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The Crux

BIMONTHLY FORUM FOR THE LABORATORIANS

True lab™

• Real Time micro PCR System •

An introduction

STOP PRESS

EVERY LABORATORY IN INDIA CAN NOW PERFORM RT/PCR TESTS

TÜV ISO 9001

True lab™

• Real Time micro PCR System •

Launched by Honourable President of India Mr. Pranab Mukherjee.

Tulip Group

True lab™

• Real Time micro PCR System •

An introduction

Q.1) Is it not true that the current diagnostic tools are adequately meeting the requirements of pathogen detection?

A) The basis for effective treatment lies in the early and correct diagnosis of the disease and identification of its causative agent. It is indeed true that the current diagnostic tools, singly or in combination, are significantly contributing to reliable detection of various pathogens. However, for various reasons discussed below, they still do not meet the requirements of ASSURED criteria completely. Hence case detection still remains a major problem, both to the patient and the society, in combating diseases. This brings into question the impact of current diagnostic interventions.

Q.2) What is ASSURED criteria?

A) ASSURED criteria prescribed by WHO requires a diagnostic test to be,

A - Affordable
S - Sensitive
S - Specific
U - User-friendly
R - Rapid & Robust
E - Equipment free
D - Deliverable to end users

A technology that comes closest to meeting these requirements would be the most appropriate and most effective in addressing disease control needs.

Q 3) What are the drawbacks of current diagnostic techniques?

A)

- Conventional **laboratory tests based on Microscopy/ Immunoassays/ serology** lack adequate sensitivity/ specificity to detect pathogens at early stage.
- **Culture methods** used to isolate pathogens are laborious, and time consuming, especially in case of organisms which are difficult to grow *in vitro*. Most importantly cultivation of many of these pathogens in the laboratory poses a grave risk to the staff. Hence these methods require high level of laboratory safety (BSL level-3) measures, possible only at specialized reference labs.
- **Current molecular diagnostic techniques,** considered most sensitive and specific, can be run only in batches, require sophisticated lab infrastructure, expensive equipment and highly skilled manpower. The reagents require transportation and storage at low temperatures (-20°C to -40°C) and have limited reconstituted stability. Assay standardization is an issue as the source of equipment and reagents are different, especially considering that mostly "home brew" reagents are used. Assay contamination is a serious risk that needs to be monitored continuously.

All these factors have ensured that molecular testing remains the domain of large reference laboratories thus limiting the routine usage of this technology for reasons of cost, accessibility and long turnaround time to results.

To meet ASSURED criteria, a molecular platform, apart from high sensitivity and specificity, needs to be,

- Affordable to acquire, maintain and run.
- Relatively "maintenance-free".
- Mains and battery operable.
- Portable.
- Simple, fast and easy to perform by any technician.
- Deployable at all levels of health care without requiring special laboratory infrastructure.
- Robust with room temperature stabile reagents (2-30°C).

- Able to automatically report results, quantitatively.
- Designed for minimizing contamination.
- Capable of single/STAT (short turnaround time) testing to enable near patient diagnosis.
- Standardized and validated.

Q.4) Does such a modern molecular diagnostic platform exist today?

A) Yes, **Molbio**™ Diagnostics, India, has just launched "**Truelab**™ **Real Time micro PCR system**", that overcomes the limitations of existing technologies. It is indeed an" *Anytime, Anywhere*" platform and promises to be a true game changer.

Truelab™ Real Time micro PCR system is based on Quantitative Real Time PCR (qPCR) technology and, is Rapid, Simple, Portable, Robust and Affordable and meets all the ASSURED criteria.

Q.5) What is PCR and what is the difference between a conventional PCR and a Real Time PCR (qPCR)?

A) The name Polymerase chain reaction comes from the enzyme DNA polymerase that is used to amplify, through a process of repeated replications, a piece of nucleic acid (DNA) by an *in vitro* process. With each cycle of replication in the "chain reaction" the amount of nucleic acid doubles leading to an exponential amplification of the starting trace nucleic acid after a short time frame.

Technically the process of PCR involves the heterothermal cycling of the nucleic acid (DNA) between two temperatures that first separates (denaturation at ~94°C) the double helix into single strands where each strand serves as a replicating template, and then the primers pair (annealing at ~54°C) with the template as well as newly synthesized complementary strands formed with the help of the DNA polymerase (extension at ~72°C) enzyme and nucleotides present in the reaction medium.

