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Complete List of Authors:	Olbrich, Laura; Ludwig Maximilians University Munich, Division of Infectious Diseases and Tropical Medicine, University Hospital, LMU Munich; University of Oxford, Department of Paediatrics Khambati, Nisreen; University of Oxford, Department of Paediatrics Bijker, Else Margreet; University of Oxford, Department of Paediatrics Ruhwald, Morten; FIND Heinrich, Nobert; Ludwig-Maximilians-Universitat Munchen, Division of Infectious Diseases and Tropical Medicine, University Hospital, LMU Munich Song, Rinn; University of Oxford, Department of Paediatrics; Boston Children's Hospital
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FujiLAM for the diagnosis of childhood tuberculosis: A systematic review

Abbreviated & running title: FujiLAM for diagnosis of childhood TB

Authors and Affiliations

Laura Olbrich, MD * [1, 2, 3]; Nisreen Khambati, MD * [3]; Else Margreet Bijker, MD PhD [3], Morten Ruhwald, MD PhD [4], Norbert Heinrich, MD 1, [2], Rinn Song, MD [3, 5]

* Joint first author

1 Division of Infectious Diseases and Tropical Medicine, University Hospital, LMU Munich, Germany;

2 German Centre for Infection Research (DZIF), Partner Site Munich, Munich, Germany;

3 Oxford Vaccine Group, Department of Paediatrics, University of Oxford, Oxford, UK

4 FIND, the Global Alliance for Diagnostics. Chemin des Mines 9, 1201 Geneva, Switzerland

5 Division of Infectious Diseases, Boston Children's Hospital, Boston, Massachusetts, USA)

Key words

Childhood TB, FujiLAM, lipoarabinomannan, diagnosis

Corresponding Author

LMU Klinikum, Division of Infectious Diseases and Tropical Medicine

Leopoldstraße 5, 80802 Munich, Germany

Phone +49 89 4400-59803, Fax +49 89 336038

olbrich@lrz.uni-muenchen.de

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Conflicts

MR is employed by FIND, the Global Alliance for Diagnostics. FIND is a not-for-profit NGO that collaborates in partnerships to develop, evaluate and implement new diagnostics for LMIC. FIND has product evaluation agreements with FujiFilm and several other private sector companies that design diagnostics and related products for treatment of tuberculosis and other diseases. These agreements strictly define FIND's independence and neutrality vis-à-vis the companies whose products get evaluated and describe roles and responsibilities.

“What is already known on this topic”

- Despite recent advances, paediatric TB remains difficult to diagnose and accurate point-of-care tests that use easily obtainable non-sputum specimens are urgently needed.
- Lateral flow tests detecting urine lipoarabinomannan (LAM), including the original AlereLAM and the recently developed FujiLAM, could improve diagnosis in children in low-resource settings.
- FujiLAM’s analytic sensitivity for the diagnosis of pulmonary TB was found to be higher compared to AlereLAM in adults.

“What this study adds”

- Compared to AlereLAM, FujiLAM has a moderate but superior diagnostic sensitivity in diagnosing childhood TB whilst maintaining a high specificity.
- Gaps in studies and points to be addressed in forthcoming evaluations are emphasised, including subgroup analyses, prospective testing, and application of rigorous reference standards.

“How this study might affect research, practice or policy”

- Whilst more paediatric studies are needed, high specificity and use of an easy-to-obtain specimen indicates that FujiLAM could be a useful rule-in test for TB

ABSTRACT

Background

Childhood tuberculosis (TB) remains underdiagnosed. The novel lateral flow FujiLAM assay detects lipoarabinomannan (LAM) in urine, but data on performance in children remain limited.

Methods

We conducted a systematic review assessing the diagnostic performance of FujiLAM for diagnosing paediatric TB, using AlereLAM as a comparator. The last search was conducted in November 2021.

Results

We included three studies with data from 698 children for FujiLAM and 619 for AlereLAM. For FujiLAM, pooled sensitivity and specificity using a microbiological reference standard (MRS) were 51% (95%CI 43-59) and 87% (95%CI 84-90), respectively, and 27% (95%CI 23-32) and 87% (95%CI 82-90) using a composite reference standard (CRS). For AlereLAM, sensitivity and specificity were 41% (95%CI 33-50) and 83% (95%CI 79-86) for MRS, and 32% (95%CI 27-37) and 88% (95%CI 84-92) for CRS. Subgroup analyses for FujiLAM suggested an increased sensitivity in children living with HIV, especially when immunocompromised. Meta-analysis was not performed due to considerable study heterogeneity.

Conclusion

This systematic review demonstrates that FujiLAM in children has a moderate but potentially superior sensitivity compared to AlereLAM, whilst still maintaining a high specificity. Although the small number of studies in children and overlapping confidence intervals between the two tests highlight that further data is needed, as an instrument-free point-of-care test that uses an easy-to-obtain specimen, FujiLAM has potential to improve TB diagnosis in children in low-resource settings. This review emphasises the points to be addressed in forthcoming paediatric evaluations, including the application of rigorous reference standards and specific subgroup analysis, to characterise its performance in different geographical locations and disease phenotypes.

