INTERNSHIP REPORT (2022-23)

Molbio Diagnostic

PRESENTED TO

Dr. Ruchira Malik Validation Dept, Molbio Diagnostics private limited, Verna Goa.

PREPARED BY

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Dated: 30/12/2022

TO WHOM IT MAY CONCERN

This is to certify that Ms. Priyanka Arun Naik a student at school of Biological sciences and Biotechnology, Goa University, have successfully completed internship programme at Molbio Diagnostics Private Limited Verna, Goa from 01st December 2022 to 31st December 2022.

During the period of her internship programme with us she was found hardworking, punctual and inquisitive.

Yours sincerely, For Molbio Diagnostics Private Limited

Denhioulows

Dushyant Bhawsar Head- HR & Administration

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INTERNSHIP REPORT

A report submitted in partial fulfillment of Degree of

MASTERS OF SCIENCE

in ZOOLOGY

As a record of work done

by

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at

MOLBIO

DIAGNOSTCS

PRIVATE LIMITED

VERNA -GOA.

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SCHOOL OF BIOLOGICAL SCIENCES AND BIOTECHNOLOGY, GOA UNIVERSITY Taleigao,Goa 2022-2023

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CHAPTER 1 - INTRODUCTION



Molbio Diagnostics Private Limited, Verna Goa, is a molecular diagnosics manufacturer specialised in synthesing innovative IVD test kits for approx 40 infectious diseases including tuberculosis, AIDS, COVID-19 etc on the battery operated, Truelab Real Time Quantitative micro PCR System, that provides sample to result within 1 hour. It is a reliable name in the industry as they aim to provide better medicine throght precise, rapid, cost effective molecular diagnosis at the Point-of-Care.

Among the other serological tests like ELISA(enzyme linked immunosorbent assay),genetic testing, smear microsopy,western blot test etc. Molbio Diagnostics focuses on using PCR technology as pathogen detection tests. The system works on disease specific, Truenat microchips/PCR micro reactors that along with Truenat Mastermix harbours a real time PCR. Initial step of extraction and purification of nucleic acids is carried out by a fully automated, cartidge based Trueprep AUTO sample prep device,that work alongside with pre-treatment pack of various buffer reagents. Bigtech Labs situated in Banglore is the Research and Development Wing of Molbio Diagnostics, which aims at commercializing novel medical devices.Truenat claims to be world's first point-of-care molecular diagnosis to get endorsed by the World Health Organization (WHO) as a replacement to smear microscopy for the diagnosis of tuberculosis.

The main intention of undertaking an internship at Molbio Diagnostics was to enhance my practical skills, understand working of an IVD manufacturing industry especially during outbreak of severe diseases, equipments employed in achieving set target.

I along with one of my batchmate were assigned under validation department. It deals with documentation of evidence which provides assurance that a system operating within specified limits will consistenly produce quality products complying to predetermined specifications. It holds the benefits of providing consistent results, improvisation in productivity due to standardisation, assurance of cost reduction and quality.



The above processes of validation are carried out in accordance with other sub sections as dissused below-

- Design Qualification (DQ) documented validation that design of new equipment will result in a system that is suitable for intended purpose. For instance, cartridge design as per URS (User Requirement Specifications) is proposed to the vendor. The machines used at Molbio Diagnostics are highly customised/designed as per their specifications.
- Installation Qualification (IQ)- involves checking if the counterparts are installed in proper loactions. For instance rotary valves of the cartridge possess smiley pattern which has to be in proper orientation when it is bought from vendors. So also it involves checking of any cosmetic defects, cross-checking contents of the package against packing list etc.
- Operational Qualifiaction (OQ)- Checks and documents that each individual function of item perform as expected.
- Performance Qualification (PQ)- checks and documents that equipmennt or a system meets the user's needs as defined in URS. It checks for system performs at real time basis.

All of these validation protocols has to abide by certain standard operating procedures proposed by higher regulatory authorities including WHO.



