

Rapid Molecular Diagnosis of Infections using Truelab™ - A Point of Care Platform

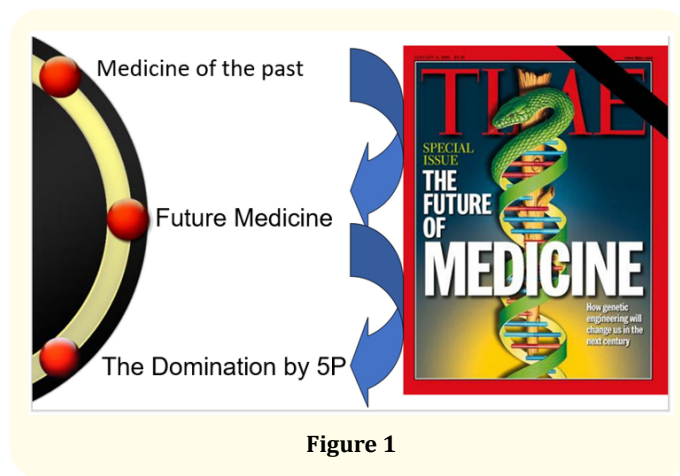
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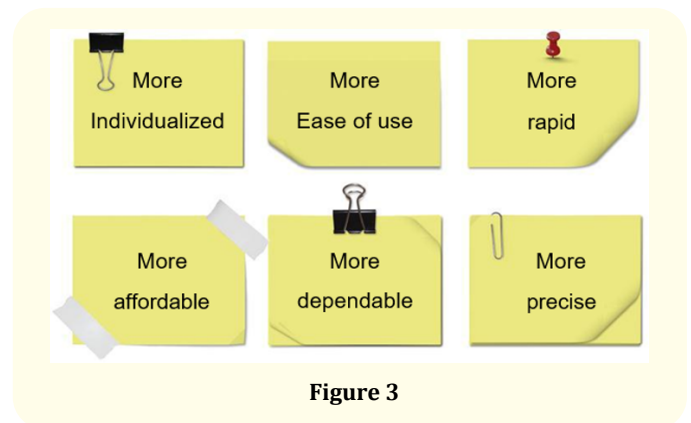
The new trend in medicine



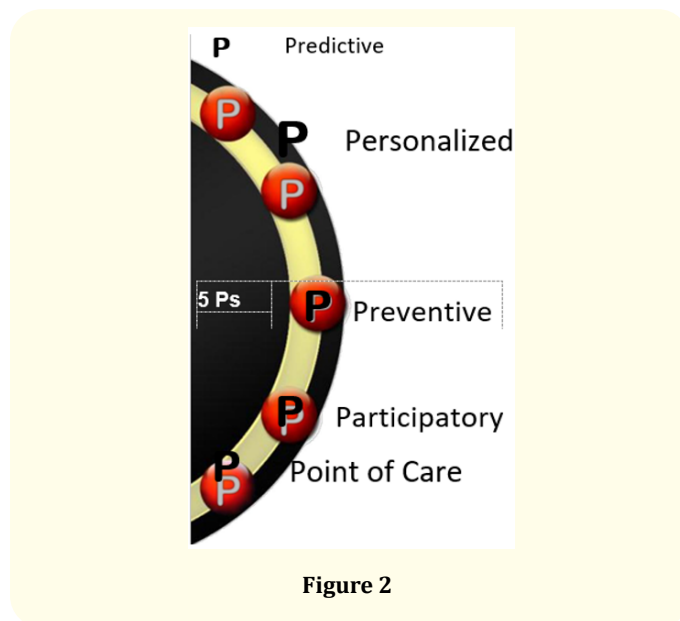
Devices	For diagnosis and treatment
Pharmacological approaches	For prevention and treatment
Information tools	For coordination, Predictive analysis and dissemination of information