Traditionally the products of a PCR reaction, also called amplicons (amplified DNA fragments) were visually analyzed by running the fragments through a gel electrophoresis system comparing them by running DNA "ladders", or fragments of DNA with known sizes. In this "end point method", before visualizing the amplicons on a electrophoresis chamber, it is assumed that the efficiency of the PCR reaction is 100% throughout the reaction. However because of certain reasons like inhibitors of the polymerase reaction found in the sample, finiteness or limitations of the reagent, accumulation of pyrophosphate molecules and self annealing among accumulating products the PCR reaction does not realistically amplify targets in an exponential manner throughout. After a timed exponential phase the PCR enters a plateau phase where the amplification is no longer exponential. This is the reason why quantification of PCR amplicons using the "end point" method is rather unreliable.

Having understood that the most significant disadvantage of the end point method of quantitating the PCR amplicons, it should be very simple to appreciate why the real time PCR method has become the method of choice today.

- Real time PCR permits analysis of products while the reaction is in progress – real time
- Only the "true" exponential phase of the PCR reaction is used to quantify the amplicons
- The technique tells us "what DNA" is present and also "how much" of it – quantitative PCR (qPCR).
- It is a very rapid assay and there is no need to resort to other procedures such as electrophoresis after the amplification reaction, so no further manipulations are required.

To truly appreciate the advantages of real time PCR one needs to understand the PCR fundamentals.

1. During the start of the PCR reaction while the reagents are in excess, the starting nucleic acid template and product is in low concentration, there is no competition between product renaturation and primer binding – thus amplification is at a constant exponential rate.
2. The point at which the exponential rate of amplification ceases, though variable, is primarily due to the competition between product renaturation and primer binding, ultimately leading to the plateau phase where very little or no more product is made.

Following are the differences between a conventional PCR and a Real Time PCR (qPCR).

Sr.	Conventional PCR	Real-Time PCR (qPCR)
1.	End point analysis of amplified DNA	Amplification monitored real-time
2.	Post-PCR processing	No post-PCR processing of products
3.	Quantification often not possible	Quantification possible
4.	Gene expression studies not possible	Gene expression studies possible
5.	Less sensitive, specific and reproducible than Real Time PCR	Most specific, sensitive, reliable and reproducible
6.	Data interpretation is very difficult	Data interpretation is easy, automated and biologically meaningful
7.	Hybridization step to confirm specificity	No hybridization step involved
8.	2-5 hrs to complete one test	1hr or <1hr for one test
9.	Ethidium bromide used to view DNA is carcinogenic	Taq Man probe technology, do not require Et Br
10.	Need Standard DNA marker	No DNA marker needed
11.	Non-automated	Totally automated
12.	No internal positive control	Internal positive control
13.	For multiplexing all target amplicons should be of different length	Multiplexing all target amplicons can be of same length
14.	Stage of infection monitoring Not possible	Monitoring of Stage of infection possible

It is for these reasons, Real Time PCR is preferred now universally

Q.6) What is Truelab™ Real Time micro PCR system? Kindly provide a brief description?

A) The **Truelab™** Real Time micro PCR system has been developed with the aim to bring real time PCR testing to near patient and point of care level to enable early diagnosis and better patient care. The system works on disease specific microchips that have pre-loaded reagents for conducting a real time PCR. The chips also carry batch specific information and standard curve values and require only 6 µl of purified nucleic acid sample for the reaction. The chips run on the fully automatic **Truelab™** Real Time micro PCR analyzer and quantitative results are available in about 30 minutes.

The sample preparation (extraction and purification) is done on a semi-automated **Trueprep™ MAG** sample prep device and reagents. The process is simple and user friendly and takes about 25 minutes.

All the reagents including microchips are stable at up to 30^0 C for one year and all the instruments are battery and mains operated. It is possible to carry the full system in a carry case for field level or near patient use.

The Truelab™ Real Time micro PCR system comprises of three components:

1) **Truelab™ Real Time micro PCR workstation:**

The Truelab™ Real Time micro PCR workstation is a **One Time** requirement for any laboratory. It consists of all equipment required to conduct PCR analysis using the **Truelab™** platform. It comprises of the following components:

a) **Trueprep™ MAG device** for **extraction** & **purification** of nucleic acids from raw samples.

b) **Truelab™ Uno** Real Time micro PCR **analyzer** that conducts automatic amplification of the pathogenic nucleic acids extracted from the sample and also displays results of the final analysis of the PCR/RTPCR run. The results are automatically saved in the system, can be shared through SMS, e-mail or printed.

c) **Truelab™** Real Time micro PCR **printer**-a Bluetooth printer that prints hardcopies of results generated by the **Truelab™** Uno Real Time micro PCR analyzer.

d) **Truepet™**- set of five fixed volume high precision **micropipettes** that take care of the entire "hands on" procedures of the nucleic acid extraction & purification process on the **Trueprep™** MAG device.