CHAPTER 2 – TRUEPREP® SECTION

The vital component here is CARTRIDGE which does the work of extraction and purification of nucleic acids from samples including blood (0.25µl), plasma $(0.5\mu l)$, serum $(0.5\mu l)$, sputum $(0.5\mu l)$ nasal and throat swab $(0.5\mu l)$, urine etc. The swab samples (as for COVID-19 testing) are collected in the VTM tube (with 1.5ml viral lysis transport medium). The sample is pre-treated with lysis buffer (2.5ml). The whole of 3ml sample is loaded in sample chamber of one time use cartridge. The sample containing cartridge is then loaded into automated device connected to the buffer packs. The rotatory valves (in red) are designed in the pattern of smileys that under electrical influence will result in opening and closing capillaries and allow or restrict flow of fluid inside the cartridges. The red rubber like grommets(silicone washers) will help build negative pressure on heating (electrical stress) which also favor the filtering out of cellular debris down in the dump chamber constituted with cellulose/Scotchbrite sponges. There are 2 plate heaters for sample chamber and matrix chamber that aid in heating internal of chamber uniformly allowing in denaturation of certain molecules. The matrix chamber comprise of matrices like cellulose, within which nucleic acids are trapped (past washing with Wash A and Wash B buffers). These nucleic acids molecules are further eluted out in the elution chamber, from which it is collected and stored in the Elution Collecting Tube (ECT).



Based on their process of

manufacturing there are 2 types of cartridges-

- Laser welded cartridges no DCM(Dichloromethane) coating so it works faster. Here the 2 halves of cartridges (male + female counterparts) are welded using laser technology.
- ✤ Ultra sonic cartridges- Welded using sonar technology .

The cartridges are also loaded with Internal control (pink solution -30µl), in the sample chamber. There are 2 types of it, one of which is an universal synthetic exogenous internal control which is not cross reactive with human DNA. This usually contains Trehalose, Poly A tail, Plasmids (encapsulated genetic matter) with pathogen target sequences etc. when extraction process initiates the mixture of plasmid DNA/RNA from IC and Human DNA/RNA will run simultaneously. When such positive controls are present is adds assurance that any samples that are negative are truly negative thus preventing false negatives. Other type is endogenous internal control that uses housekeeping genes to report the presence of genetic material from the sample. This control type is not placed in the designated sample chamber. Example for Covid-19, using throat swab the genes like RPLP-1 can serve as housekeeping gene. Following 2 products are designed to pre-treat the samples-

- Trueprep® Universal Sample Pre Treatment Pack that contains buffer reagents to treat human samples involving blood, plasma, serum, swab, urine etc.
- Trueprep® MTB Sample Pre Treatment Pack the major buffer reagents composition is the same, with only difference of containing Liquification Buffer (9ml). Usually it contains the mucolytic agent like DTT(Dithiothreitol) which acts by reducing the disulphide bonds of the mucus proteins of the sputum sample used in testing of MTB (*Mycobacterium tuberculosis*), thereby decreasing the thickness.

The 3 buffer pouches contained in both universal and MTB sample prep kit are as follows-

- WASH A BUFFER (with green cap)- comprising of
 - Guanidine Hydrochloride :is used as strong denaturant, it break the hydrogen bonds between amino acid residues and thus, 3D conformation of protein is unfolded. The aqueous solubility of proteins is highly increased.
 - Trizma Base/Tris base : is basic buffer -Best at maintaining pH in the range of 7.0-9.0 (Trizma-trademark name)
 - T-CEP (2 carboxy-ethyl phosphyl hydrochloride) : used as reducing agent (as replacement for DTT) to break disulphide bonds between various proteins including nucleases.
 - Ethanol: amount of 70% is commonly used to induce nucleic acid precipitation.
- WASH B BUFFER (with blue cap)– comprising of
 - Sodium Acetate : For ethanol precipitation of DNA from solution it needs to have high salt concentration, NaAc is best salt for this purpose. Positively charged Na+ from NaAc competes with positive charge of water to bind with negative charge of DNA and RNA. Thus decreasing hydrophilicity of nucleic acids.
 - HEPES (N-2-hydroxyethyl piperazine-N'-2-ethanesulfonic acid) buffer : Better at maintaining physiological pH(7.5) despite aerobic activities or in carbon dioxide conc.
- ELUTION BUFFER (with black cap)- comprising of
 - Trizma HCl/Tris HCl : is an acidic buffer -Best at maintaining pH in the range of 7.0-9.0.
 - EDTA (Ethylene Diamine Tetraacetic Acid) : to inactivate metal ion requiring enzymes like DNAses and RNAses
 - Sodium Azide : general preservative to avoid microbial contamination which would degrade substrate present in sample.