INTRODUCTION

Childhood tuberculosis (TB) is a major contributor to morbidity and mortality worldwide (1). The very young are disproportionally affected in case load and mortality, contributing to approximately 50% of all paediatric TB cases (2) and 80% of deaths (2, 3). The burden and mortality of paediatric TB are likely underestimated, as confirmation of disease remains challenging. There is an unmet need for accurate and easy-to-use diagnostic tests for children.

The World Health Organisation (WHO) has defined target product profiles (TPP) for new non-sputum-based point-of-care (POC) diagnostics for TB in children(4). Promising candidates include lateral flow assays detecting lipoarabinomannan (LAM), a glycolipid found in the mycobacterial cell-wall, secreted in urine. The first commercially available test was the Alere Determine TB LAM Ag (AlereLAM; Abbott, Palatine, IL, USA), which is the only instrument-free POC LAM test currently recommended by the WHO (5, 6). According to a systematic review in adults, pooled sensitivity of the AlereLAM is 42% (7) and increases to 54% in PLHIV with CD4 ≤100 cells/μL (7, 8). Recently, Fujifilm developed the Fujifilm SILVAMP TB LAM assay (FujiLAM; Fujifilm, Tokyo, Japan), a novel lateral flow test detecting LAM in urine (9, 10). Initial studies in hospitalised HIV-infected adults with TB showed a significantly higher sensitivity of 70% (9). A recent modelling study also suggested that conducting FujiLAM in adults presenting with TB symptoms averted 30% of TB deaths and 18% of incident cases (5, 11).

In contrast to the increasing number of publications in adults (5, 8, 12-15), few studies have explored the performance of FujiLAM in children and none have modelled its impact on clinical outcomes. In children, diagnostic yield, which represents both the diagnostic accuracy of a test and feasibility of obtaining a specimen (16), is improved by the availability of a specimen such as urine, compared to sputum. FujiLAM could therefore have a positive impact to reduce the burden of childhood TB. A systematic review on FujiLAM in children has not yet been performed. Here, we reviewed diagnostic test accuracy studies that assessed the sensitivity and specificity of FujiLAM to diagnose TB in children.

METHODS

The protocol for this systematic review was registered at PROSPERO (CRD42021270761). Reporting was according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (17).

Search strategy and study selection

We identified studies via PubMed and EMBASE and registration of past and ongoing studies (clinicaltrials.gov, WHO trial registry). Additionally, we consulted experts in TB diagnostics to identify relevant publications. There were no restrictions on language or time of publication. The full search strategy incorporated various terms (text words, keywords, and medical subject headings) related to lipoarabinomannan, tuberculosis, and children, and is presented in the Supplementary material. The last search was conducted on 10th November 2021.

Any original data study that reported diagnostic accuracy estimations on the performance of FujiLAM in children (defined as less than 18 years) for TB was included. The index test was FujiLAM, the comparator where available was AlereLAM. We excluded animal studies, conference proceedings, editorials and reviews. The eligibility assessment was performed by two investigators (LO, NK), who independently screened titles and abstracts followed by full text review. Any disagreement was resolved through discussion with a third reviewer.

Risk of bias assessment

Two independent investigators (LO, NK) assessed the quality of included studies using the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) framework (18), all standard items were applied (19). Consensus was achieved through discussion and consultation with a third reviewer if necessary. RevMan (version 5, The Cochrane Collaboration, 2020) (20) was used for visualisation.

Data collection

The following information was extracted from the original publications by LO and NK independently and exported (XML), with any discrepancies discussed with a third reviewer:

i. Characteristics of cohort (including age, clinical presentation, country of origin, HIV-status)

ii. In- and exclusion criteria

iii. Reference standards

iv. Diagnostic accuracy measures

Summary measures and data analysis

The outcome measures were sensitivity and specificity of FujiLAM to diagnose active TB in children, using a microbiological reference standard (MRS; culture and/or WHO-endorsed nucleic acid amplification tests - NAAT) or a composite reference standard (CRS). Sensitivity was defined as probability of a positive test in diseased children, while specificity represented the probability of a negative test result when the disease was absent. Analyses were performed using RevMan (version 5, The Cochrane Collaboration, 2020) (20).

Patient and Public Involvement

Being a systematic review, this research was done without patient or public involvement.

RESULTS

Study results

The search identified 149 unique records from which 24 full texts were reviewed for eligibility and 3 studies met inclusion criteria (**Supplemental figure 1**). No further registered trials, or publications on preprint servers were identified**. Table 1** shows the characteristics of the studies. The clinical settings differed; two studies were conducted in sub-Saharan Africa and one in Haiti. Studies also varied in the healthcare level for recruitment and proportion of children with microbiologically confirmed TB. In all studies, enrolment was prospective, but the index test FujiLAM was evaluated on biobanked samples. Only the African studies assessed the performance of AlereLAM. Due to study heterogeneity, a meta-analysis was not done.