2) **Trueprep™ sample preparation kits (nucleic acid extraction kits):**
These contain reagents for extraction and purification of nucleic acids from samples of patients**.** Reagents along with the sample are added in tubes provided and are put into **Trueprep™ MAG Sample Prep Device** for extraction and purification of nucleic acids.

3) **Truenat™ chip based micro PCR test platform:**
It consists of a reaction chamber and circuitry for control of PCR reaction by the analyzer. The reaction chamber has pre-dispensed dried down disease specific PCR mastermix in it. Extracted nucleic acid sample is pipetted into the reaction chamber of **Truenat™** chip for PCR reaction.

Q.7) Are there any consumables required for Truelab™ Workstation?

A) Yes, the only consumables required for the **Truelab™** Real Time micro PCR work station are

1) **Printer paper rolls** for the **Truelab™** Real Time micro PCR printer.

2) Appropriate filter barrier **Micropipette tips** to fit the **Truepet™** high precision micropipettes.

Both the consumables can be procured from **Molbio™** Diagnostics-India.

Q.8) How does Truelab™ Real Time micro PCR system function?

A) The basic functioning of **Truelab™** Real Time micro PCR system is outlined below:

The **Truelab™** Real Time micro PCR system works on four simple processes that can be completed within an hour's time.

1) *Sample Collection:* as per standard laboratory practices or as instructed.

2) *Sample extraction and purification* using **Trueprep™ MAG Sample Prep kit, Trueprep™ MAG Sample Prep Device** and **Truepet™** fixed volume precision **micropipette** for transferring samples.

3) *Automatic sample Analysis* using test/disease specific **Truenat™ Real time micro PCR chip** which runs on the **Truelab™ Uno Real Time micro PCR analyzer**.

4) *Reporting the results* displayed on the screen of **Truelab™ Uno Real Time micro PCR analyzer**. Results can be shared via SMS, e-mail or printouts of the same can be taken using the **Truelab™ micro PCR printer**.

The PCR analysis and reporting, from sample to result is completed in less than 1 hour.

Sample Collection.

Q.9) Are standard laboratory samples used for testing and analysis in this system?

A) Yes, this system is designed to fit into the current practice, and not to increase but to decrease human effort.

Clinical specimen such as whole blood/serum/plasma/other body fluids and sputum incase of TB are collected fresh as per standard laboratory procedures in the tubes provided.

Nucleic acids are then extracted from the samples, analyzed and results are generated through **TrueLab™** micro PCR system.

☞ Frozen specimen can also be used but must be brought to room temperature before starting extraction and analysis procedure.

Sample extraction and purification

Q.10) Why is extraction and purification process necessary?

A) The extraction and purification of nucleic acids from a clinical specimen is necessary. It frees the extracted nucleic acids from potential PCR inhibitors and cell debris. If PCR inhibitors are present in nucleic acid sample it will give false negative results.

Sample preparation is therefore done using **Trueprep™** MAG Sample Prep kit which comprises reagents and accessories necessary for the purpose.

Q.11) How many reagent kits does one have to buy for performing Real Time PCR / RTPCR tests using the Truelab™ Real Time micro PCR system ? What are the details?

A) The answer lies in the scientific & innovative design of the **Trueprep™ MAG** kits.

Only Two Types of kits are required for a wide range of samples.

Trueprep™ MAG Sputum: is the reagent kit for the extraction of *Mycobacterium tuberculosis* (MTB)–DNA from sputum samples.

Trueprep™ MAG Blood: is the reagent kit used for the extraction of DNA/ RNA from either bacterial or viral pathogens present in blood. This kit is *not disease specific* and is common for all disease types.

Q.12) What are the contents of Trueprep™ MAG kits and what are their functions? Does it contain all the reagents and accessories necessary for nucleic acid extraction from samples?

