Real-Time micro-PCR platform that brings molecular testing right to the point of care. Thereby enabling early and accurate diagnosis of TB and drug resistance diagnostics. This Platform works as a combination of portable fully automated battery-operated machines or room-temperature stable RT-PCR reagents with minimum training. This can be deployed at various peripheral Laboratories with minimum infrastructure and minimum technician training. The technician can perform the MTB test and report the result within 1 hour of sample collection. Truenet is a WHO-endorsed test that provides indoor testing for diagnosing tuberculosis and drug resistance diseases. These kits are manufactured at Goa Mall bio–Diagnostic Centre.

On day 3 we were taken to the QC and instrumentation room, after which we read the SOP for the testing procedure for the finished product. Later Mr. Dhananjay and Ms.Samata took us to the instrumentation room wherein the FTIR (Fourier transform infrared spectrophotometer) was demonstrated to us. FTIR of model IRSPirit is used to identify functional groups and the identification of bonds. FTIR is of two types solid sample analysis the important one and the gases sample analysis. The energy source used is Infrared light, which creates a hibernating source causing excitation in the electrons causing excitation in the electron orbital. The criteria for loading the sample on the FTIR is that it should have a carrier molecule in this case it was HBr (Potassium bromide). The sample to be loaded must be moisture free before loading. FTIR spectrometers rely on the same basic principle as NDIR analyzers, i.e., the fact that many gases absorb IR radiation at species-specific frequencies. However, FTIR spectroscopy is a dispersed method, which means that measurements are performed over a broad spectrum instead of a narrow band of frequencies.

Fig.13-FTIR Spectroscopy

On day four we discussed the validation SOPs and standard operating procedure for truemix tubes. The objective is to prepare product-specific Truenet/Truemix tubes. The scope of this is to produce products applicable for carrying out real-time PCR of the nucleic acids obtained from patient samples to detect disease Truenet/truemix tubes. Is also used in combination with a truenet chip on the truelab real-time PCR analyzer. The procedure to prepare the chip is that personal protection is within the required range and all the material equipment within the set lies within the set of criteria and all the material used for the preparation and arrangement is done from the QC approved Ultra-pure water for washing the bungs and show that the temperature within the required range with material and equipment as per the SOP. Then repeat this step three times, and keep the bungs in the hot air oven for 20 minutes at room temperature at 80 degrees Celsius. After drying and sorting the chips and bungs are kept in the UV chamber for 20 minutes. Later after washing and drying, treated bungs are to be placed in a bag and then must be labeled. For prebatch master mix testing materials will have to get verified by QA before commencing the purpose of pre-batch testing is to ensure that the batch prepared shall pass QC specification. Lamina air flow must be thoroughly wiped with 70% alcohol and all the things added to the laminar air flow must be sterilized then it is taken to the lyophilization room and post-lyophilization is done by the QC and standard curve generalization by QC is done then are sent for treatment tube sorting the preparation of through net. To describe this procedure for washing, drying, and UV treatment of the true net balance chips is done by preparation of 10 mg/ml of BSA. This preparation of Universal Truenat chips which in combination with the specific truenat tubes help detect disease using a real-time micro-PCR analyzer that can be used to treat different types of diseases.

On day 5 we discussed the product's packaging and transport process and validated the AutoV2 pouches. To maintain stability and protection the packages are packed with ABS frame, which is a plastic supporter used inside the box and some are packed with fitment cardboard paper. There are three different types of buffers in the box: wash buffer A wash buffer B, elution buffer, and the primary waste bottle. Wash buffer A and wash buffer B are used to wash inhibitors from the sample and the primary waste bottle is used to purge residual liquid from the tubing. This package is called the auto V2 and comes with a reagent reset card, indicating the reagent is over. This particular box set can give 50 cycles. As and when the reagent gets over the cycles are reduced.

On day 6 we discussed the testing procedure SOPs for ultrasonic cartridges. The objective is to lay down a procedure for testing incoming cartridges and internally transferred cartilage

used into trueprep auto/trueprep and auto V2 Universal cartridges based on sample pre-kit. We also discussed the classical Diagnostic culture techniques sensitivity of the auto V2 machine regarding TB diagnosis and we also discussed the different types of RT-PCR (LAMP -PCR, BOX-PCR NAT-PCR), upcoming research topics on proteomics, and the uses of proteomics in artificial testing.