Quality

Figure 1 summarises the risk of bias and applicability of included studies, there were no disagreements between reviewers. Two studies had some concerns regarding risk of bias relating

to patient selection. One study enriched their cohort by specifically including known microbiologically confirmed CLHIV (21). Another study recruited healthy controls, which tends to over-estimate diagnostic performance and did not explicitly state that samples were taken consecutively (22). The index test domain was generally at low risk of bias, with all studies reporting blinded interpretation by two readers. Due to the challenges in confirming TB disease in children, interpreting the true value of a novel test remains problematic, especially for those without microbiological confirmation (23-25). Accordingly, all studies explicitly described the reference standards and tested the index test against both an MRS and a CRS. One study, was deemed at high risk of bias as the MRS only included Xpert MTB/RIF and not culture (22). The risk of bias was low for all studies regarding patient flow and timing.

Test accuracy

Table 2 outlines the in- and exclusion criteria, microbiological investigations, reference standards, and case definitions of studies. All studies applied an MRS, being roughly equivalent to the National Institutes of Health (NIH) clinical case definition of "confirmed TB" (25), requiring microbiological confirmation of MTB , although underlying tests and testing algorithms varied between the studies. CRS were also used, with both microbiologically confirmed and clinically diagnosed TB defined as CRS positive, however, underlying clinical information varied between studies.

The total number of children included in this analysis was 698 for FujiLAM, with 152 (21%) confirmed by MRS and 396 (54%) by CRS. For AlereLAM, data from 619 children (89%) were available, with 147 (24%) confirmed by MRS and 341 (55%) by CRS. The sensitivity and specificity of index tests across the studies are shown in **Table 3** and **Figure 2**.

Microbiological reference standard (MRS)

When applying the MRS, sensitivity of FujiLAM was estimated at 60% (95%CI 15-95), 42% (95%CI 31–53), and 63% (95%CI 50–75) for the three studies respectively (pooled sensitivity 51% (95%CI 43-59)). For specificity, estimations were 93% (95%CI 85-98), 92% (95%CI 85–96), and 84% (95%CI 80–88; pooled specificity 87% (95%CI 84-90)) (**Figure 2**). For the comparator AlereLAM,

sensitivities were 50% (95%CI 39–61) and 30% (95%CI 19–43) for the individual studies (pooled sensitivity 41% (95%CI 33-50)). While FujiLAM sensitivity was significantly higher compared to AlereLAM in the study by Nkereuwem et al., this was neither the case for the study by Nicol et al., nor the pooled analysis. AlereLAM specificity estimates differed between the two African studies with 66% (95%CI 57–74) and 89% (95%CI 85–92; pooled specificity 83% (95%CI 79-86)), which was not significantly different from FujiLAM.

Composite reference standard (CRS)

When applying the CRS, sensitivity of FujiLAM was 11% (95%CI 4-22), 27% (95%CI 20-34), and 33% (95%CI 26-40; pooled sensitivity 27% (95%CI 23-32)). Specificity estimates were 92% (95%CI 73-99), 97% (95%CI 87-100), and 85% (95%CI 79-89; pooled specificity 87% (95%CI 82-90)). For AlereLAM, sensitivity estimates differed considerably between studies with 44% (95%CI 37-52) and 20% (95%CI 15-27; pooled sensitivity 32% (95%CI 27-37)). AlereLAM specificity was 74% (95%CI 58-87) and 90% (95%CI 86-94; pooled specificity 88% (95%CI 84-92)).

Results stratified by HIV

The Haitian study excluded CLHIV, therefore only the African studies assessed performance in this subgroup. Data only allowed for comparison to the MRS (**Figure 2**). For CLHIV, sensitivity of FujiLAM tended to be higher with 60% (95%CI 39-79) and 53% (95%CI 27-79; pooled sensitivity 57% (95%CI 41-73)) compared to AlereLAM with 36% (95%CI 18-57) and 33% (95%CI 12-62), although confidence intervals were wide and overlapped. Specificity of FujiLAM in CLHIV was 93% (95%CI 68-100) and 76% (95%CI 61-87; pooled specificity 80% (95%CI 68-89)). Neither sensitivity nor specificity showed a statistically significant difference between FujiLAM and AlereLAM in CLHIV, but the number of included children was too low to draw robust conclusions. Only one study demonstrated test performance stratified by CD4-count, suggesting a higher sensitivity of 80% (95%CI 38-96) in children with CD4-counts <200/uL, compared to 55% (95%CI 34-74) in children with CD4-counts >200/uL. In contrast, specificity was higher in children with CD4-counts >200/uL (100%, 95%CI 74-100; compared to 75%, 95%CI 30-95), but wide confidence intervals limit the interpretation of observed differences.