A) **Trueprep™ MAG Sputum** & **Blood kits** are self-sufficient and contain the following:

- *Lysis buffer* - Decontamination and lysis of clinical samples.
- *Binding Reagent A* - Alcohol based sample denaturation reagent.
- *Binding Reagent B* - Nanoparticles that specifically bind nucleic acids.
- *Wash Buffer A & B* - Washing reagent to purify nucleic acids.
- *Elution buffer* - Reagent to elute nucleic acids from nanoparticles.

☞ All the reagents in the kits have specially designed color coded bottle caps that uniquely identify the reagents.

Q.13) How is extraction and purification process carried out in Trueprep™ MAG device?

A) **Trueprep™** MAG device works on advanced paramagnetic nanoparticle based mechanism for extraction & purification of nucleic acids.

The Extraction Tube (EXT) containing the lysis reagent and the sample is placed in the holder of the **Trueprep™** MAG Sample Prep Device. The device guides the user through a simple step by step menu driven process.

1) Nucleic acids, from the sample are released by chemical and thermal lysis of cells.
2) When binding reagent containing paramagnetic nanoparticles is added, nucleic acids bind to their surface in the presence of magnetic field.
3) Subsequently the captured nucleic acids are then washed with buffers to remove the PCR inhibitors, cell debris etc.
4) Finally nucleic acids are separated from the nanoparticles, using the elution buffer.
5) The elute, containing nucleic acids, is then pipetted out using **Truepet™** fixed volume precision micropipette into the Elute Collection tube (ECT) provided in the accessories pack. In this form nucleic acid is ready for analysis.

☞ The entire extraction process is quick (25 minutes max), reliable and user friendly.

☞ Step by step instruction driven process control, does not require highly skilled staff. Any technician capable of performing correct pipetting procedures can easily perform the extraction.

Q.14) Kindly explain the features and benefits of Trueprep™ MAG device.

A) Features and benefits of **Trueprep™** MAG device is explained in this table:

Sr.	Features of Trueprep™ MAG device	Benefits
1	Extraction uses Magnetic nanoparticles.	Innovative, well researched and well proven mechanism for extraction & purification of the nucleic acids. Increases specificity.
2	Sample preparation in 20-25 mins.	The entire extraction process is quick, reliable and user friendly.
3	4 line alpha-numeric LCD display Screen	Step by step instruction driven process control & does not require highly skilled manpower.
4	Runs on rechargeable Lithium Ion Battery Pack 7.4V,4.4Ah	The device can be taken to remote areas having no power supply. And can perform about 8 extraction processes after one full battery recharge.
5	Weight: 1.6 kgs.	Light weight and portable.
6	Size: 210mm x 155mm x109 mm	The device can sit comfortably on any bench.
7	Operating Environment conditions : Temperature: 15- 35°C, RH: 10-80%	No special laboratory/ testing conditions required.

Q.15) Why the Truepet™ Micropipettes?

A) Accurate pipetting is an essential and critical component of Good Laboratory Practice (GLP).

- ☞ The **Truepet™** Precision Micropipettes, a set of ***five*** fixed volume micropipettes ensures that the user performs each and every step of the extraction procedure precisely and accurately.
- ☞ Further each **Truepet™** Precision Micropipette is colour coded to match the coloured caps of the **Trueprep™** MAG reagent bottles it is meant for. This ensures that the right pipette gets used with the correct reagent thereby acting as a crosscheck for the user.
- ☞ All the **Truepet™** Precision Micropipettes are autoclavable allowing the user to decontaminate them in case of contamination.

Q.16) What is the evidence of having a good yield of extracted nucleic acids, using the Trueprep™ MAG kits?

A) Indeed both the quality and quantity of extracted nucleic acids from the sample is crucial for the proper performance of Real Time PCR/RTPCR assays.

Internal validation data is available which supports good yield of disease specific nucleic acids using the **Trueprep™** MAG kits. A summary of validation is as follows:

- Samples were taken from TB patients at a hospital in South East Asia, whose results were confirmed and cross-checked with both smear microscopy and culture technique.
- The sample panel containing 100 specimens, when cross-checked with both smear microscopy (S) and culture technique(C), following are the results obtained:
  40 were S+C+ i.e.(sputum smear positive & culture technique positive)
  40 were S- C+ i.e.(sputum smear negative & culture technique positive)
  20 were S- C- i.e.(sputum smear negative & culture technique negative)
- The nucleic acid was extracted from sputum specimen using **Trueprep™** MAG Sputum Sample Prep Kit on the **Trueprep™** MAG Sample Prep Device.
- One set of the purified nucleic acids were tested using **Truenat™** MTB chip based real time PCR test.