Table 1

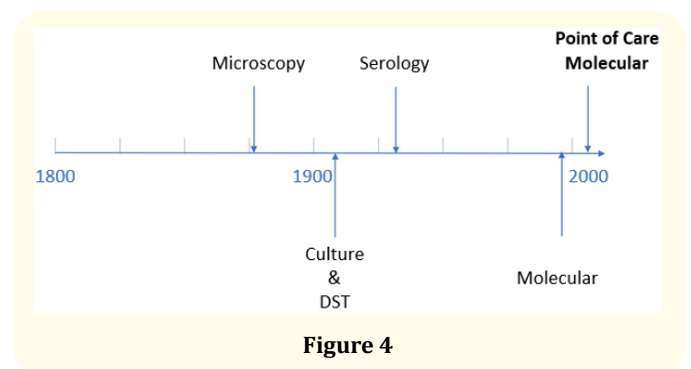
The distinct shift in focus



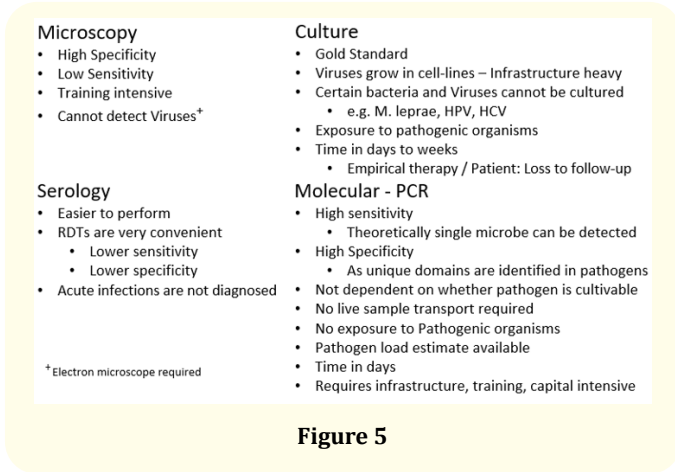
The new 5Ps of medicine



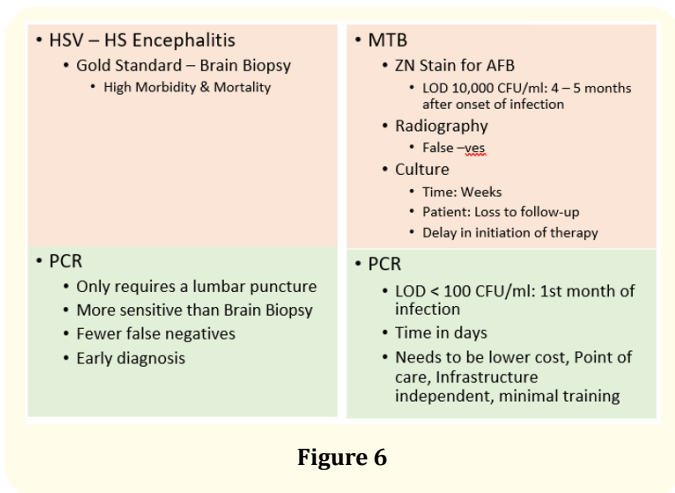
Evolution of the microbiology Lab



Techniques Comparison



Examples of PCR based diagnosis applicability



What is PCR

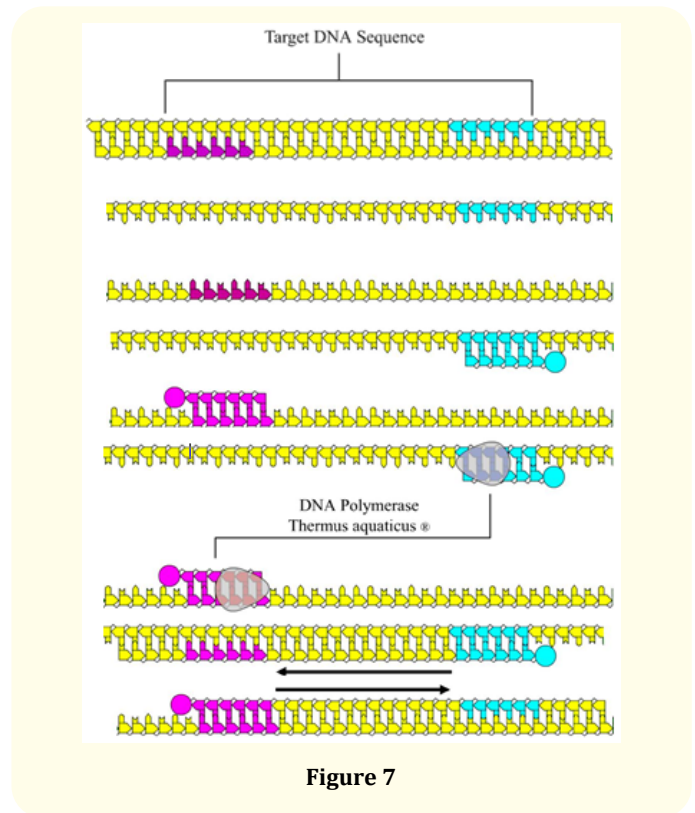
- Determine which sequence we need to amplify
- Locate Primers Upstream and Downstream of gene

