“TB -LAMP: New validation study and its importance to find the “missing millions”

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Introduction

- Tuberculosis- very high morbidity and mortality among infectious disease
- 10 million cases globally in 2018.
- India has the highest burden of TB with ~25% of global TB (~2.8 million new cases/year).

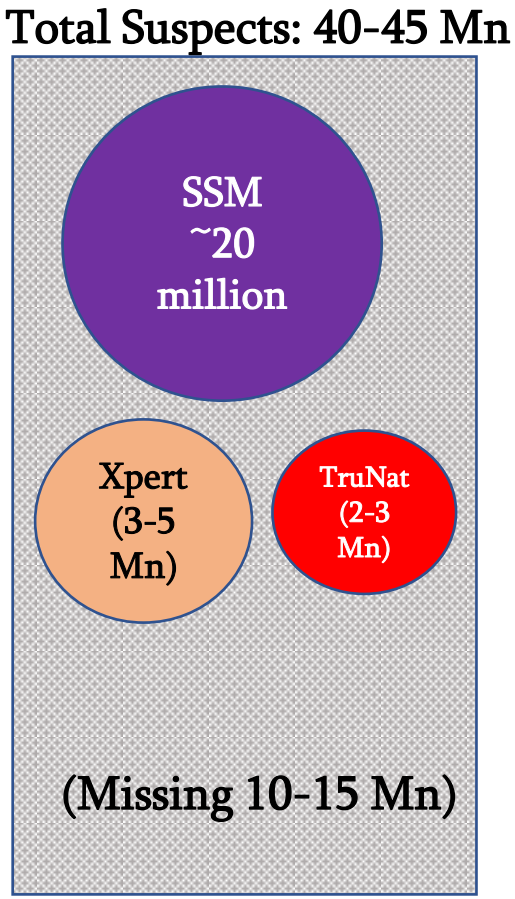
WHO TB Report 2017

- In India, problem is more complex due to
  - Higher incidence
  - Inadequate diagnostic service in difficult to reach areas

Need an accurate diagnostic test which is simple, cost effective, rapid and can be performed at peripheral settings.

The Problem of "Missing the Millions"

- Though, patients enrolled under DOTS program increased, yet it is not reaching to 100% target
- Many people are escaping diagnosis due to insufficient capacity even after instituting ACF campaign
- In 2018, the diagnostic coverage was able to screen ~30 Million suspects
- 15-20 million more suspects which need to be screened for TB than what the Public/Private Program is not able to reach



India's End TB 2025

Which test is actually required for TB diagnosis ?

1. Can be performed at peripheral settings
2. Minimal requirement of training
3. Better or equal sensitivity and specificity than the existing tests at peripheral health settings
4. Rapid and cost effective , simple - help in treatment initiation under TB control programme