- The second set was performed on a commercial real-time PCR machine using MTB specific probe and primers.
- Both the experimental sets showed results similar to the original known result.
  This shows that **Trueprep**™ MAG Sputum is able to isolate MTB DNA efficiently for use in PCR reactions.
  The recovery of the internal positive control (IPC) DNA was also tested.
- A known amount of IPC DNA was added into 5 different sputum specimens.
- The specimens were processed with **Trueprep**™ MAG Sample Prep Device using tubes devoid of IPC.
- The isolated nucleic acids were then subjected to IPC DNA specific real-time PCR.
- Also a pure IPC DNA without any other DNA was subjected to Real Time PCR.
- The Ct values obtained from the IPC DNA added sputum specimens were in agreement with Ct values obtained using pure IPC DNA.

Automatic Sample Analysis

Q.17) How is sample analysis done in Truelab™?

A) Automatic sample Analysis is done using disease specific **Truenat™ Real time micro PCR chip** that runs on the **Truelab™ Uno Real Time micro PCR analyzer**.

Q.18) So what exactly are the Truenat™ micro PCR chips?

A) The Patented **Truenat**™ micro PCR chip is the key component of the micro PCR system.

- It consists of a reaction chamber and circuitry for control of PCR reaction.
- Reaction chamber has pre-dispensed disease specific PCR mastermix immobilized in it. The PCR reaction is carried out by addition of the extracted nucleic acid samples to the well of the chip and loading the chip into the **Truelab™** Uno Real time micro PCR analyzer.
- The **Truenat**™ micro PCR chips have high RAMP (heating and cooling cycle) rates – i.e., have the ability to switch from one temperature to the other quickly (heating 5°C/sec & cooling 1.5°C/sec). This allows completion of 40 PCR cycles in approximately 30-35 minutes.
- Further each chip comes with a lot specific calibration-ensuring that the user does not have to waste a single rupee on calibration costs.
- It is pre-programmed with lot specific data as well as data that prevents the chip from being used for a second time.

Q.19) Kindly elaborate about the reaction chamber.

A) **Truenat**™ micro PCR chip consists of a reaction chamber which has the disease specific PCR mastermix, immobilized in it.

- Extracted nucleic acids are pipetted into this chamber using **Truepet™** precision micropipette. The **Truenat**™ micro PCR chip is then inserted into **Truelab™** Uno Real time micro PCR analyzer for performing PCR.
- This reaction chamber is fabricated using Low Temperature Cofired Ceramics (LTCC) technology, that acts as a heater and is monitored by an embedded thermistor.
- The reaction well is covered by a *unique barrier* that prevents evaporation of the sample/mastermix and also makes the chip contamination free after the PCR reaction is over.

Q.20) And what about the MASTERMIX?

A) The Patented **Truenat**™ micro PCR chip has disease specific mastermix immobilized in the reaction chamber. The MasterMix contains:

- Primers for both the pathogen nucleic acid (target) and the internal positive control (IPC).

- TAQ probes for both the pathogen nucleic acid (target) and IPC.
- dNTP's with all the bases.
- DNA Polymerase
- Standardized buffers.
  - ☞ The entire mastermix is immobilized by freeze drying in the reaction well of the chip which makes the chip stable at room temperature (2-30°C) for one year.
  - ☞ The mastermix is covered with a *unique barrier* coating. This not only prevents evaporation of sample during PCR but also protects the mastermix from external elements and prevents post PCR contamination.
  - ☞ It also ensures that the extracted sample comes in contact with the master mix only when loaded in the **Truelab**™ Uno Real Time micro PCR analyzer. When the temperature is raised to 94°C, this unique barrier melts thus allowing the extracted nucleic acid to react with the components of the mastermix.

Q.21) What about assays with RNA as the target nucleic acid?

A) In case of assays with RNA as the nucleic acid, reverse transcription PCR (RTPCR) is performed.

The **Truenat**™ pouch contains entire mastermix along with the RT enzyme in a sealed tube in a freeze dried format.

Six µl of the sample is introduced into the tube to hydrate the reaction mix. Then the entire content is transferred to the reaction chamber of the **Truenat**™ chip for the reaction to proceed in **Truelab**™ Uno Real Time micro PCR analyzer.