60 seconds @ 94°C

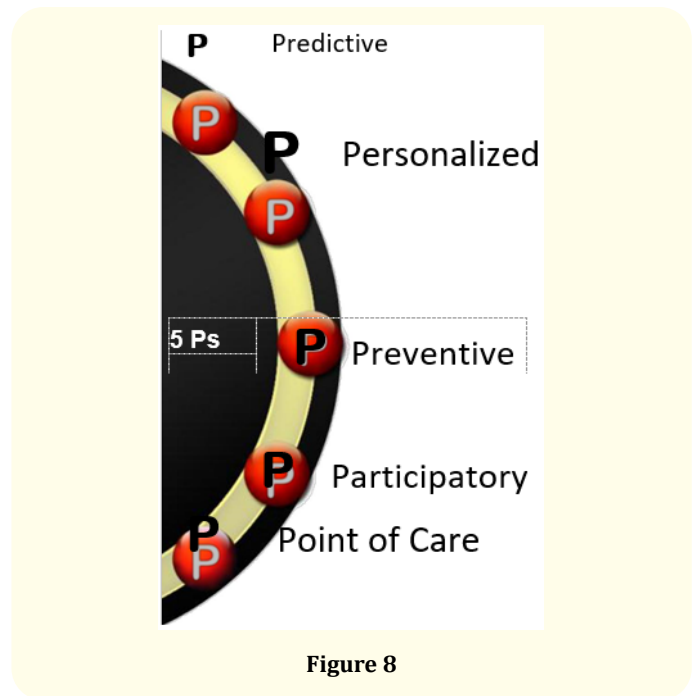
60 seconds @ 60°C

Forward and Reverse Primers – Annealing

2 minute at 72°C DNTP's



The exponential amplification of the gene in PCR.



Potential Pitfalls of PCR

False Positives

- Due to the techniques inherent sensitivity
- Sample Contamination
- Dead Bacteria
- Broad Range PCR – DNA in reagents

False Negatives

- Not so much of a problem
- Can happen due to sample inhibition/Improper Prep

Infrastructure Dependence

- Typically needs a full-fledged PCR lab/ Conditioned Power / Air conditioning/ Deep Freezers

Training

- Intensive technician training
- These can be addressed with good laboratory practices and appropriate training / infrastructure



Figure 9

The new buzz – Lab on chip

Technologies which permit operations that normally require a laboratory to function on a very miniaturized scale, within a portable or handheld device.

Benefits = POC [Point of Care] diagnosis

- Sample analysis takes place where the samples are generated, rather than being transported around to a large laboratory facility.
- Small scale fluid dynamics = Enhanced molecular interaction, shortening process time.

Limitations and Expectations with Lab on Chip

- Efficacy on par with the best in the world
- Design and fabrication on a very small scale but yet be functional and cost-effective
- Must have Reproducibility
- Must be User-friendly
- Must operate in Resource limited settings
- Must be communication friendly

Example of application of Lab on Chip

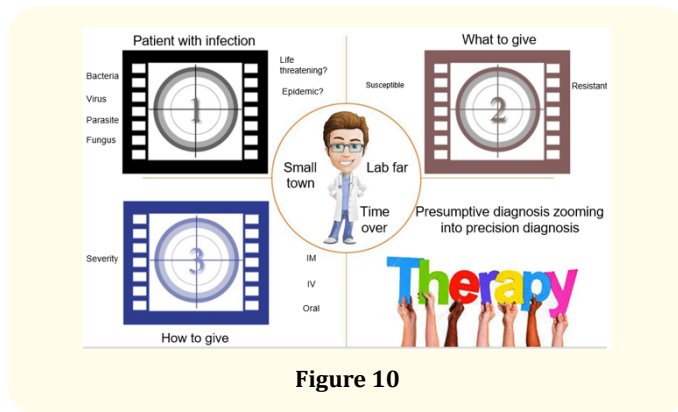


Figure 10

Is it possible to integrate and introduce such technologies in India?

Who we are and what we do

- We have developed the world’s first commercially available Point of Care molecular diagnostics platform for use in resource limited settings
- Our aim is to decentralize diagnostics and enable the move from presumptive to precise diagnostics for infectious diseases