In HIV-negative children, estimates on FujiLAM performance differed considerably: while one study showed a lower sensitivity, 34% versus 60% in CLHIV, another stated a sensitivity of 67% (compared to 53% in CLHIV). Specificity estimates were less ambiguous with 93% (95%CI 85-98), 91% (95%CI 84-96), and 87% (95%CI 82-90), and overall comparable estimates in CLHIV. Performance estimates for AlereLAM in HIV-negative children were divergent: one group reported a sensitivity of 56% (compared to 36% in CLHIV), another a sensitivity of 30% (compared to 33% in CLHIV). Specificity estimates differed more markedly (69% (95%CI 59-77) and 90% (95%CI 86-93)).

DISCUSSION

We examined the accuracy of the recently developed FujiLAM to diagnose paediatric TB across the available literature. While there are numerous studies evaluating FujiLAM for diagnosing TB in adults (5, 8, 12-15), there are only three paediatric publications (21, 22, 26), two of which used AlereLAM as a comparator (21, 26). We estimated a pooled sensitivity of 51% and specificity of 87% for FujiLAM, which was higher compared to 41% and 83% for the AlereLAM, when applying an MRS. Confidence intervals for the point estimates between the two types of lateral flow tests overlapped, therefore conclusions must be interpreted with caution, especially given the small number of paediatric studies. However, the higher specificity of FujiLAM is promising. Compared to sputum where collection is difficult, urine can mostly be obtained within the first 24h of admission and benefits for diagnostic yield and potentially clinical outcomes are likely (27, 28). FujiLAM could have particular utility when used in combination within a diagnostic algorithm to rule-in TB in children with a high pre-test probability, like CLHIV or malnourished children in high endemic settings (29).

The estimated sensitivity of FujiLAM here is comparable to results from a multicenter diagnostic accuracy study in HIV-negative adults (53%) (5). In this study, a strong association of sensitivity with bacterial load was observed, which likely impacts performance in children, as they generally have paucibacillary disease. Diagnostic evaluations for TB in children remain difficult, as available reference standards are imperfect. While the MRS might miss TB cases, a CRS potentially includes

children not ill with TB, both hampering the interpretability of sensitivity estimates. Using an MRS is likely to underestimate specificity because the number of children with TB and therefore the proportion of true negatives are underestimated. How studies define their reference standards may also contribute to hetereogeneity in accuracy estimates. While all studies applied the NIH clinical case definitions for intrathoracic tuberculosis (25), underlying clinical and microbiological investigations varied. For example, one study did solely perform NAAT but not culture, and only in cases with positive smear microscopy or abnormal chest Xray, potentially misclassifying microbiologically confirmed cases and subsequently underestimating sensitivity (22). This heterogeneity of classifications outlines the necessity of applying standardised diagnostic classifications rigorously to enable cross-comparisons and meta-analyses (23-25).

Patient cohorts (and therefore pre-test probabilities) also differed considerably between studies. Participants were recruited from different levels of health care, reflecting real-life variation, which is favorable for the generalizability of results (30). However, all tests were performed on biobanked specimen in research laboratory settings. Broger and colleagues compared test read-outs of fresh vs. biobanked samples from adult patients, and while categorical agreement was high, a reduction of positive percentage agreement was observed (31). Studies using FujiLAM on fresh specimens, prospectively, and in real-life settings need to be conducted in children.

Subgroups of highest priority include CLHIV and the very young, who are at high risk of dying from TB (2). We found that FujiLAM's sensitivity was slightly higher in CLHIV, but reliable conclusions are difficult to draw due to small numbers. Analyses stratified by age were only performed in the African studies, but different age cut-offs were used (21, 26), and direct comparison was not possible. Estimates in the original publications suggest a similar sensitivity, but a pronounced decrease in specificity in younger children. An explanation could be contamination in nappy-wearing children, with specificity potentially compromised due to corynebacteria, dust, soil, and stool (29, 32, 33). Future studies should follow strict collection criteria to prevent contamination and describe them in detail. Finally, data on LAM-assays in extrapulmonary cases (EPTB) remain scarce and reported sensitivities of FujiLAM range from 47-94% in adults (34). Extrapulmonary manifestations are more

common in children, but only one study recruited those cases and did not show subgroup analysis (22).

All included studies, and thus this review, have limitations and data gaps. In addition to those already discussed, the geographical distribution of cohorts was limited to sub-Saharan Africa and Haiti and results may not be generalisable to other regions. Lastly, important subgroup analyses could not be performed across cohorts due to unavailablility of data and variable application of definitions, such as test performance in EPTB, CD4-count in CLHIV (except for one study), and specific age-groups.

This review summarises the current evidence of FujiLAM in comparison to the original AlereLAM assay, with the high specificity demonstrating its potential as a POC rule-in test for diagnosing paediatric TB. It reflects the current state of knowledge, highlighting that more data on FujiLAM in children are needed to more accurately understand the diagnostic value of this test at scale and suggests the points to be addressed in forthcoming evaluations. Late inclusion of children into evaluations of new technologies limits progress in childhood TB and this study stresses the demand for more prospective assessments in real-life settings from several geographical regions with different subgroups, including CLHIV and extrapulmonary disease.