The patients suffering from severe COVID-19, COPD, Cystic Fibrosis etc. are

administered with various anticoagulants one of which is Heparin. Heparin can seriously complicate PCR, so to remove off such inhibitors washing with above discussed reagents is critical step.

There is one more empty pouch entitled Priming waste (with red cap) – which will collect all the debris content from the dump section of cartridge.

Attached to this sample prep kit is REAGENT RESET CARD – which is to be inserted while using device for the first time. The device will read the QR Code from the card and display the number of tests done. For instance, for the kit with 50T (50 Tests), after performing 2 tests, on insertion of card it will display number 48, from this one can make out if reagents are over._

Following are the products of Trueprep® system manufactured at Molbio Diagnostics-





CHAPTER 3 - TRUENAT SECTION

The main components here are the microchip/micro PCR reactor and lyophilized Master mix tube.

The Truenat® chip works on Nucleic Acid based testing technology and is species specific it is composed of network of resistors, memory and a reaction well. The white coloured reaction well is a low temperature co-fired ceramic (LTCC) substrate, manufactured by adding glass matter to ceramic substrates. Once the chips are issued from the store they are counted and placed in chip tray and subjected for alternate washings with EDI (Electrodeionized) and UP (ultrapure) water on Automatic PCB Air dry system conveyor in the chip washing area. Later they are dried at 80° c in the hot air oven for 20minutes, followed by UV treatment. Later these are transferred into MG filling room for the dispensing of BSA coat and hydrophilic coat respectively, which is done by Biodot reagent dispensing machine. Below enlisted are functions of each constituent –

• BSA coat $(10\mu l)$ – increases PCR yields from low purity templates + to create smooth surface, by cementing pores of substrate..

• Hydrophilic coat(6µl)-

- Trehalose- a potent PCR enhancer, it aids in lowering of DNA melting temperature and thermal stabilization of taq pol.
- MgCl2- acts as a cofactor that enhances the enzymatic activity of Taq pol.
- Tween 20 help disperse the molecules from reaction well so that it can bind to probe.
- Glycerol it enhances the specificity of the primers and reduces formation of 2° structures formed by GC- rich regions.
- DDW to act as nuclease free solvent.

After dispensing each layer the chips are dried thoroughly in Vaccum Tray Dryer(VTD) and stored in Dryzone with moisture less than 5%. It is critical step to check moisture of the chips after dispensing. For this Karl Fischer Coulometer is used-that monitors the moisture.

Later chips are transferred in polymer dispensing room, wherein around 5.8-6.2mg

of wax is dispensed using Biodot Polymer/wax dispensing machine. Since it dries very fast no vacuum drying is required. Later chips are passed into chip sorting room , where visual inspection is undertaken to check for concave nature of wax coat. Wax coat is essential protective layer. The elute +master mix (6µl) will be loaded directly on the wax coat. During thermal cycling the reaction will heat up, resulting in turning of wax coat-upside down, so that the reaction mixture comes in proximity of hydrophilic + BSA coat and wax coat will form protective domelike layer.

Final step is synthesizing the microchip is flash writing, here the disease ID, product specific details including mfg. date, lot details, slope values is fed in the memory of the chip. Using multimeter they even check for resistance values if they are in acceptable range as per standard operating protocols.

Master mix tube-

It involves preparation of master mix which is mixture of reagents like buffers, enzymes, dNTP, primers, probes etc. is required amount. The amount of 6μ l is dispensed in the tubes and sent for lyophilization. Lyophilization involves process of freeze drying wherein the matter turns from solid to gaseous phase directly, such that when 6μ l of elute is dispensed in the tube the master mix content is reconstituted. The buffers are added to maintain consistent pH throughout the reaction. The probes are basically flouroprobes like SYBR green etc. which add color to the template and make detection easier. The control and sample DNA will exponentially pick up the flour probe and this is sensed by the analyzer and displayed in the form of optical graph.

Finally the lyophilized tube, coated chip and nuclease free pipette tip are packed into a pouches containing silica gel desiccant.