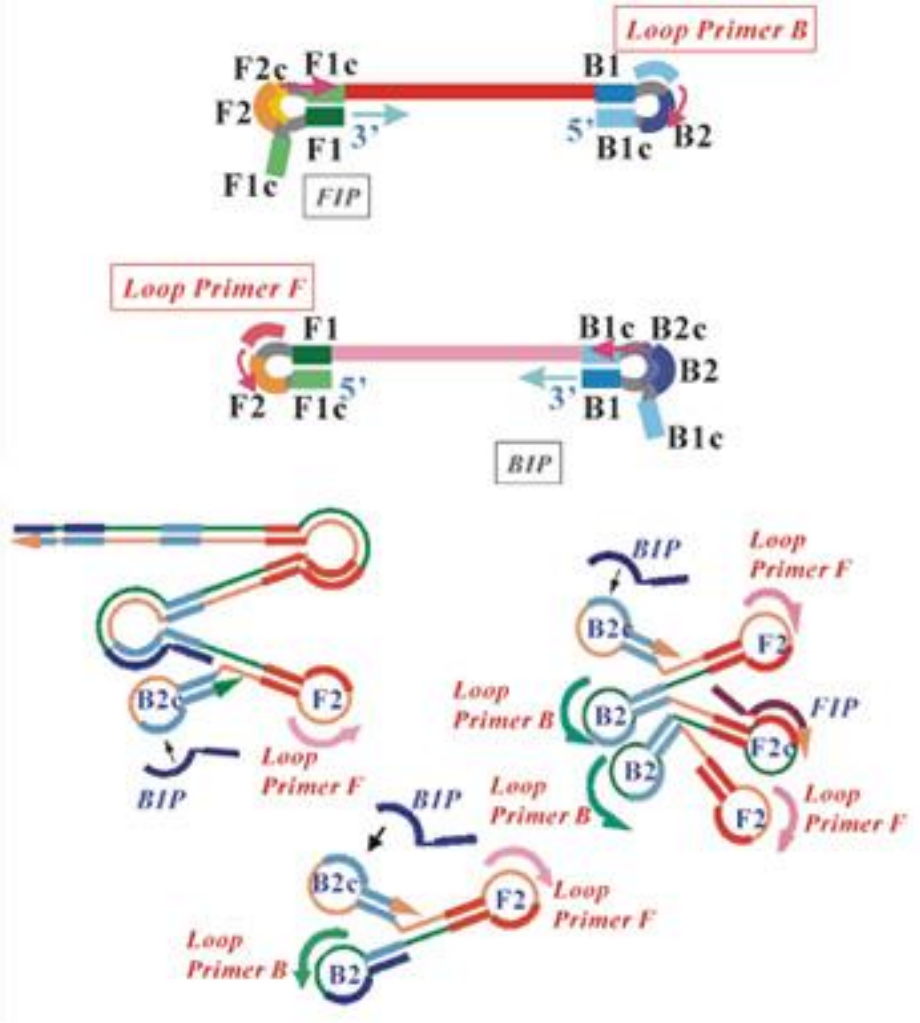
Current TB diagnostics tests

- Smear Microscopy - 50-60% sensitive and misses almost every alternate case
- Culture - 2 to 4 weeks to become positive
- The NAAT's(PCR based) like Xpert MTB/RIF/ True Nat(Mol bio)-
  - Needs a costly infrastructure
  - Trained staff
  - Limited to tertiary and secondary care centers

Test	Sensitivity	Essential Requirements	Penetration	Limitations	Cost / instrument	Cost of Diagnosis
Smear Microscopy	50-60% (LED Microscopy is not widely used)	Sputum Processing, Staining, Manual intervention, (LOD 10000 cfu/ml)	Until the CHC (approx. 20000 DMC) covering ~ 15-20 Million suspects	•Subjective , • tedious sample processing - requires two samples	Rs 1-1.5 lakhs	Rs 75-100/test
GeneXpert	~ 95%	Dedicated infrastructure, cold chain, highly trained manpower (LOD 130 cfu/ml)	At the District level (~ 1500 sites) 3-5 Million suspects	Specialized infrastructure, Transportation of samples , High cost of instrumentation , Recalibrated issue Cost of the test	Rs 15 lakhs	Rs 1100/test (with RIF resistance)
CB NAAT (TruNat)	~ 95%	Cold chain, complex sputum processing, trained manpower (LOD 130-150 cfu/ml)	District level (2-3 Million suspects)	Complex sputum processing, single test per run, samples have to be brought to installation site, cold chain reqt, low thruput (6-8 samples/day)	Rs 6-8 lakhs	Rs 800/test (with RIF resistance)
TB LAMP	~95%	Similar infrastructure as microscopy, minimal trained manpower, no sputum processing (raw sputum can be used directly), high throughput (1 - 60-70 samples/day) (LOD 100 cfu/ml)	As alternative to of Smear microscopy (WHO recommendation), until DMC level (can cover 20-25 Million suspects)	Don't detect RIF resistance	Rs 3 lakhs	Rs 800/test (which will come down to half as volumes build up)

The Proposed Solution: TB-LAMP Assay

- Isothermal amplification Method
- High sensitivity and specificity
- Visible detection of positive samples (through fluorescence)
- No cold chain requirement and can be performed in room temperature conditions