Figure 11

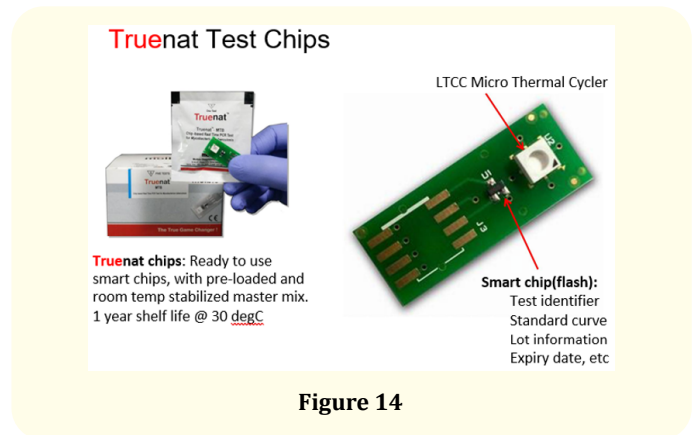


Figure 14

Test Result – Graphical View

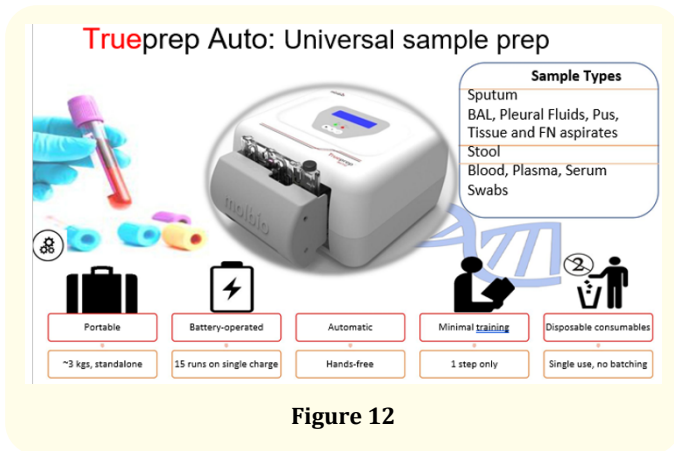


Figure 12

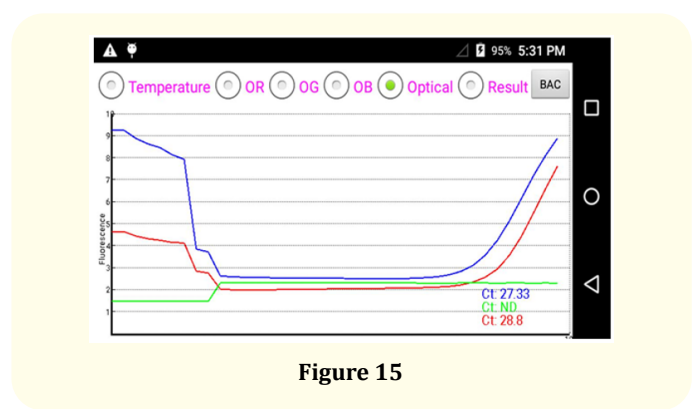


Figure 15

t result view

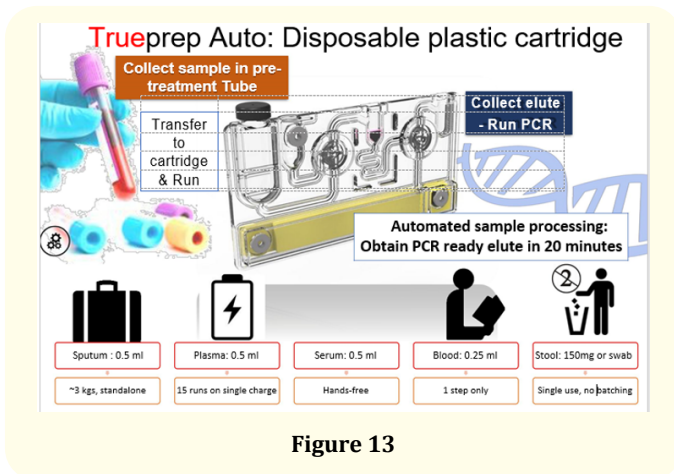


Figure 13

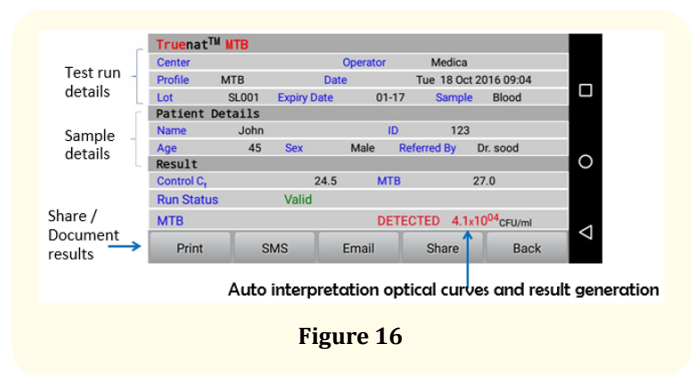


Figure 16

Truenat™ HBV

Test for the quantitative monitoring of Hepatitis B virus in human blood/serum/plasma

Test detects all known genotypes of HBV

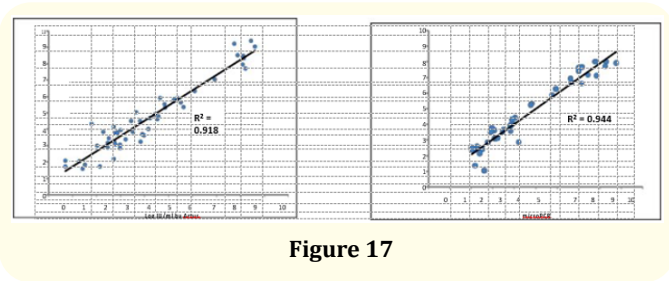


Figure 17

- Correlates well with both a CE- marked kit and a US FDA marked kit for detection of Hepatitis B virus.
- For the positive samples, a Bland-Altman analysis found no significant difference between viral loads obtained by the two methods.
- Standardized to HBV 4th International Standard from NIBSC