So initially the sample is processed using Trueprep® AUTO universal/MTB sample prep kit and device. The 6µl of elute from the ECT is pipetted into master mix tube, and incubated till the mixture is colorless. The whole content is then loaded in to LTCC well of the chip kept for run on the PCR analyzer.

Plate below displays the Truenat® microchip, pouch containing mastermix tube and pipette tip. 6µl of elute goes in the mastermix tube till it turns colourless. Same is pipetted into LTCC well of the chip for further processing.



CHAPTER 4 - TRUELAB® ANALYZERS

Following are the PCR analyzers put up by Molbio Diagnostics for rapid, real time sample to result analysis.



Following is the optical graph for the Covid-19 RT-PCR test, here the red curve marks the Ct value for control (From the IC). The blue and the green curve marks the ORF and E-gene (gene encoding for envelope protein) respectively. The functional range for Ct value of control is 26-29. Same result can be printed using Truelab micro PCR printer. The screen of the above discussed analyzer will give the real time result of ongoing run including number of cycles completed for each microchip. It ha touchscreen feature of selecting the species specific test, enter patient details etc. Since it is a RT-PCR analyzer , the RNA from the patient's sample is first treated with Reverse transcriptase enzyme turning RNA into cDNA (complimentary DNA), this DNA further undergoes normal PCR steps including denaturation, annealing and extension.

The Ct value, short for Cycle threshold is the number of cycles needed in the test, after which the sample reaches a detectable level. Ct value is inversely proportional to the amount of viral load present is the human body.



Optical graph for COVID-19 RT-PCR analysis

CHAPTER 5-OUTLINE OF WEEKLY INTERNSHIP ACTIVITIES

	DATE	DAY	NAME OF THE MODULE COMPLETED
1 st WEEK	01/12/22	Thursday	 Introduction To the Validation Department and its role by Dr. Ruchira ma'am
	То	То	 Introduction to the Cartridge coating machine, the role of IC ,evaluation of Trueprep® section by Prakash sir and Saurav sir.
	03/12/22	Saturday	 Discussion about various standard operating protocols followed by the department for validation purpose.

- 1/	DATE	DAY	NAME OF THE MODULE COMPLETED
2 nd WEEK	04/12/22	Monday	 Demonstration of FTIR including discussion of its working principle, applications etc. by Dhananjay Sir, Samata ma'am and Aishwarya ma'am in the QC Instrumentation room.
	То	То	• Evaluation of the Truenat® section, including walkthrough chip coating, master mix tube building by Shabana Ma'am and Goraksh sir.
	10/12/22	Saturday	 Discussion about various spectroscopic methods, Gel doc, Gel electrophoresis, types of PCR, TB diagnostics by Dhananjay Sir.

	DATE	DAY	NAME OF THE MODULE COMPLETED
	12/12/22	Monday	 Validation of the Cartridge coating machine under supervision of Saurav sir
WEEK	То	То	 Validation of embossing of truenat® pouches/sleeves under supervision of Shabana miss
3 rd	17/12/22	Saturday	 Continuing reading of various SOPs(standard operating protocols)

	DATE	DAY	NAME OF THE MODULE COMPLETED
4 th WEEK	20/12/22	Tuesday	 Validation of silicone bungs including leak test using methylene blue under supervision of Goraksh sir
	То	То	 Validation of the Reagent pouches in the sample prep kit, checking for spillages, breakages(cosmetic defects) if any.
	23/12/22	Saturday	 Validation involving monitoring the pH of the buffer stock solutions using Eutech pH tutor
			 Continuing reading of various SOPs(standard operating protocols) for

-			
	DATE	DAY	NAME OF THE MODULE COMPLETED
	26/12/22	Monday	 Reading and discussing the SOPs and STPs Cleaning, operation and Calibration of UV-Vis Spectrophotometer, Trueprep® AUTO V2 Universal Sample Prep Device ETIR
×	То	То	Spectrophotometer, Gel Rocker, Incubator shaker, Pass box, Laminar air flow Conductometer.
5 th WEE	31/12/22	Saturday	 Visit to the Quality Control section – Sample loading and extraction room, PCR room , Stability Room – Induction by Santosh and Rinoj sir
0			 Performing performance tests, leak tests and volume verification test for cartridges in the QC
			 Performing COVID -19 RT-PCR using own throat swab.