Our Experience

Evaluation of In-House Loop-Mediated Isothermal Amplification (LAMP) Assay for Rapid Diagnosis of *M. tuberculosis* in Pulmonary Specimens

Sethi S et al. JCLA, 2013

	IS*6110*				
Group	Positive	Sn. (95% CI)	Sp. (95% CI)	PPV (95% CI)	NPV (95% CI)
Group A S+C+ (65)	60	92.3 (82.9–97.4)	100 (88.4–100)	100 (93.9–100)	85.7 (69.7–95.1)
Group B S+C– (5)	1	20 (0.5–71.2)	100 (88.4–100)	100 (2.5–100)	88.2 (72.5–96.7)
Group C S–C+ (13)	8	61.5 (31.6–86)	100 (88.4–100)	100 (62.9–100)	85.7 (69.7–95.1)
Group D S–C– (20)	5	25 (8.17–49.1)	100 (88.4–100)	100 (47.9–100)	66.7 (51–79.9)

	LAMP				
Group	Positive	Sn. (95% CI)	Sp. (95% CI)	PPV (95% CI)	NPV (95% CI)
Group A S+C+ (65)	64	98.4 (91.7–99.7)	100 (88.4–100)	100 (94.3–100)	96.7 (83.2–99.5)
Group B S+C– (5)	3	60 (15.4–93.5)	100 (88.4–100)	100 (30.5–100)	93.8 (79.2–99.5)
Group C S–C+ (13)	10	76.9 (46.2–94.7)	100 (88.4–100)	100 (68.9–100)	90.9 (75.6–97.98)
Group D S–C– (20)	10	50 (27.2–72.7)	100 (88.4–100)	100 (68.9–100)	75 (58.8–87.2)

First Indian Study on in-house TB-LAMP assay

Combination of adenosine-deaminase and nucleic acid amplification assays for diagnosing tuberculous pleural effusion

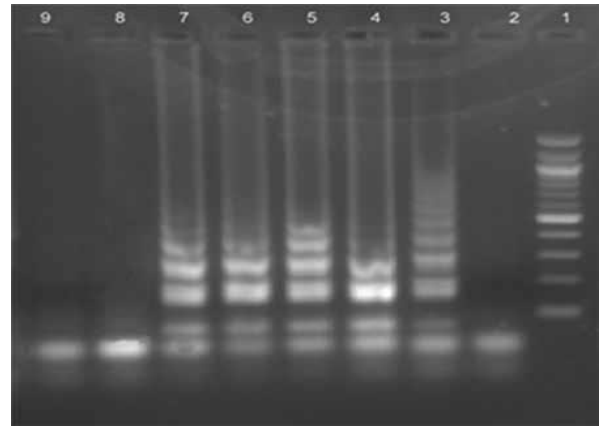
Sethi S et. al. J Infect. 2014

Patient group	ADA % (95% CI)	LAMP % (95% CI)	PCR % (95% CI)	Culture % (95% CI)	Smear (95% CI)
Confirmed TPE (31)	25	17	12	15	4
Sensitivity	80.6 (62.5–92.5)	58.4 (36–72.7)	38.7 (21.9–57.8)	48.4 (30.2–66.9)	12.9 (3.7–29.8
Specificity	71.4 (41.9–91.4)	100 (88.7–100)	100 (88.7–100)	100 (88.7–100)	100 (76.7–100)
PPV	86.2 (68.3–96)	100 (80.3–100)	100 (73.4–100)	100 (78–100)	100 (40.2–100)
NPV	52.5 (35.5–84.7)	50 (30.6–69.3)	42.4 (25.5–60.8)	46.7 (50.7–79.1)	34.1 (20.1–50.6)
Probable TPE (61)	42	18	8	0	0
Sensitivity	68.8 (55.7–80)	29.5 (18.5–42.6)	13.1 (5.85–24.2)	–	–
Specificity	71.4 (41.9–91.4)	100 (76.7–100)	100 (76.7–100)	–	–
PPV	91.1 (78.8–97.5)	100 (81.3–100)	100 (62.9–100)	–	–
NPV	34.4 (17.9–54.3)	24.6 (14.1–37.7)	20.9 (11.9–32.6)	–	–
Total (92)	67	35	20	15	4
Sensitivity	72.8 (62.5–81.6)	38 (28.1–48.7)	21.7 (13.8–31.6)	16.3 (9.4–25.5)	4.3 (1.2–10.7)
Specificity	71.4 (41.9–91.4)	100 (76.7–100)	100 (76.7–100)	100 (76.7–100)	100 (76.7–100)
PPV	94.3 (86.2–98.4)	100 (89.9–100)	100 (83–100)	100 (78–100)	100 (40.2–100)
NPV	28.6 (14.7–46.3)	19.7 (11.2–30.8)	16.3 (9.2–25.8)	15.4 (8.7–24.5	13.7 (7.7–21.9)