High inter-user, inter-analyzer and inter-lot reproducibility was observed (Average standard deviation= 0.25Ct)

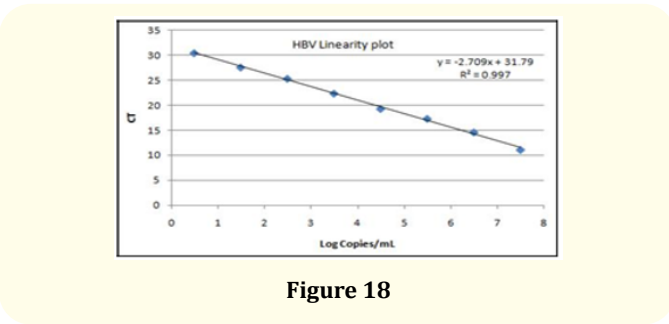


Figure 18

The assay was found to be linear over 5 orders of magnitude.

LOD of 55.92 IU/ml

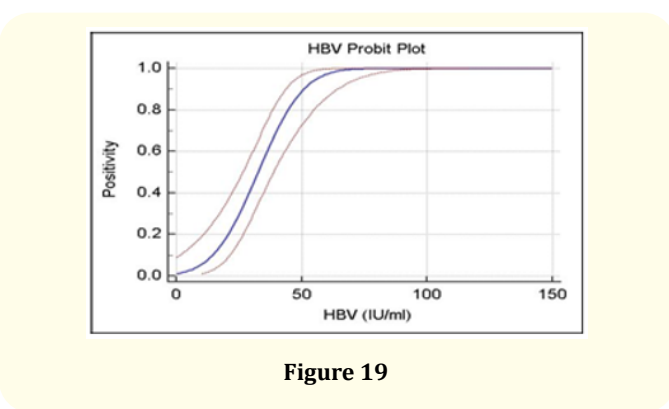


Figure 19

Truenat™ Dengue

Test for the quantitative detection and diagnosis of Dengue virus in human blood/serum/plasma

- Test detects all 4 virus types- DENV1, 2, 3 and 4 - verified with characterized virus isolates from NIV, Pune
- High inter-user, inter-analyzer and inter-lot reproducibility was observed (Average standard deviation= .5Ct)

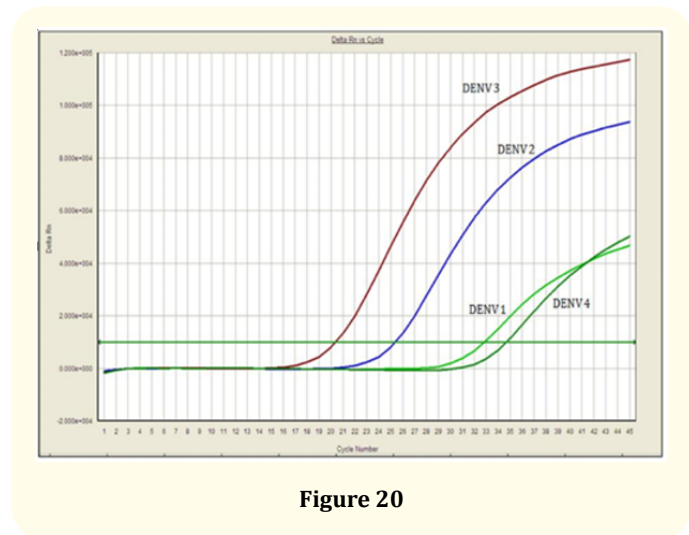


Figure 20

100 serum samples characterized by NS1/IgG/IgM duo ICT assay at a tertiary care hospital in Bangalore were independently tested on Truenat™ Dengue- leading to a sensitivity and specificity of 100% for Truenat™ Dengue.

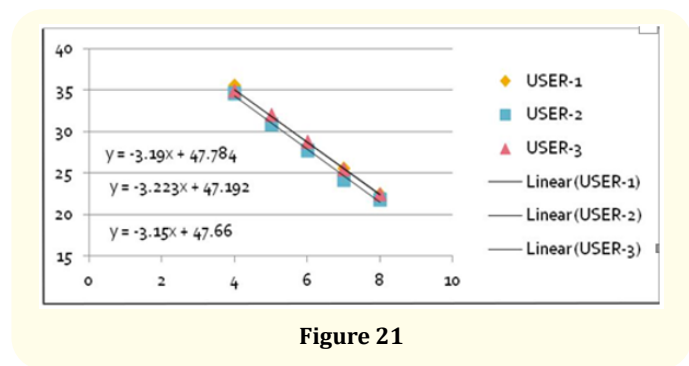


Figure 21

The assay was found to be linear over 5 orders of magnitude and could detect as low as 10 copies of RNA per reaction