On day 7 we discussed the process of RT-PCR in detail, and the preparation of Truemix for Real-Time RT-PCR reconstitution buffer, and negative control and its dispensing and labelling. This protocol helps prepare Truemix for real-time PCR test reconstitution buffer, Truemix Real-time negative control they are dispensing, and their labeling. Preparation of different buffers (100Mm) mg of MgCl both liquid and solid according to the need of the reaction. We also discussed the overprinting process which helps lay down the procedure for the printing of Truenet/Truemix o materials. The scope of the objective is that the primary printing process and for proper identification and storage of Truemix procedure includes the issuance of receiving of required material, intimation for the primary process monitoring of the temperature of the area, printing process, QC testing, and in house printing and of Kit labels.

Later, we discussed the operating cleaning, and maintenance of the biodot reagent dispensing machine. The biodot dispensing machine is to be cleaned before use with 70% alcohol or 5% sodium hypochlorite solution and after every 3 months, the machine has to be checked for maintenance. We also discussed the operation and cleaning of the biodot wax dispensing machine.

On day 8 we discussed the safety and precautions and the objective was to lay down the standard operating procedure for the safety and precautions of the person appointed in the quality control department the scope of the study was to produce an application for the entry of safety and precaution, in addition, conduct all quality control personal appointments in the quality control department. Furthermore, a Discussion about the procedure for handling and cleaning of Bio Hazard material was accomplished. The objective is to lay down procedures for the handling of raw materials in the department The scope is applicable for handling the cleaning of biohazardous material. There are different types of colour codes that are given which indicated the function of each box kept in the industry, Yellow indicates that soiled waste such as blood or body Fluids, chemical liquid such as cleaning soaps or dish washes, and microbiology/biotechnology or other clinical waste should be added in the yellow color bin. Red indicates contaminated waste such as lysis buffer tubes, bottles, cartridges, syringes, and

urine bags. At last, the blue indicates broken or discarded and contaminated tips, metals, needles, syringes, cutters, burners, and blades. Before using any of the department's machines safety precautions such as wearing gloves and wiping the area with 5% Sodium hypochlorite or ethanol must be carried out. Later in the afternoon section, we were taken into the cartridge room wherein the cartridge coating machine was demonstrated. This machine is connected to 20 pumps which are used to increase or decrease the IC into the cartridge. It is also controlled by F2(start) and F3(stop) and F4(main settings) buttons displayed on the screen. The prime will drain all the solution and remove air bubbles from the pipes. There is also positive dispensing, also called the Metering pump which dispenses 30 µl of the solution or the IC into the cartridges.

On day 8 we discussed the procedure for the contamination of cultures culture glassware and pathological samples it helps us to lay down procedure the for the contamination of cultures using media and other pathological samples used in the QC department lab. Machines such as autoclaves should be maintained at 121 degrees Celsius for 30 minutes at 15 PSI and material must be autoclaved before use. the used cultures are then sent for decontamination in the autoclave.