Loop-mediated isothermal amplification assay for detection of *Mycobacterium tuberculosis* complex in infertile women

Sethi S et al. Indian J Med Microbiol. 2016

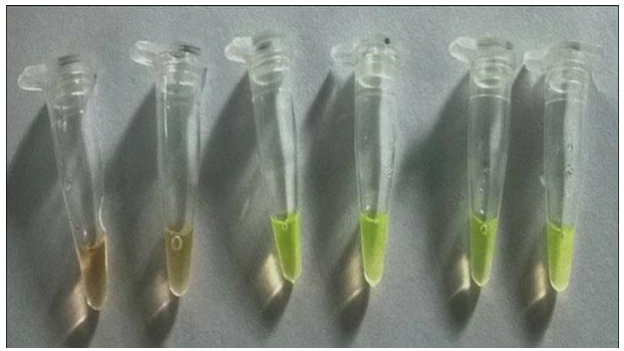
- Sensitivities of ZN smear, culture, HPE, PCR and LAMP were 2.94%, 10.29%,8.82%, 95.59% and 66.18%, respectively.
- Concordance between PCR and LAMP was 63%, which shows a good agreement.



A loop-mediated isothermal amplification assay for the diagnosis of pulmonary tuberculosis

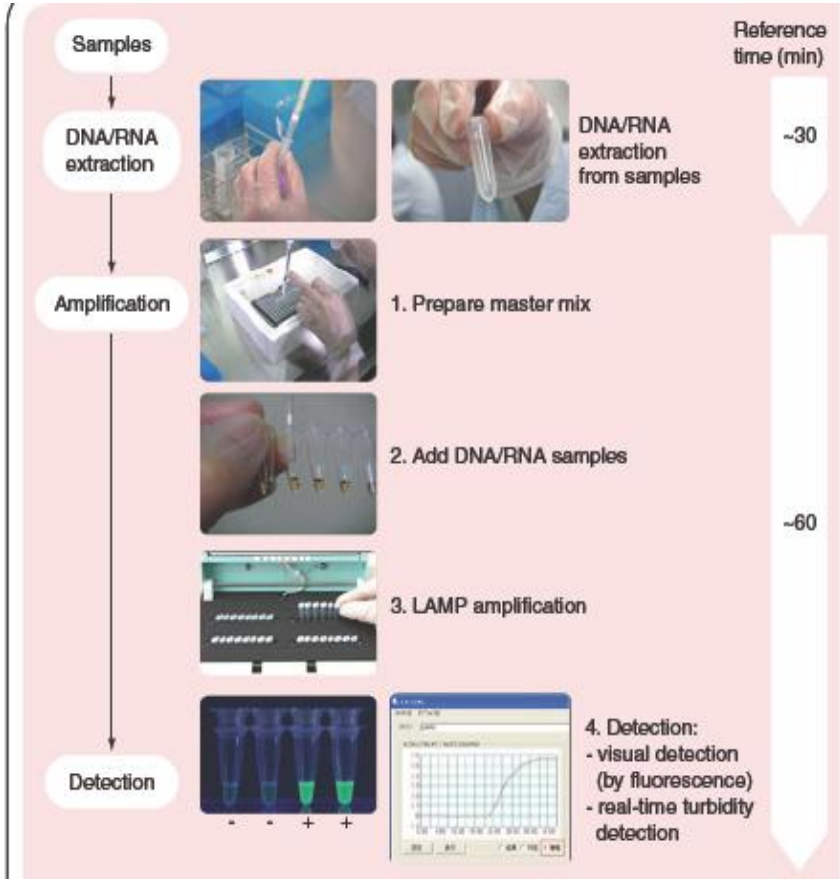
Lett Appl Microbiol. 2019

- A LAMP assay based on the mpt64 gene sequence was developed
- Analytical Sensitivity of 1 pg per ml (or 200 copies of M.tb genome) in a 40-min reaction



The WHO Endorsed TB-LAMP Assay (Eiken, Japan)

- TB-LAMP (Eiken, Japan) – WHO endorsed in 2016
- Based on isothermal amplification and strand displacement principle, no requirement of costly PCR based instrument
- High sensitivity (>95%) and specificity (~98%)- (LOD of 100 CFU/ml)
- 15% more patients than Smear Microscopy, 40% additional smear negative cases- Can replace or add on test to sm microscopy.
- High throughput- 14 samples in 1.5 hours, easy to perform



TB-LAMP Workflow – Quick and Reliable!

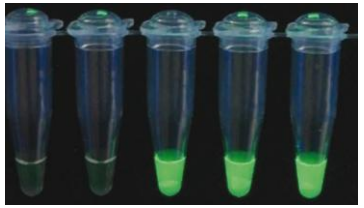
1. Sample transfer & lysis
2. Pure DNA extraction
3. Reaction mix preparation
4. LAMP reaction

Result reading