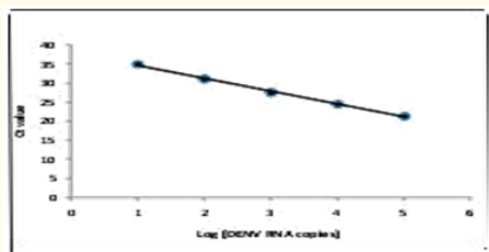


Figure 22

Truenat™ Chikungunya

Test for the quantitative detection and diagnosis of Chikungunya virus in human blood/serum/plasma

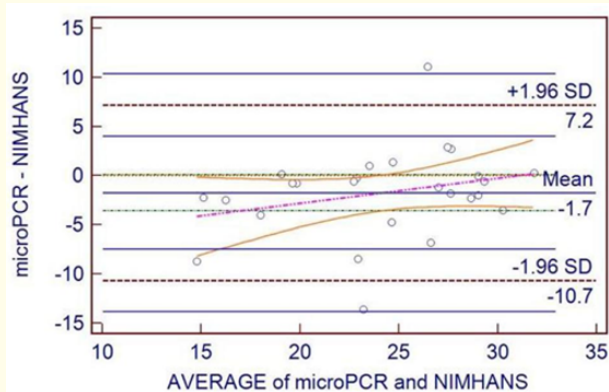


Figure 23

60 serum samples characterized by reference in-house PCR at a tertiary care hospital in Bangalore were independently tested on Truenat™ Chikungunya- leading to a sensitivity and specificity of 100% for Truenat™ Dengue.

The assay was found to be linear over 5 orders of magnitude and could detect as low as 10 copies of RNA per reaction.

High inter-user, inter-analyzer and inter-lot reproducibility was observed (Average standard deviation= 0.8Ct)

Truenat™ Malaria Pf/Pv

Test for the quantitative detection and diagnosis of Plasmodium falciparum and vivax from human blood/serum/plasma

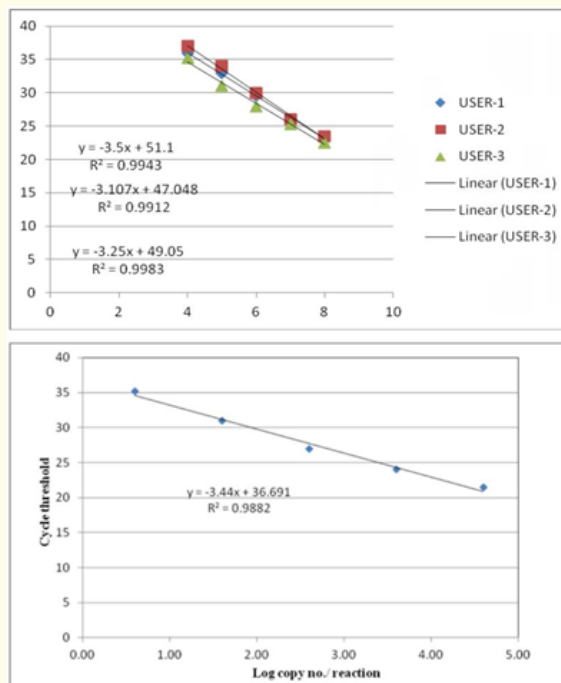


Figure 24

- Quantitative detection of malaria causing parasites - Plasmodium falciparum and vivax
- Analytical study as per CLSI NAT assay validation guidelines: Clinical sensitivity 100% and Clinical specificity 100% when evaluated against WHO nested PCR protocol (WHO SOP 5.8)
- Limit of detection <5 parasites/ul.
- Clinical sensitivity 99.3% (n =301) in study done with NIMR, Bangalore comparing Truenat, microscopy and RDT's. Data submitted for publication

	Truenat Malaria	RDT
Agreement with microscopy positive samples at species level	139 (of 140)	131 (of 140)
Sensitivity (%) using microscopy as reference	99.3 [95.5-99.9]	93.6 [87.8-96.8]

Table 2

Truenat™ H1N1

Chip based Real-Time PCR test for H1N1

Test for the diagnosis of H1N1 virus in human throat and nasal swabs.

The results are in agreement with established Centers for Disease Control and Prevention (CDC) guidelines for H1N1 detection.

Pilot studies

50 throat/nasal swabs were processed and analyzed at NIV, Pune according to CDC guidelines for H1N1 detection. The microPCR sensitivity was found to be 96% and the specificity was found to be 95.6%.

115 throat/nasal swabs were processed by the Department of Virology at NIMHANS, Bangalore. Sensitivity and specificity found to be 97.8 and 100% respectively.

Multi-centric validation over 4 centres in India conducted by ICMR – sensitivity and specificity consistently > 95% in the field.

AP Govt has a Truenat device in every district hospital Now planning 225 such installations for TB elimination.