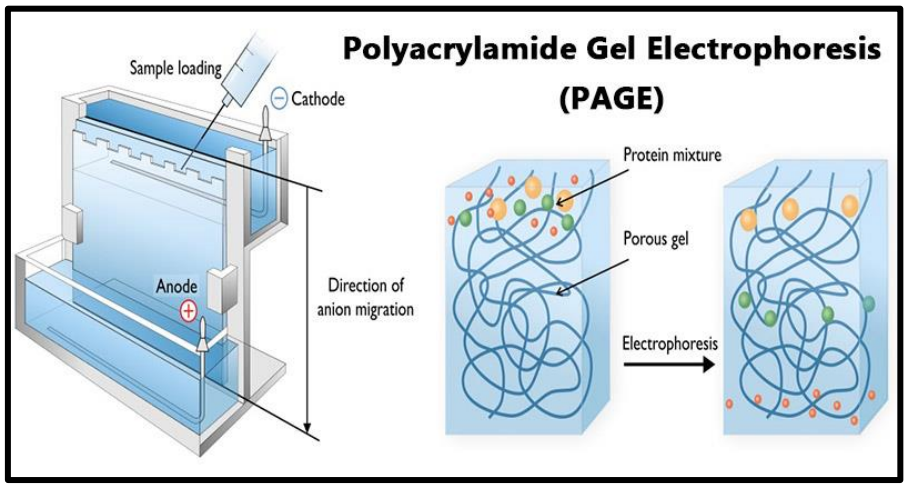
The next day we discussed labelling. This procedure is applicable for the identification of all raw material or raw chemical packaging that is involved in the process material for the finished product or printed on the packaging material. We also discussed the workers' training procedure which strictly says that any person entering any of the departments must wear the lab courts provided to them. The objective to identify any worker or officer using the lab will be known by the coat color. We also discussed the guidelines for handling chemicals and allotting the Year number to lay out procedures for a lot of A.R. NOS products in the future department. In the afternoon session, we were taken to the cartridge dispensing room for the validation of the cartridge coating machine. The cartridge coating machine normally dispenses 30 µl of the solution inside the cartridges but the validation was carried out to check whether the machine was exactly dispensing 30 µl or not this was checked by two different methods the first method was to weigh the tube's initial weight and then The Final weight by adding the IC and the second was method was to check the volume by using the pipette. Five different sets were prepaid and the data was analysed and validated.

On day 11 we discussed the sampling of incoming material to check for the incoming material, in this process we check for a lot size, sample, sample size, and sampling plan for packaging the material.

Number of samples from each package= Sample size number

No. of packages received

We also discussed the procedure for sampling inspection and acceptance for inspections by attributes. The consumer risk is known as the beta and the producer risk is called the Alpha risk. In addition, PAGE (Polyacrylamide gel electrophoresis) was also dissuaded by Mr.Dhanajay. PAGE is a technique widely used in biochemistry, forensic chemistry, genetics, molecular biology, and biotechnology to separate biological macromolecules, usually proteins or nucleic acids, according to their electrophoretic mobility. Which is based on the principle of an anionic detergent called sodium dodecyl sulfate (SDS) is used to bind to proteins and give them a negative charge. Proteins are then separated electrophoretic ally according to their size using a gel matrix made of polyacrylamide in an electric field.



Furthermore, the difference between SDS-PAGE and PAGE was also discussed. SDS PAGE is a separation technique that separates proteins on the basis of their mass. Native PAGE is an electrophoretic technique that separates proteins on the basis of their size and charge. The gel is denatured. The gel is not denatured. Additionally, Agarose gel electrophoresis was also discussed. It is commonly used to separate DNA fragments following restriction endonuclease digestion or PCR amplification. Fragments are detected by staining the gel with the intercalating dye, ethidium bromide, followed by visualization/photography under ultraviolet light.

In the third week of December, Ms.Shabana gave us a tour of the labeling room where the sealing machine was demonstrated to us. We also discussed about the labeling procedure of the reagent agent bottles then we also perform the validation for Silicon bunks. We also performed validation for the cartridge dispensing machine we check whether the machine is responding or not. Later, along with Mr.Goraksh we performed the validation for the Silicon bunks. 50 bottles of reagent what taken and 1 ml of methyl Blue dye was added the bottles work placed inverted for a week to check for any leakage. Mr.Goraksh demonstrated the peristaltic pump which is a pump used to add a particular amount of chemical inside the reagent which works on the principle of RPM later in the afternoon we also performed the validation for the cartridge dispensing machine we were also informed about the sampling plan for finished product based upon the SOP's. later in the afternoon, we validated reagent boxes' ABS frames were broken or not. We also validated the fitment of paper frames.

In the third week of the month, we performed the validation for software of UNoDx flash lighting for the chips the purpose of this validation is to perform Cross verification so that all the damaged chips will be sent back or discarded then this data will be sent to the QC for data verification. Later we were taken to the lyophilization room to explain the lyophilization process and the different types of kits used at Molbio diagnostic center for example for Malaria testing, master mix tubes are filled with low glycerol hydrophilic Coat and VTD is not used but instead lyophilizer is used for drying.