Time to result approx.1-1,5h
16 tests/run

How TB-LAMP will help in diagnosis of Missing millions

- Can reach up to CHC level, making it the only option which gives fast and accurate diagnosis
- Through battery/solar power, can reach to even remote areas (thus making access/sample collection easy)
- Increase of scale will reduce the cost/test to < Rs 600/test, much lower than any other similar test

TB-LAMP Test Performance -WHO Policy Guideline 2016

PURE-LAMP-TB	N	Sensitivity Smear +	Sensitivity Smear-	Specificity (Culture-)	Treatment Status
Ou et al. (2014)[1]	1329	92.1% (152/165)	53.8% (113/210)	98.3% (938/954)	Before (spot sputum)
		88.8% (333/375)		96.8% (924/954)	Before (spot/morning/night sputum)
Kaku et al. (2016) [2]	472	99.1% (113/114)	52.1% (21/41)	98.4% (312/317)	Before (sample analysis)
	209	100% (47/47)	56.5% (13/23)	97.8% (136/139)	Before (patient analysis)
Gray et al. (2016)[3]	1745	97.2% (243/250)	62 % (88/142)	96.6% (1307/1353)	Before
Bojang et al. (2016)[4]	261	100%	90.3%	100% (Smear+); 99% (Smear-)	Before
	156	100%	71.3%	63% (Smear+); 93% (Smear-)	Follow up

Gray et al. study featured 2 Indian sites – PGIMER, Chandigarh and MGIMS, Wardha

TB-LAMP Test Performance -WHO Policy Guideline 2016

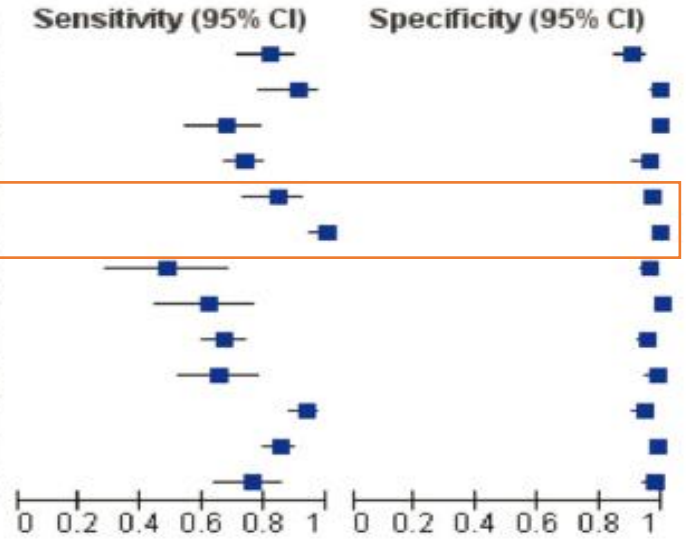
Table 3. TB-LAMP as a replacement test for smear microscopy: Eligible and included patients according to reference standard and study site.