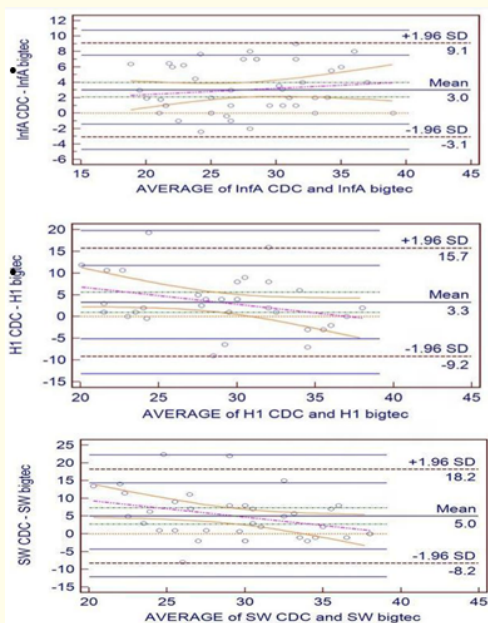


Figure 25

MTB: Methods of Diagnosis

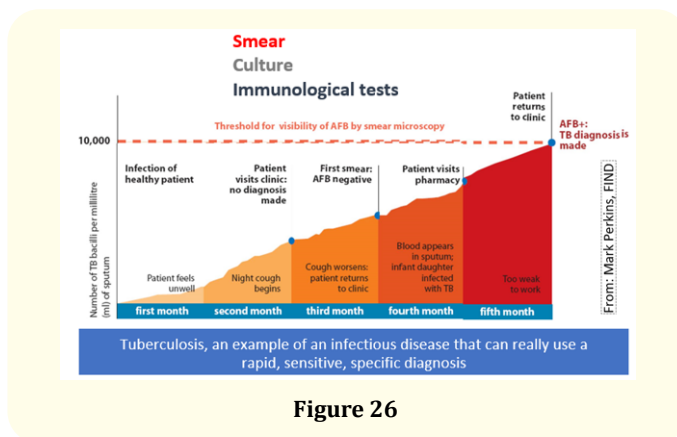


Figure 26

Truenat™ MTB : Clinical evaluations

Study 1: Performed with Hinduja Hospital, published in PLOS one journal

- Sensitivity: S+C+: 99.12 (Archived panel, n=226). Specificity in “Non-TB” cases (based on CRS) = 100% (35/35). More sensitive than in-house reference PCR assay

Truenat vs culture

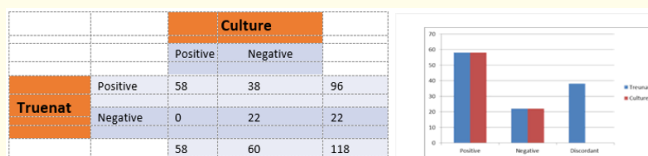


Figure 27

Specificity against Comprehensive Reference Standard = 100% [1]

Truenat™ MTB : Clinical evaluations

Study 2: Performed with Hinduja Hospital, published in International Journal of Mycobacteriology

- Performance equivalent to WHO endorsed Xpert MTB/RIF (96% concordance)

Sensitivity as compared with GeneXpert

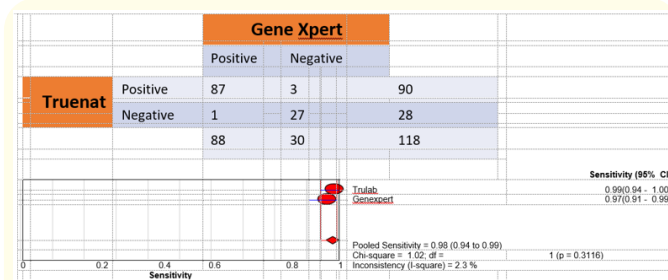


Figure 28

Sensitivity

- GeneXpert: 96.7%(CI:90.6-99.3)
- TruLab: 98.9%(93.8-100.0)
- Pooled sensitivity: 97.8%(94.3-99.4) [2]

Studies with ICMR

Multicentric National Laboratory Studies at NIRT, JALMA, NITRD and AIIMS

- Phase 1 for detection - 4000 samples
- Phase II for RIF Resistance - 2400 samples

In-country field deployment in 10 states, 10 districts per state, 125 workstations (Assam / Tripura from NE)

- 18000 samples

Studies conducted successfully and recommended by ICMR to MoH for appropriate introduction in RNTCP

Enabling Point of care molecular diagnostics

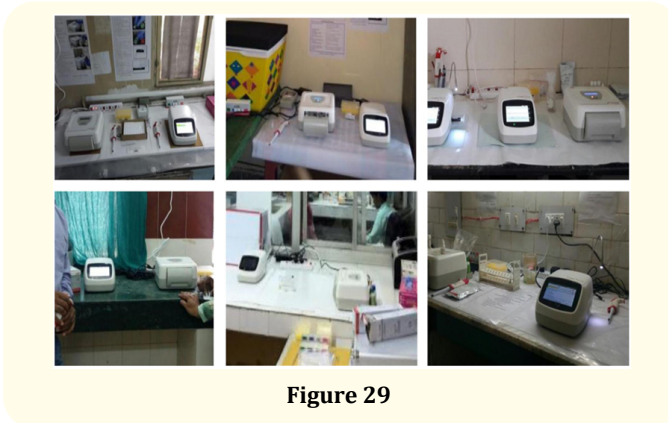


Figure 29

Images from a few of the 100 Designated Microscopy Centers running Truelab for pulmonary TB and RIF diagnostics as part of the ICMR-RNTCP in country study.