Validation of Cartridge coating machine-

The cartridge coating machine does the work of dispensing 30µl of internal control (pink solution) into the sample chamber. It contains network of 20 dosing / positive displacement pumps, one part of it (inlet) is connected to the Reservoir (containing dummy solution/blank) and other part (outlet) is connected to the dispensing head. which needs to be aligned at proper scale to dispense off required amount. At a time it can dispense IC into 20 cartridges. It is attached with software wherein one can manage time interval between each dispense. To check whether each nozzle was dispensing required concentration validation was carried out by 2 methods-The pipetting method that involved use of stoppered pipette and checking volume dispensed each time (volume verification approach). If the volume was more than required then the dosing pumps was turned in clockwise fashion to lower the volume and vice versa.

The second method involved weight determination of small tubes, before and after dispensing of IC (initial weight – final weight). More than 200 tubes were determined using this method following single dispense and multiple dispense, undertaking Trial and error hypothesis. Once the IC is loaded, the cartridges are sent for performance testing in QC section.

Validation of Embossing of Truenat® pouches

Embossing is the process of creating raised relief patterns against the background. It is done to help one company brand communicate elegance and reflect greater value. The validation process involve checking proper sealing of the pouches, inspecting whether the label can be visualized properly. For this Pouch sealing machine is used which runs at the speed of 10 and at $</=261.3^{\circ}$ c. The high pressure and temperature of the machine aids in the embossing. A set of 20 pouches was checked each time ,of which 10 were sent to QC section for dye Leak test. In first set the label- MDI-5 was checked and in the second set (other week) the label of MDI-10 was checked.

Validation of the silicone bungs-

Silicone bungs is basically a cork or stopper which is added to the buffer bottles to prevent the liquid chemical leaks or escape the container. This can occur only if the bung fit tightly to the container's opening for which the dimension of rubber bung is of concern. For the validation purpose the set containing 50 bottles was loaded with diluted methylene blue solution. The bottles were placed upside-down on the blotting paper, and inspected after every week, for any spillage. The leakage count was documented.

Validation involving inspection of reagent pack

The shrink wrapped cartons containing Trueprep Auto V2 Universal Cartridge Based Sample Prep Kit was validated for any cosmetic defects. These defects included, the breakage of the EBS frame (black frame) used to keep the pouches in intact orientation, the fitment board is having any bends, if the pouches are leaking, if the plug in connector is loose etc. 30 plus, such boxes were checked for validation purpose.

Validation involving inspection of pH fluctuation

The pH is the negative logarithm of the molar hydronium ion concentration, the letters pH stand for potential of hydrogen. In the extraction and amplification of nucleic acids maintaining stable pH as that of the physiological pH is critical step. The process involved checking of pH fluctuation if any among the stock solution of Wash Buffer A , B and Elution buffer, each of which were maintained at ~9.75pH. The inspection was carried out 4 times a month, based on which stability report was provided. Stability study involved checking the shelf life of the buffer stock solutions with pH fluctuations.

Analysis of Truenat® COVID-19 chip using own throat swab sample.'

The extraction and amplification was carried out in QC section. Using soft swab tip, sample was collected from back of the throat and the swab tip was inserted in the VTM tube. 0.5ml of aspirate was taken from VTM and transferred into lysis buffer with2.5ml volume. With another pipette the mixture was triturated, following addition into sample chamber (whole 3ml), of the cartridge. The

cartridge was kept for run on prep device. 1.5ml elute was collected from the elute chamber and transferred into ECT. Nucleic Acid amplification for COVID-19 diagnosis was carried out in PCR section, using Truelab® Quattro real time micro PCR analyser.

Leak test and volume verification test for incoming cartridges

Dye leak test included pouring 3mlof diluted methylene blue solution in the sample chamber of the cartridges to check for the leakages if any. Prior to this volume verification is carried out using micropipette. The elute quantity is checked to match with required quantity of 1.5ml. Around 50 cartridges were sampled for these analyses.