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TABLE AND FIGURES

Table 1: Cohort characteristics

		Nicol et al	Nkereuwem et al.	Barrio et al.
Cohort size		241	415	79
Study design		Prospective enrolment Plus enrichment of CLHIV	Prospective enrolment	Prospective enrolment Plus control cohort
Index test	**Comparator**	AlereLAM	AlereLAM	none
	Sample storage	Yes, -80°C	Yes, -80°C	Yes, -20°C
Country		South Africa	Gambia, Mali, Nigeria, Tanzania	Haiti
Health care level of recruitment of study participants		Tertiary hospital	Mixed (community, Tertiary hospital, urban comprehensive health care)	Reference hospital
Age in months (median) Median (IQR)		45.2 (21.2 – 88.8)	67.2 (27.6-111.6)	76 (58–121)
Age categories	**< 5 yrs.**	118 (58%)	194 (47%)	24 (30%)
	≥ 5 yrs.	86 (42%)	221 (53%)	55 (70%)
Male sex		111 (54%)	225 (54%)	51 (65%)
TB status	**Confirmed TB**	84 (41%)	63 (15%)	5
	Unconfirmed TB	81 (40%)	113 (27%)	50
	Unlikely TB	39 (19%)	239 (58%)	24
HIV status	**HIV infected**	40 (20%)	61 (15%)	excluded
	CD4 cells/uL median (IQR)	552 (206–849)	-	-
Malnutrition	**Stunted**	73 (40%)	134 (32%)	12 (21%)

Table 2: Definitions of reference standards and diagnostic classifications

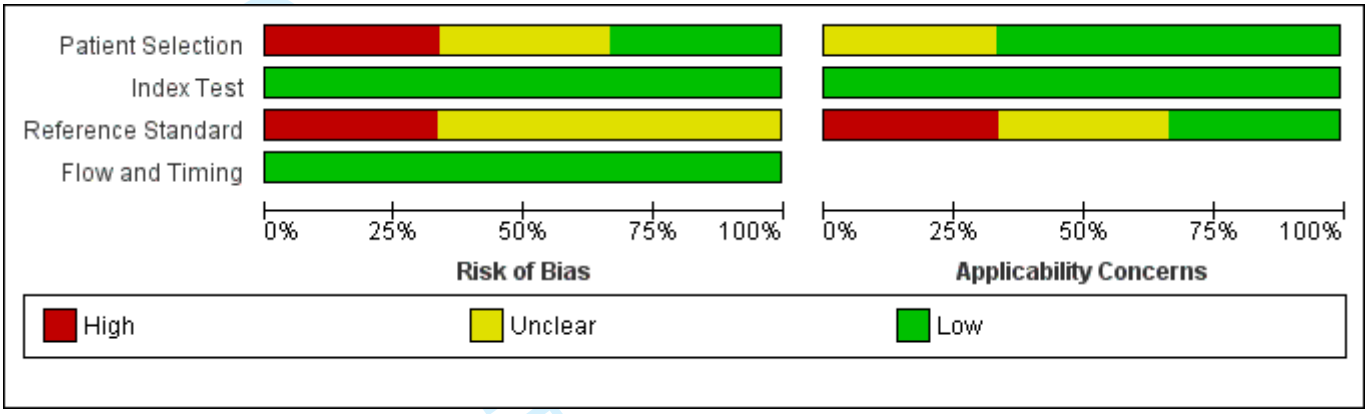
		Nicol et al	Nkereuwem et al.	Barrio et al.
Enrolment criteria	**Inclusion criteria**	- Symptoms suggestive of TB (pulmonary)	- Symptoms suggestive of TB (pulmonary)	- Symptoms suggestive of TB (pulmonary and extrapulmonary) - Controls: negative TST and QFT-GIT, and no signs or symptoms of TB
	Exclusion criteria	- More than 72 hours of TB treatment or prophylaxis - Not a resident in Cape Town	- Not specified - Presense of mediastinal lymphadenopathy alone	- Anti-TB treatment for two or more weeks before enrolment - HIV positivity or other known immunodeficiencies or immunosuppressive treatment
Symptoms of TB		- Cough of any duration and at least one of the following: - Household contact with an infectious tuberculosis source case within the preceding 3 months - Loss of weight or failure to gain weight in the preceding 3 months - Positive tuberculin skin test (TST) - Suggestive CXR	Symptoms suggestive of pulmonary tuberculosis: - Persistent or unremitting cough for more than 2 weeks - and either weight loss, failure to thrive, or persistent unexplained fever	Not specified
TB sampling & microbiological investigations		At least one induced sputum Xpert MTB/RIF or Xpert MTB/RIF Ultra ® & MGIT	At least one induced sputum Xpert MTB/Rif Ultra® (all sites) & MGIT/LJ (not Nigerian site)	Three consecutive sputum samples (induced or nasopharyngeal/nasogastric aspiration) Smear microscopy, Xpert MTB/RIF if positive smear microscopy OR abnormal Xray
TB case classification	**Confirmed TB**	Any induced sputum culture or Xpert MTB/RIF positive for M. tuberculosis	Bacteriological confirmation of Mycobacterium tuberculosis (culture, Xpert MTB/RIF assay, or both) from at least one respiratory specimen	Any sputum Xpert MTB/RIF positive for M. tuberculosis
	Unconfirmed TB	All children not defined as confirmed or unlikely TB	Bacteriological confirmation not obtained, and at least two of the following: - Symptoms or signs suggestive of tuberculosis - Chest radiograph consistent with tuberculosis - Close tuberculosis exposure - Positive response to tuberculosis treatment (requires documented positive clinical response to tuberculosis	Bacteriological confirmation not obtained And positive TST/QFT-GIT and at least one of the following - X-rays consistent with TB - signs and symptoms of TB - close TB exposure, or - positive response to TB treatment - OR if TST/QFT-GIT negative at least - two clinical criteria - X-rays consistent with TB - signs and symptoms of TB - close TB exposure, or - positive response to TB treatment
	Unlikely TB	All of the following: - TB culture negative - No tuberculosis treatment given - Documented improvement of symptoms and signs at follow up visit	Bacteriological confirmation not obtained and criteria for unconfirmed tuberculosis not met	Only evidence of M. tuberculosis infection OR presented only one clinical criterion compatible with TB Controls: negative TST and QFT-GIT, and no signs or symptoms of TB
Definition of reference standards	**Microbiological reference standard (MRS)**	Positive = confirmed TB Negative = unconfirmed & unlikely TB	Positive = confirmed TB Negative = unconfirmed & unlikely TB	Positive = confirmed TB Negative = unconfirmed & unlikely TB + controls
	Composite reference standard (CRS)	Positive = confirmed & unconfirmed TB Negative = unlikely TB	Positive = confirmed & unconfirmed TB Negative = unlikely TB	Positive = confirmed & unconfirmed TB Negative = unlikely TB + controls