The platform has been recommended for use in RNTCP

Live data on TB detection and MDR status

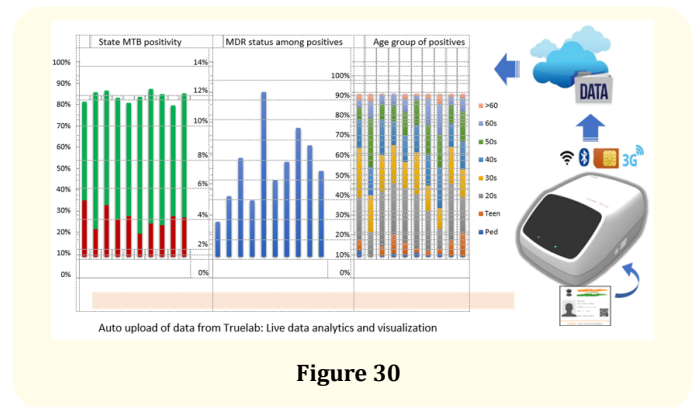


Figure 30

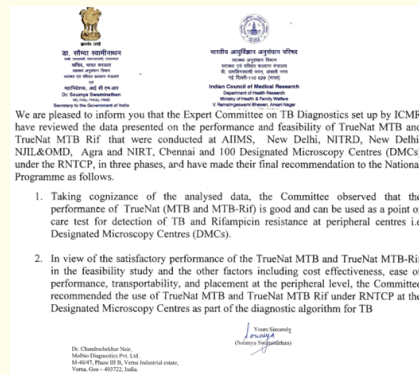


Figure 31

Enhancement of throughput: For high load sites

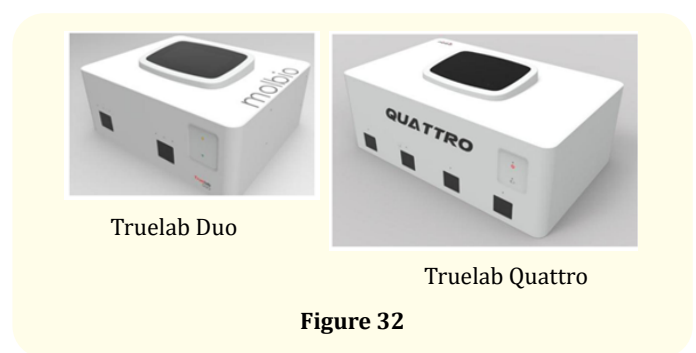


Figure 32

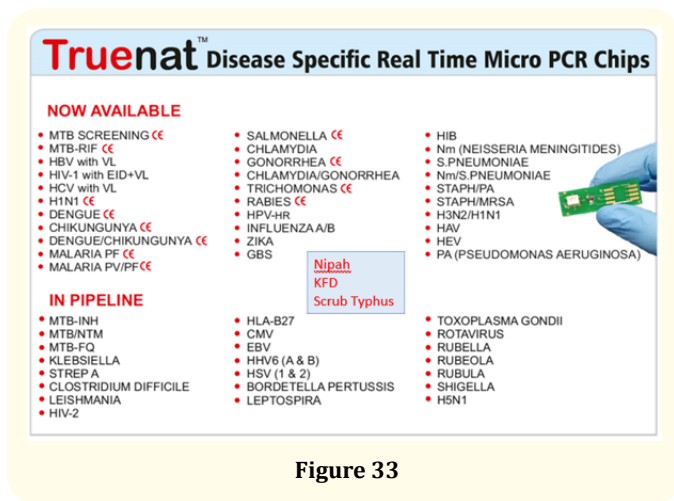


Figure 33

The bottom line

- All tests have their advantages and limitations
- No two patients can be similar
- Nothing can replace the clinical acumen of the treating physician

Laboratory can however be an able partner in diagnosis and treatment.

Bibliography

1. Nikam C., et al. "Rapid Diagnosis of Mycobacterium tuberculosis with Truenat MTB: A Near-Care Approach". *PLoS ONE* 8.1 (2013): e51121.
2. C Nikam., et al. "Evaluation of the Indian TrueNAT micro RT-PCR device with GeneXpert for case detection of pulmonary tuberculosis". *International Journal of Mycobacteriology* (2014).

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