In the last week of December, we discussed the STPs for and process testing of negative control and positive control. We also disscused about the procedures for stability study of the through net positive control and cleaning operation in collaboration of the Truelab real-time micro-PCR analyzer. The micro-PCR sample Uno DX real-time micro-PCR analyzer machine is used for the validation of the Truenet chips. The machine is switched on by pressing the power button of the analyzer. A touch screen provider is used to switch on the screen this is done by using the Jack portion holding for two seconds. The power LED indicates that the UNO is switched ON and a low battery LED light will indicate that the battery is low. The charging LED begins when the charging of the power cable is connected to the circuit. In-use LED indicated that Uno is running the test. Screen wake/Back zone will awaken the machine from sleep mode to active mode.

Furthermore, we discussed the cleaning procedure cleaning operation of the conductometer as well as the magnetic Nanoparticle. A nanoparticle is a small particle that ranges between 1 to

100nm in size and is undetectable by the human eye. Nanoparticles can exhibit significantly different physical and chemical properties to their large material count of parts magnetic properties of the nanoparticles are used for drug delivery therapeutic treatments contrast agents for MRI imaging bio speciation and in vitro diagnostic. These nanoparticle-sized particles are superparamagnetic properties resulting from the tiny size of only a few nanometers. Superparamagnetic nanoparticles are not magnetic but are located in the zero magnetic fields but they quickly become magnetized when the magnetic field is applied. when returned to zero magnet field they quickly revert to a nonmagnetic state. Superparamagnetic is one of the most important properties of nanoparticles used for biomagnetic separation.

Later in the afternoon session, we discussed the cleaning operation, collaboration and validation of the laminar airflow, and the SOP for automated cartridge testing zig machine operation and cleaning procedure. Basically, the lick errors are detected in the Zig test machine for example SN leak 1 (simple nozzle) label indicates a higher leak. SN leak indicates a medium leak. SN leak 3 indicates a lower leak. Also discussed the preparation of internal control and MAG sputum. MAG sputum was a technique used by the moll biodiagnostic a few years ago which is now replaced by the UNO DX machine. MAG sputum was used for the separation of the sample. There are four different types of IC prepared,1) MTB-IC,2) HCV-IC,3) HBV-IC and,4) HIV-IC. MTB-IC is using sputum samples whereas the others use blood plasma and serum, normally 250 µl of each blood sample is taken and the analysis is carried out. We also discussed the cleaning operations for the UV spectrophotometer and also further incubators.