Table 3: Diagnostic accuracy estimates as reported

	Nicol et al									Nkereuwem et al.									Barrio et al.								
	Total	TP	FP	FN	TN	Sens		Spec		Total	TP	FP	FN	TN	Sens		Spec		Total	TP	FP	FN	TN	Sens		Spec	
	(n)	(n)	(n)	(n)	(n)	%	95%CI	%	95%CI	(n)	(n)	(n)	(n)	(n)	%	95%CI	%	95%CI	(n)	(n)	(n)	(n)	(n)	%	95%CI	%	95%CI
Overall																											
MRS																											
FujiLAM	204	35	10	49	110	41.7	31.7–52.3	91.7	85,3–95,4	415	40	55	23	297	64.9	43.7–85.2	83.8	76.5–89.4	79	3	5	2	69	60	17–93	95	73-100
AlereLAM	204	42	41	42	79	50.0	39.5–60.5	65.8	57.0–73.7	415	19	40	44	312	30.7	8.6–61.6	87.8	79.0–93.7	-	-	-	-	-	-	-	-	-
CRS																											
FujiLAM	204	44	1	121	38	26.7	20.5–33.9	97.4	86.8–99.5	415	58	37	118	202	32.9	24.6–41.9	83.3	71.8–91.7	73	6	2	49	22	11	523	92	72-99
AlereLAM	204	73	10	92	29	44.2	36.9–51.9	74.4	58.9–85.4	415	36	23	140	216	20.2	12.3–29.4	90.0	81.6–95.6	-	-	-	-	-	-	-	-	-
HIVpositive																											
MRS																											
FujiLAM	40	15	1	10	14	60.0	40.7–76.6	93.3	70.2–98.8	61	8	11	7	35	54.8	28.7–81.5	75.9	61.8–86.9	-	-	-	-	-	-	-	-	-
AlereLAM	40	9	8	16	7	36.0	20.2–55.5	46.7	24.8–69.9	61	5	9	10	37	36.6	13.8–70.4	80.4	66.3–91.0	-	-	-	-	-	-	-	-	-
CRS																											
FujiLAM	-	-	-	-	-	-	-	-	-	61	14	5	30	12	31.9	18.9–47.0	71.4	46.8–91.5	-	-	-	-	-	-	-	-	-
AlereLAM	-	-	-	-	-	-	-	-	-	61	13	1	31	16	29.3	16.3–44.6	92.8	72.6–99.8	-	-	-	-	-	-	-	-	-
HIVnegative																											
MRS																											
FujiLAM	164	20	9	39	96	33.9	23.1–46.6	91.4	84.5–95.4	344	31	40	15	258	67.5	41.8–88.0	85.9	79.2–91.0	-	-	-	-	-	-	-	-	-
AlereLAM	164	33	33	26	72	55.9	43.3–67.8	68.6	59.2–76.7	344	14	30	32	268	26.6	1.2–66.4	89.1	80.7–94.7	-	-	-	-	-	-	-	-	-
CRS																											
FujiLAM	-	-	-	-	-	-	-	-	-	344	43	28	86	187	33.2	23.7–43.5	85.7	76.2–92.2	-	-	-	-	-	-	-	-	-
AlereLAM	-	-	-	-	-	-	-	-	-	344	23	21	106	194	15.3	1.7–37.5	89.3	81.0–94.7	-	-	-	-	-	-	-	-	-
Age	**<2yrs**									**<5yrs**																	
MRS																											
FujiLAM	59	9	7	13	30	40.9	23.3–61.3	81.1	65.8–90.5	194	16	35	10	133	61.8	36.6–85.5	78.5	69.1–86.0	-	-	-	-	-	-	-	-	-
AlereLAM	59	16	18	6	19	72.7	51.8–86.8	51.4	35.9–66.6	194	9	32	17	136	38.8	0.4–98.9	80.5	68.3–89.4	-	-	-	-	-	-	-	-	-
CRS																											
FujiLAM	-	-	-	-	-	-	-	-	-	194	28	23	55	88	33.3	19.8–48.3	78.4	66.5–87.2	-	-	-	-	-	-	-	-	-
AlereLAM	-	-	-	-	-	-	-	-	-	194	20	21	63	90	23.3	10.0–39.9	81.4	70.4–90.4	-	-	-	-	-	-	-	-	-
Age	**>2yrs**									**<5yrs**																	
MRS																											
FujiLAM	145	26	3	36	80	41.9	30.5–54.3	96.4	90.0–98.8	221	24	20	13	164	67.1	40.1–90.2	88.8	82.1–93.6	-	-	-	-	-	-	-	-	-
AlereLAM	145	26	23	36	60	41.9	30.5–54.3	72.3	61.8–80.8	221	10	8	27	176	26.9	7.2–54.8	95.2	89.7–98.3	-	-	-	-	-	-	-	-	-
CRS																											
FujiLAM	-	-	-	-	-	-	-	-	-	221	30	14	63	114	32.7	22.4–44.4	88.2	76.0–96.4	-	-	-	-	-	-	-	-	-
AlereLAM	-	-	-	-	-	-	-	-	-	221	16	2	77	126	17.3	9.6–27.4	98.1	93.7–99.9	-	-	-	-	-	-	-	-	-