Study	Total	Eligible[2]	Included		
			Standard 1[1]	Standard 2[1]	Standard 3[1]
Brazil (EVAL)	266	239	237 (99%)	237 (99%)	237 (99%)
Peru (EVAL)	199	198	198 (100%)	198 (100%)	198 (100%)
South Africa (EVAL)	259	240	237 (99%)	237 (99%)	238 (99%)
Vietnam (EVAL)	312	304	304 (100%)	304 (100%)	304 (100%)
India (DEMO)	619	598	–	559 (94%)	586 (98%)
India (RFA)	530	504	–	–	446 (89%)

Pooled sensitivity-77-80%
Specificity-97-98%

Standard 3

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Brazil (Eval)	62	16	14	145	0.82 [0.71, 0.90]	0.90 [0.84, 0.94]
Peru (Eval)	39	1	4	154	0.91 [0.78, 0.97]	0.99 [0.96, 1.00]
South Africa (Eval)	42	1	20	175	0.68 [0.55, 0.79]	0.99 [0.97, 1.00]
Vietnam (Eval)	149	4	53	98	0.74 [0.67, 0.80]	0.96 [0.90, 0.99]
India (Demo)	53	15	10	508	0.84 [0.73, 0.92]	0.97 [0.95, 0.98]
India (RFA)	65	3	0	378	1.00 [0.94, 1.00]	0.99 [0.98, 1.00]
Vietnam (RFA)	13	14	14	320	0.48 [0.29, 0.68]	0.96 [0.93, 0.98]
Malawi (RFA)	24	0	15	195	0.62 [0.45, 0.77]	1.00 [0.98, 1.00]
Tanzania (RFA)	122	23	60	427	0.67 [0.60, 0.74]	0.95 [0.92, 0.97]
Uganda (RFA)	38	2	20	130	0.66 [0.52, 0.78]	0.98 [0.95, 1.00]
Ivory Coast (RFA)	140	18	10	283	0.93 [0.88, 0.97]	0.94 [0.91, 0.96]
Madagascar (RFA)	161	6	28	321	0.85 [0.79, 0.90]	0.98 [0.96, 0.99]
Haiti (Unpublished)	50	3	16	134	0.76 [0.64, 0.85]	0.98 [0.94, 1.00]



1) two negative cultures on two different sputum specimens (Standard 1); 2) two negative cultures on the same or different sputum specimens (Standard 2); or 3) at least one negative culture (Standard 3).

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Evaluation of the TB-LAMP assay for the rapid diagnosis of pulmonary tuberculosis in Northern India

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Table 1 Performance of diagnostic tests for the detection of *Mycobacterium tuberculosis*

	Sensitivity % (95%CI)	Specificity % (95%CI)	PPV % (95%CI)	NPV % (95%CI)
TB-LAMP	100 (94.8–100)	99.2 (97.8–99.8)	95.83 (88.3–99.1)	100.00 (99–100)
Xpert® MTB/RIF	75 (63.7–84.2)	96.8 (94.5–98.34)	82.6 (71.5–90.6)	95.05 (92.4–97)

CI = confidence interval; PPV = positive predictive value; NPV = negative predictive value.

