LOOP-MEDIATED ISOTHERMAL AMPLIFICATION ASSAY TO DIAGNOSE TUBERCULOSIS IN RURAL UGANDA

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ABSTRACT

Background*: The loop-mediated isothermal amplification assay (TB-LAMP) can be staged with minimal infrastructure and is rapid, low cost and detection can be with the naked eye. We assessed feasibility and accuracy of Eiken TB-LAMP test in TB diagnosis in a high prevalence TB/HIV rural setting in Uganda.*

Setting: *Kiboga District where smear microscopy is the available routine diagnostic option.*

Methods: *TB-LAMP test was performed on sputum specimens from outpatient presumptive TB adults at the district hospital and two low-level health centers . A simple room without biosafety cabinet set up at Kiboga hospital was used. MTB sputum cultures were used as reference.*

Results*: Of the 233 presumptive TB (126 hospital; 107 low-level health centers); 113 (48.5%) HIV-infected; 55% male; median age 40 (IQR 30-53). Compared to MTB culture, overall sensitivity and specificity of TB-LAMP were 55.4% (95% CI 44.1-66.3) and 98.0% (95% CI 94.3-99.6) respectively. Among HIV-infected participants, TB-LAMP sensitivity and specificity were 52.3% (95% CI 36.7-67.5) and 97.1% (95% CI 89.9-99.6) respectively. Among smear-negatives overall, TB-LAMP sensitivity and specificity were 26.5% (95% CI 14.9-41.1) and 98.6% (95.2-99.8) and 33.3% (95% CI 17.3-52.8) and 98.5 (95% CI (92.1-100) respectively in HIV-infected.*

Conclusions: *In this high HIV/TB prevalence rural setting, TB-LAMP performs better than conventional smear microscopy in the diagnosis of MTB among presumptive TB patients. TB-LAMP can easily be performed following a short training period and in the absence of sophisticated infrastructure and expertise.*

BACKGROUND

TB is underdiagnosed in most resource-limited settings (RLS) due to lack of inexpensive and accurate diagnostic methods and threatens global TB control[1,2].

Smear microscopy remains the main method for diagnosis in most high prevalence TB/HIV RLS despite its low sensitivity. Rapid tests with better sensitivity such as Xpert MTB/RIF can only be implemented at sites with electricity or solar panel infrastructure[3].

TB-LAMP molecular method can be staged with minimal infrastructure, is rapid, low cost and detection can be with the naked eye[4]. TB-LAMP could be a true point-of-need molecular technique at peripheral laboratories.

AIM

We sought to determine feasibility and accuracy in comparison to liquid and solid MTB cultures of the commercial Eiken TB-LAMP test performed in a rural peripheral setting in Uganda with high rates of TB and HIV co-infection.

METHODS

Study Site: Kiboga hospital and 2 low-level health centers (Bukomero HC III and Ntwetwe HC III) in Kiboga district

Study period: October 2013 -February 2014

Study Design: A cross-sectional diagnostic study

Participants: Clinically suspected to have TB (presumptive TB) were;

- ≥ 18 years old
- Adequate left-over sputum from 2 sputum specimens submitted to the lab for TB testing (Z-N or FM) during routine care
- Not on TB treatment for ≥ 2 days in past 60 days

METHODS

Study Procedures

- Left-over spontaneously expectorated sputum specimens submitted to the lab for routine TB testing were used (2 spot specimens are routinely collected)
- Study tests including TB-LAMP, Xpert MTB/Rif test, MGIT & LJ MTB cultures performed on the left-over sputum specimens
- Species identification on all TB positive cultures performed
- All TB-LAMP testing was performed at Kiboga hospital by a technician without prior experience in the technology after a week of training
- A simple room without biosafety cabinet prepared by technician used for TB-LAMP testing
- TB suspect form with de-identified clinical information: TB symptoms, socio-demographics and medical history were filled
- Routine smear-microscopy TB test results were recorded and linked to results of the study tests

Data management and statistical methods

- *Data handling was by DataFax system at IDI and analysis was by use of STATA Version 12.0*
- *Accuracy of the Eiken TB-LAMP test in the diagnosis of TB among presumptive TB patients, was calculated using LJ and MGIT MTB cultures (and Xpert MTB/Rif) as reference standard*
- *Sensitivity, specificity, predictive values and likelihood ratios of TB-LAMP were calculated*

RESULTS

Study participants characteristics

- 233 were eligible; 100% outpatients, 44.6% females, median age 40 years(IQR 30–53), 9.9% had previous TB treatment **(Figure 1)**
- 48.5% (113/233) were HIV-infected with 55.8% taking ART
- Overall smear microscopy positivity rate was 18.4%: 17.5% for Kiboga Hospital and 19.6% for the low-level health centers
- 83/233 (35.6%) had ≥ one positive MTB cultures for M. tuberculosis complex by MGIT and/or LJ TB culture
- TB-LAMP was positive in 46 (55.4%) of the 83 MTB culture-positive participants

Table 1: **Diagnostic accuracy of TB-LAMP test in the diagnosis of PTB using MTB culture (and Xpert MTB/Rif) as reference**