Figure 1: Assessment of study quality of FujiLAM paediatric studies using the QUADAS-2 framework. Risk of bias and applicability concerns graph (A) and summary (B): review authors' judgements about each domain presented as percentages across included studies

(A)



(B)

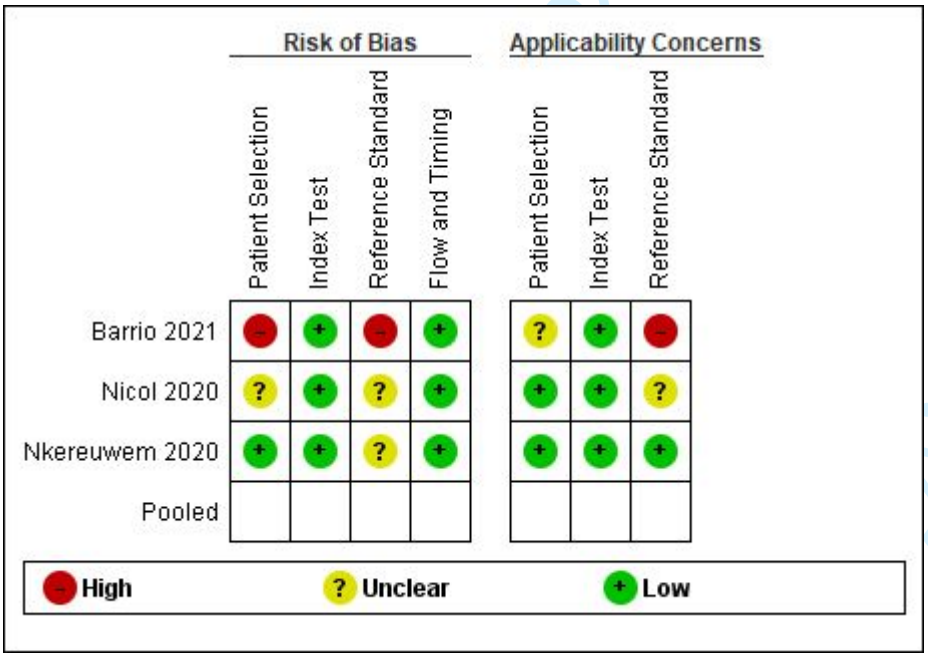
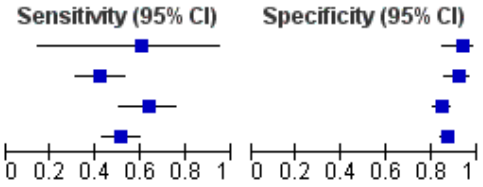


Figure 2: Forest Plots of performance of lateral flow LAM assays against MRS and CRS.