Determination of diagnostic accuracy of TB - LAMP assay in pulmonary tuberculosis patients from North India

- Total samples(n)= 234
- 120 TB suspects+ 117 pulmonary disease(other than TB)

Group	Name of the Test	Sensitivity	Specificity	Positive predictive value	Negative Predicative Value
Overall	Xpert MTB/RIF	98.1% (89.7-99.9%)	97.2 %(93.1-99.2%)	92.7%(82.9-97.1%)	99.3 % (97.8-99.8%)
	TB LAMP	94.2% (84.1-98.8%)	96.6% (92.1-98.9%)	90.7% (80.5-95.9%)	97.9% (93.9-99.3%)
No TB cases	Xpert MTB/RIF	-	99.2% (95.4-99.98%)	-	-
	TB LAMP	-	99.2%(95.4-99.98%)	-	-

Unpublished data

TB-LAMP assay for diagnosis of pediatric tuberculosis: A prospective cohort study

- Respiratory samples(n)= 187
- (GA/GL/BAL/Sputum)

	Sensitivity	**Specificity**	**PPV**	**NPV**
TB-LAMP	80% (95% CI, 59.3-93.2%)	96.1% (95% CI, 91.6-98.5%)	76.9%(95% CI, 59.8-88.2%)	96.7%(95% CI, 93-98.5%)
Xpert MTB/RIF ultra	84%(95% CI, 63.9-95.5%)	97.4% (95% CI, 93.4-99.3%)	84%(95% CI, 66.3-93.3%)	97.3%(95% CI, 93.7-98.9%),

Unpublished data

TB-LAMP is part of WHO EDL

World Health Organization

List of Essential In Vitro Diagnostics (EDL)

The first edition of the EDL is presented by health care facility level in two tiers:

I Primary health care; with section a for general IVDs; and section b for specific diseases

II Health care facilities with clinical laboratories, with section a for general IVDs; and section b for specific diseases,

As follows:

I List of Essential In Vitro Diagnostics (EDL): For primary health care

Includes IVDs for health posts, community health centres, doctors' offices, outreach clinics and ambulatory care.
Typically, self-testing and rapid diagnostics tests are available, but there are either no laboratories, or only small laboratories with trained health care personnel but no trained laboratory technicians.
In case laboratory facilities are available in a primary health care facility, please refer to the IVDs described in the next tier.
It should be noted that in some cases sampling can take place where there are no laboratories, and then processed in the next tier.

I.a General IVDs for primary health care
Note: See list of WHO supporting documents at the end.

	Diagnostic test	Test purpose	Assay format	Specimen type
i)	Tuberculosis	Smear for AFB	Sputum, CSF or any other specimen	Manual
		Capillary- based Nucleic Acid Amplification Test	Sputum	PCR
		Mantoux	Skin test	Manual
		TB LAMP test	Sputum	LAMP
		TB culture (liquid)	Sputum, fluid etc.	Automated
		TB DST (liquid)	Sputum, fluid etc.	Automated

Still need high quality studies under programmatic conditions in India at peripheral level

"Multi-centric validation of WHO approved TB-LAMP assay for detection of *Mycobacterium tuberculosis* in suspects of Pulmonary tuberculosis in peripheral settings"

Objectives of the study-

1. To elucidate the diagnostic accuracy of TB-LAMP assay in sputum samples of suspected tuberculosis patients at peripheral centres in different pats of India

2. To assess the feasibility of TB-LAMP assay at the peripheral level for diagnosis of tuberculosis.

Methodology

- A total of 2400 TB suspects will be enrolled at 5 peripheral sites in different states.