Q.22) What is the function of the Truelab™ Uno Real Time micro PCR analyzer?

A) The **Truelab**™ Uno Real Time micro PCR analyzer is a portable unit housing a touch screen, a sliding chip tray for the **Truenat**™ micro PCR chip, optical detection system and electronics for controlling all aspects of the unit. The analyzer controls the PCR process on the chip, reads the fluorescence signals and reports results.

Q.23) Kindly enumerate the features and benefits of Truelab™ Uno Real Time micro PCR analyzer.

A) The features and benefits of **Truelab**™ Uno Real Time micro PCR analyzer is explained as follows:

Sr.	FEATURES	BENEFITS
1	Real Time Micro PCR based system	High sensitivity and specificity
2	Fluorescence, Dual wavelength	Allows for internal control
3	High ramp rate	Speed, 40 cycles of PCR/ 35 mins.
4	Wi-Fi, Bluetooth, GPRS	Enables wireless communication of results to computers, printers and other compatible devices.
5	Android based	Easy to operate.
6	Autocalibration	No extra effort required for calibrating.
7	High storage Memory	Store upto 5000 test results for future reference.
8	Operating Environment: Temperature:15-35°C, RH10% -80%	No need for special infrastructure
9	AC/DC adapter or Rechargeable Lithium Ion Battery Pack 7.5V, 2.2Ah	Mains and battery operated - can run 8 PCR analysis from one full battery recharge.
10	Size: 210 mmm x 140 mm x109 mm	The device can sit comfortably on any bench.
11	Weight: 900 gms	Light weight and portable.

Q.24) So where is the thermal cycler in the Truelab™ Real Time micro PCR system?

A) There is no conventional thermal cycler in the **Truelab**™ Real Time micro PCR system. The reaction chamber of the **Truenat**™ micro PCR chip itself plays the role of the cycler, controlled by the electronics of the **Truelab**™ Uno Real Time micro PCR analyzer.

Q.25) How is the quality of extraction of nucleic acid and the performance of the PCR run maintained and validated?

A) As mentioned earlier both **Trueprep**™ MAG Sputum and Blood reagent kits have extraction tubes in their respective accessory packs. The extraction tubes contain pre-dried Internal Process Control (IPC) that undergoes all the extraction and PCR steps along with the sample.

The amplification of the IPC within its specified Ct range in cases of negative samples is a validation of the entire extraction, purification and PCR run process. Thus the IPC serves as a full process control. The IPC amplification enables the user to differentiate between "True negative" samples from "False negative" samples, that can occur due to various PCR/RTPCR inhibitors.

Q.26) Then, what in the case of positive samples with regards to IPC?

A) Normally, IPC is co-amplified along with the target. However, in some cases, especially when the target load is high, IPC may not amplify. However the results are still considered "VALID".

Q.27) Apart from the IPC , does Molbio™ provide for QC material for system validation?

A) YES – **Molbio**™ offers Universal control Kit for use on the **Truelab**™ Real Time micro PCR system. It is advisable to run controls under the following circumstances:

- Whenever a new shipment of test kits is received.
- When opening a new test kit lot.
- If the temperature of the storage area falls outside of 2-30°C.
- By each new user prior to performing testing on clinical specimen.

Reporting the results

Q.28) How is the reporting of results done in this Truelab™ system?

A) Results are displayed on the screen of **Truelab**™ Uno Real Time micro PCR analyzer which is automatically saved in its memory. The results can be shared via SMS, e-mail or by connecting it wirelessly (via Wi-Fi & Bluetooth) to various devices like computers and printers. Printouts of the results can be taken using the **Truelab**™ micro PCR printer.

Q.29) What about the Truelab™ Real Time micro PCR printer?

A) **Truelab**™ micro PCR printer is a portable, mains as well as rechargeable battery operated, Bluetooth and USB compatible printer for printing results from the **Truelab**™ Real Time micro PCR analyzer. The printer works for over 10 hours with a fully charged battery.

Q.30) How are the results interpreted?

A) The results are automatically reported based on the screen. However, it is possible to monitor progress of the reaction for manual interpretation.

Positive test result:

- Two amplification curves are displayed on the **Truelab**™ Uno Real Time micro PCR analyzer screen when optical plot is selected to indicate the progress of the test.
- Both the target (pathogen DNA) and the internal positive control (IPC) curves will take a steep, exponential path when the fluorescence crosses the threshold value(Ct). The Ct will depend on the number of target genomes in the sample.