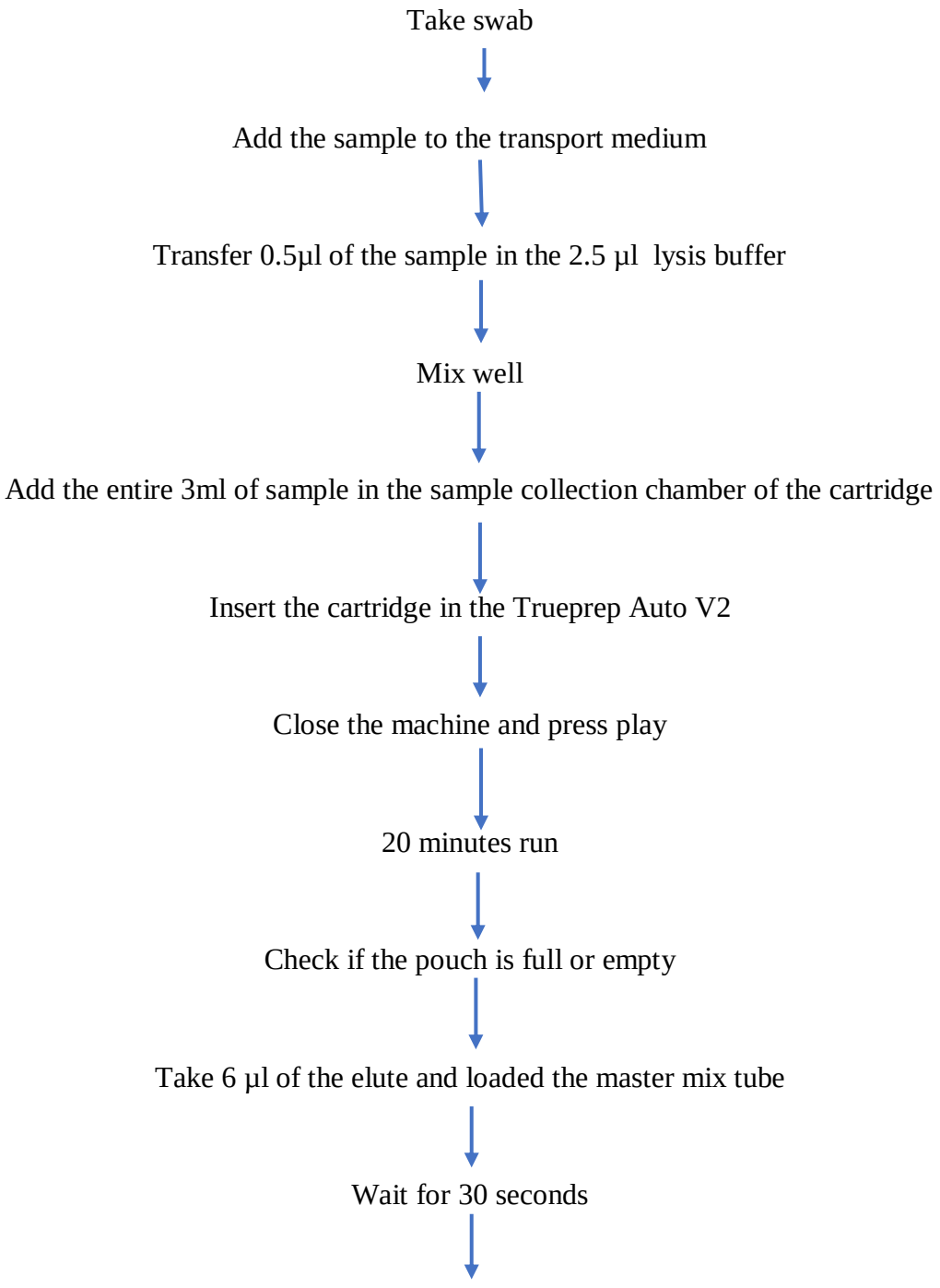
On the second day of the last week we perform the validation for wash buffer .A stock solution We started the validation of wash buffer A stock solution on the 8th of December till the last week of December which is the 22nd of December. Ms. Shabana explained to us how the buffers are prepared. Basically, 1M TCEP solution was prepared and the pH was maintained at 7.9. Performance testing is also done by the QC department for different samples. The positive test samples and negative samples and based on the CT value. The CT of the positive and negative samples is taken and compared to the buffer. Based on this experiment we can conclude that the stock solution must be used within one week after the preparation. If not then the pH of the stock solution fluctuates.

In the last week of December, we were taken to the QC. The QC room has different departments such as the extraction room, the sample storage room, the sample handling, room, and the micro PCR room. The Trueprep consists of two main departments the cartridges and the buffer or

Reagent preparation rooms, The Trrueprep also has two different machines the auto and the auto V2 the difference between the both is that auto tax 18 minutes for extraction, and auto V2 takes 20 minutes for extraction this is because the auto V2 machine cans the details on the cartridge of kids and the USB t the MSBT can produce 25 to 50 test where it can produce 50 test . The MSBT kit is only used for MTB samples.

We were given hands-on training on how to perform RT-PCR.

❖ Procedure for RT-PCR.



Add 6 µl of the sample onto the chip and start the run.

Result: Not detected.

❖ Conclusion

During the course of 30 days of internship at Moll Bio Diagnostic Centre, I was able to gain a lot of important information on the Diagnostic methods and different kits manufacture to diagnose different diseases such as mainly COVID-19, HBV, HIV, MTB, Zika virus, Rotavirus-virus, Scrub typhus, Mucormycosis, RSV and Leishmania, etc. These kits are validated at Moll bio-Diagnostic center. I also got to learn the functioning of a Diagnostic company and how it works on an industrial scale production and all the associated sectors, such as the vendors for the chips and cartridges. the designer for the machines and the customers. Hands-on practical training on how to operate various laboratory devices helped me master a new skill and also enhanced my interest in research and development. As a part of a validation team, they trained us on how to validate different machines or products and incoming material. In addition, showed us how the SOPs and STPs are designed for the testing of different products manufactured at the Moll Bio diagnostic center. It also provided us insight information on professional practices such as the role of a HOD and an officer. Over all experience at Moll Bio diagnostic center was very pleasant.