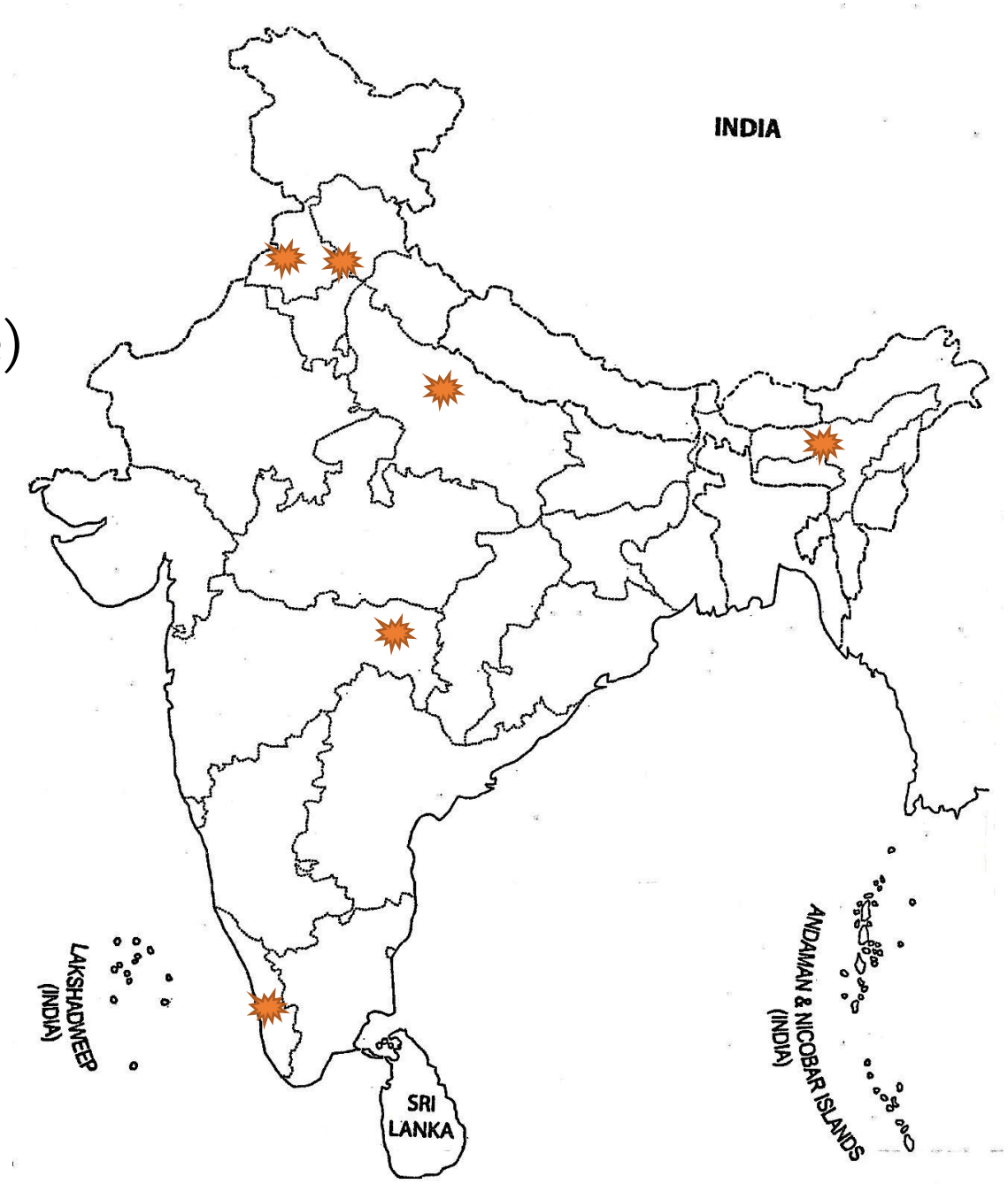
(The sample size was calculated taking the sensitivity of 78% and specificity of 98% with a desired precision of 7% at 95% confidence interval. Using these parameters, the sample size is 2251. If we took 5% of sample loss during the study the total sample size would be 2364. so we are taking a sample size of 2400, and will be divided into equal for each site)

- The samples will be tested as per standard protocols (as per WHO guidelines) for

  - Smear Microscopy
  - Liquid Culture
  - Gene Xpert
  - TB-LAMP

Participating Sites

- PGIMER, Chandigarh (Parent Institute)
- MGIMS, Wardha
- Lucknow
- Guwahati, Assam
- Kochi, Kerala
- Punjab



Level of Training required

- TB-LAMP can be operated by a minimally trained technician – could be paramedic, lab technician or a physician.
- Such people need to be trained on sputum sample collection and handling in a bio-safety environment
- 3-day hands-on training which will be imparted by the PGIMER on the use of TB LAMP and running the test

Thanks