Reading and discussing the standard operating protocols and standard testing protocols

Reading the SOPs, understanding theoretical part of machine and equipment operation, followed by demonstration of each working was helpful in understanding the basis. Following are the protocols covered throughout internship period

1.Preparation of Trunat® chips

2. Preapration of product specific Truenat® tubes

3. Preparation of Truemix[™] Real time PCR Test Reconstitution buffer, negative control and its dispensing and labeling

4. Operation and cleaning of Biodot wax dispensing machine

5. Operation and cleaning of Biodot Reagent dispensing machine

6.over printing process

7. Procedure for handling and cleaning of Bio hazardous material

8.Sampling of incoming material

9. Guidelines for handling of chemicals

10.Procedure for sanitization in QC area

11.Procedure for decontamination of culture , glasswares and pathological samples.

12. Testing procedure for ultrasonic cartridges

Demonstration of FTIR

FTIR is short for Fourier Transform Infra Red spectroscopy is a technique used to obtain an infrared spectrum of absorption or emission of solid, liquid or gas.it collects high resolution spectral data over wide spectral range unlike dispersive spectrometer. Fourier transform is a mathematical process to convert raw data into actual spectrum. The FTIR spectrometer used for demonstration was of Shimadzu company with IRSpirit i.e., solid sample analysis module. Here the FTIR grade KBr(Potassium Bromide) powder was used as blank. The spectrometer is based on the principle of change in the vibrational frequencies of various atoms. The lambda max can be accounted as the peak point for the intensity rise. In case of FTIR of glycerol, the peaks will be displayed for O-H group and C-H group. So basic applications of FTIR holds , identification of various functional groups and bonds in the molecule under study. The solid samples need to be ground finely before using spectrometer.Lab Solutions software is used to identify and analyse the peaks. IR quartz cuvettes are used

CHAPTER 6 - CONCLUSION

In a nutshell, this one month internship has been an excellent and rewarding experience. I can conclude that there have been a lot I've learnt from my work at Molbio Diagnostics. Related to my study I learned more about biology involved in designing of PCR kits for diagnosis purposes of various pathogens including MTB, Brucella, Nipah, HIV, HBV, HCV, SARS-CoV-2 etc. Not only did I gain practical skills but I also had an opportunity to make new connections with people from different academic fields.

This period helped me understand how Diagnostics Company works at economic level, in conjunction with other sectors.

It was very constructive exposure as it enabled me to strengthen existing knowledge and construct new skills throughout the period of internship.

CHAPTER 7- BIBLIOGRAPHY

- Diagnostics,M.(2022,August 25). Truenat A Point of Care RT PCR Test for COVID-19 [Video]. YouTube. Retrieved January 7,2023,from https://www.youtube.com/watch?v=rPuSk4quQT4&feture=youtu.be
- Diagnotic cartridges cleanly and precisely welded by laser. (n.d.). Medtech.plus. <u>https://www.medtech.plus/en/c/diagnostic-cartridges-cleanly-and-precisely-welded-by-laser.9046</u>
- Liger, Dominique. (2015). Re: What is the role of sodium azide in a DNA extraction from saliva and what should be its concentration?. Retrieved from: https://www.researchgate.net/post/What_is_the_role_of_sodium_azide_in_a_D NA extraction from saliva and what should be its concentration/54c91611 d039b14b6b8b4567/citation/download.
- Molbio Diangostics Pvt.Ltd. (n.d.).
 https://www.molbiodiagnostics.com/products-listing.php
- Oda, Y., Sadakane, K., Yoshikawa, Y., Imanaka, T., Takiguchi, K., Hayashi, M., Kenmotsu, T., & Yoshikawa, K. (2016). Highly Concentrated Ethanol Solutions: Good Solvents for DNA as Revealed by Single-Molecule Observation. ChemPhysChem, 17(4), 471–473. <u>https://doi.org/10.1002/cphc.201500988</u>
- Sairkar, P., Chouhan, S., Batav, N., & Sharma, R. (2013). Optimization of DNA Isolation Process and enhancement of RAPD PCR for low quality genomic DNA of Terminalia arjuana. Journal of Genetic Engineering and Biotechnology, 11(1), 17-24. <u>https://doi.org/10.1016/j.jgeb.2013.02.001</u>
- Spiess AN,Mueller N,Ivell R. Trehalose is a potent PCR enhancer:lowering of DNA melting temperature and thermal stabilization of taq polymerase by the disaccharide trehalose.Cli Chem.2004 Jul;50(7):1256-9.doi:10.1373/clinchem.2004.031336.PMID:15229160