- At the end of the test run, the results screen will display "DETECTED".
- The result screen would also display the Ct value and the quantity of pathogen load for the positive specimen.
- The result screen also displays the validity of the test run as "VALID" based on the Ct of IPC.

Note: Generally IPC co-amplifies in most positive cases, but in some specimens because of high target (pathogen DNA) load, the IPC may not get amplified, however the test run is still considered valid.

Negative test result:

- The target (pathogen DNA) curve will remain horizontal throughout the test duration.
- But the IPC curve will always take an exponential path, regardless of positive and negative test.
- At the end of the test run, the result screen will display "NOT DETECTED".
- The result screen also displays the validity of the test run as "VALID" based on the Ct of IPC.

Discrepancies:

- In case the IPC curve remains horizontal in a negative sample, the test is considered as Invalid.
- The result screen displays the validity of the test run as "INVALID".
- Invalid samples results have to be repeated with fresh specimen from the sample preparation stage.

Final Verdict!

Q.31) Why should one use the Truelab™ Real Time micro PCR system in routine?

A) The **Truelab™** Real Time micro PCR system satisfies all the *ASSURED* criteria of a diagnostic technique:

Truelab™ Real Time micro PCR system allows you to:

- Run one test at a time without having to wait to create a batch as a result of which the patient report can be reported in one hour of sample collection.
- Real Time Technology enables quantification of viral and bacterial loads. No post PCR processes.
- The system is eminently portable and has a small footprint-ideal for near patient testing.
- Extremely user friendly, step by step protocol-no need for high level of technician skills.
- Can be used *anywhere, anytime*-no need for infrastructure or special laboratory conditions.
- System works on mains & also on battery-ideal for resource limited settings.
- Enhanced memory-5000 test results can be stored.
- Patented Technology.

Trueprep™ MAG Reagents:

- Ready to use reagents.
- Only two reagent kits for the entire gamut of diseases.
- Colour coded reagent bottles.
- Comes with all accessories such as extraction tubes, elute collection tubes- no additional costs.
- Universally accepted magnetic nanoparticle based nucleic acid extraction & purification method.

Truenat™ Disease Specific Chips:

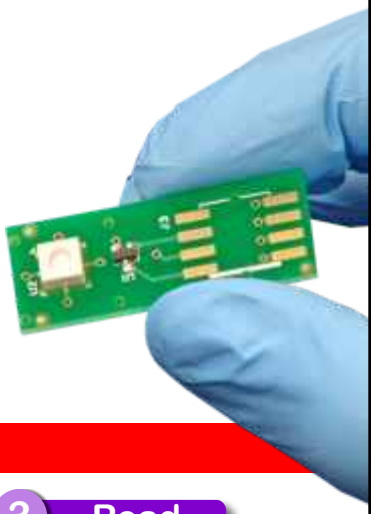
- Convenient pack sizes.
- Packaged with 6µl micropipette tip to ensure contamination free addition of sample to chip.
- Comes with lot specific calibration-rules out any additional calibration cost.
- Fast PCR-with tremendous RAMP rates-shortens run time.
- Hot start PCR-prevents formation of primer dimers & other nonspecific amplification of nucleic acids.
- Immobilized mastermix stable at room temperatures (2-30°C) – no cold storage requirements.

Truelab™

• Real Time micro PCR System •

Sample to Real Time PCR Results in Under One Hour !

- Rapid
- Simple
- Portable
- Affordable
- Robust

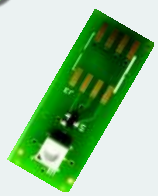


Simple Test Procedure

1 Extract

Trueprep™ MAG

2 Load



Truenat™

3 Read

Truelab Uno™

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Truenat™ Disease Specific RT PCR micro Chips

Now Available	In Pipeline
• MTB (Mycobacterium Tuberculosis)	• MTB - DR (Drug resistance)
• H1N1 (Swine Influenza)	• HIV - RNA (Viral load)
• HBV (Hepatitis - B)	• HCV - RNA (Viral load)
• Chik V (Chikungunya)	• HPV - Cervical Cancer
• Pf (Falciparum Malaria)	• VL - Visceral Leishmaniasis
• DenV (Dengue Fever)	• Chlamydia - STD
• Salmonella Spp. (Typhoid Fever)	• Gonorrhoea - STD

ANY WHERE, ANY TIME Real Time micro PCR

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