Population	Accuracy Index	TB-LAMP accuracy % (95% CI)	
		LJ and /or MGIT MTB as reference standard	MTB culture and GeneXpert as reference
Overall accuracy, N=233	Sensitivity	46/83, 55.4 (44.1-66.3)	47/95, 49.5 (39.1-59.9)
	Specificity	147/150, 98.0 (94.3-99.6)	136/138, 98.6 (94.9-99.8)
	PPV	46/49, 93.9 (83.1-98.7)	47/49, 95.9 (86.0-99.5)
	NPV	147/184, 79.9 (73.4-85.4)	136/184, 73.9 (66.9-80.1)
Smear-negatives, N=197	Sensitivity	13/49, 26.5 (14.9-41.1)	13/60, 21.7(12.1-34.2)
	Specificity	146/148, 98.6 (95.2-99.8)	135/137, 98.5 (94.8-99.8)
HIV-infected, N=113	Sensitivity	23/44, 52.3 (36.7-67.5)	24/50, 48.0 (33.7-62.6)
	Specificity	67/69, 97.1 (89.9-99.6)	62/63, 98.4 (91.5-100)
Smear-negative HIV-infected, N=98	Sensitivity	10/30, 33.3(17.3-52.8)	10/35, 28.6 (14.6-46.3)
	Specificity	67/68, 98.5 (92.1-100)	62/63, 98.4 (91.5-100)
Hospital, N=126	Sensitivity	23/37, 62.2% (44.8-77.5)	23/42, 54.8 (38.7-70.2)
	Specificity	87/89, 97.8% (92.1-99.7)	82/84, 97.6 (91.7-99.7)
Health Centre IV, N=107	Sensitivity	23/46, 50.0% (34.9-65.1)	24/53, 45.3 (31.6-59.6)
	Specificity	60/61, 98.4% (91.2-100)	54/54, 100 (93.4-100)

Fig 1: Participant flow diagram

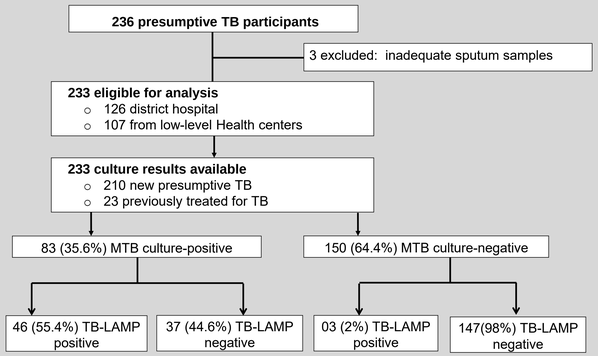


Table 2: **Diagnostic accuracy of TB-LAMP, smear microscopy & Xpert MTB/Rif test in PTB diagnosis using MTB culture as reference**

	LAMP accuracy (95% CI)	FM/ZN accuracy (95% CI)	Xpert MTB/Rif test (95% CI)
Sensitivity	**46/83, 55.4 (44.1-66.3)**	**34/83, 41.0 (30.3-52.3)**	**54/83, 65.1 (53.8-75.2)**
Specificity	147/150, 98.0 (94.3-99.6)	148/150, 98.7 (95.3-99.8)	138/150, 92.0 (86.4-95.8)
PPV	46/49, 93.9 (83.1-98.7)	34/36, 94.4 (81.3-99.3)	54/66, 81.8 (70.4-90.2)
NPV	147/184, 79.9 (73.4-85.4)	148/197, 75.1 (68.5- 81.0)	138/167, 82.6 (76.0-88.1)

SUMMARY OF RESULTS

- 48.5% of the study population was HIV-infected, 55.8% taking ART
- TB-LAMP sensitivity & specificity were 55.4% (44.1-66.3) & 98.0% (94.3-99.6) respectively overall, and 52.3 % (36.7-67.5) & 97.1% (89.9-99.6) respectively among HIV-infected **(Table 1)**
- Among smear negatives, sensitivity & specificity were 26.5% (14.9-41.1) & 98.6% (95.2-99.8) overall and 33.3% (17.3-52.8) & 98.5% (92.1-100) in HIV-infected.
- TB-LAMP had lower sensitivity (50%) in low-level health centers when compared to hospital setting (62.2%)
- TB-LAMP sensitivity was higher than that of smear microscopy (p=0.0013) but lower than that of Xpert MTB/Rif test (p=0.0114, McNemar's test) **(Table 2)**

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

- TB-LAMP performs better than conventional smear microscopy in TB diagnosis
- Overall sensitivity of TB-LAMP is lower than that reported by WHO (77.7%)
  - ✓ May be due to a higher HIV prevalence in our study
- Lower sensitivity of TB-LAMP at the low-level health centers compared to the hospital setting:
  - ✓ Probably due to sample transportation from low-level health centers to Kiboga hospital for TB LAMP testing which could have lowered the quality of samples

Recommendation

- In this rural setting, TB-LAMP can easily be performed following a short training period and in the absence of sophisticated infrastructure and expertise

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