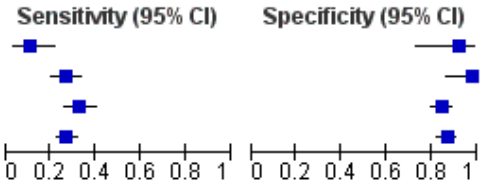
FujiLAM vs MRS

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Barrio 2021	3	5	2	69	0.60 [0.15, 0.95]	0.93 [0.85, 0.98]
Nicol 2020	35	10	49	110	0.42 [0.31, 0.53]	0.92 [0.85, 0.96]
Nkereuwem 2020	40	55	23	297	0.63 [0.50, 0.75]	0.84 [0.80, 0.88]
Pooled	78	70	74	476	0.51 [0.43, 0.59]	0.87 [0.84, 0.90]



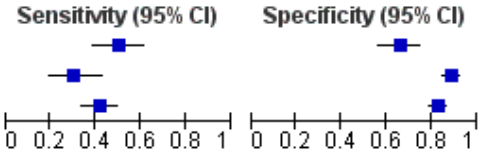
FujiLAM vs CRS

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Barrio 2021	6	2	49	22	0.11 [0.04, 0.22]	0.92 [0.73, 0.99]
Nicol 2020	44	1	121	38	0.27 [0.20, 0.34]	0.97 [0.87, 1.00]
Nkereuwem 2020	58	37	118	202	0.33 [0.26, 0.40]	0.85 [0.79, 0.89]
Pooled	108	40	288	262	0.27 [0.23, 0.32]	0.87 [0.82, 0.90]



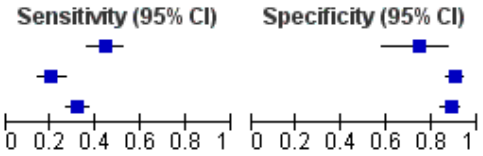
AlereLAM vs MRS

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Nicol 2020	42	41	42	79	0.50 [0.39, 0.61]	0.66 [0.57, 0.74]
Nkereuwem 2020	19	40	44	312	0.30 [0.19, 0.43]	0.89 [0.85, 0.92]
Pooled	61	81	86	391	0.41 [0.33, 0.50]	0.83 [0.79, 0.86]



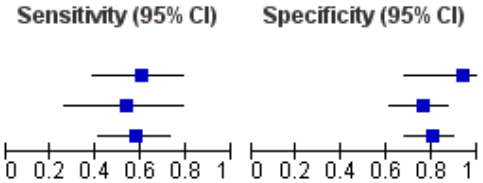
AlereLAM vs CRS

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Nicol 2020	73	10	92	29	0.44 [0.37, 0.52]	0.74 [0.58, 0.87]
Nkereuwem 2020	36	23	140	216	0.20 [0.15, 0.27]	0.90 [0.86, 0.94]
Pooled	109	33	232	245	0.32 [0.27, 0.37]	0.88 [0.84, 0.92]



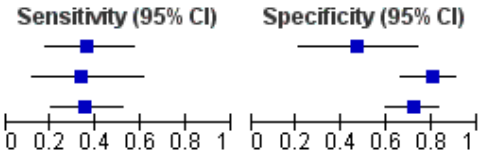
FujiLAM vs MRS in HIV +

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Barrio 2021	0	0	0	0	Not estimable	Not estimable
Nicol 2020	15	1	10	14	0.60 [0.39, 0.79]	0.93 [0.68, 1.00]
Nkereuwem 2020	8	11	7	35	0.53 [0.27, 0.79]	0.76 [0.61, 0.87]
Pooled	23	12	17	49	0.57 [0.41, 0.73]	0.80 [0.68, 0.89]



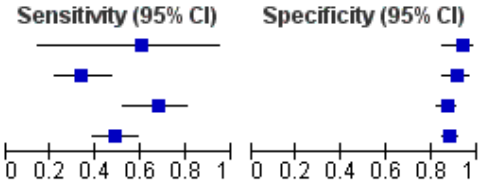
AlereLAM vs MRS in HIV+

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Nicol 2020	9	8	16	7	0.36 [0.18, 0.57]	0.47 [0.21, 0.73]
Nkereuwem 2020	5	9	10	37	0.33 [0.12, 0.62]	0.80 [0.66, 0.91]
Pooled	14	17	26	44	0.35 [0.21, 0.52]	0.72 [0.59, 0.83]



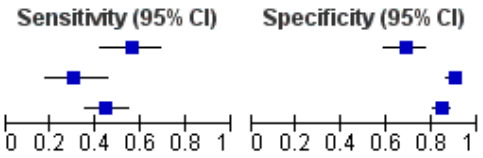
FujiLAM vs MRS in HIV-

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Barrio 2021	3	5	2	69	0.60 [0.15, 0.95]	0.93 [0.85, 0.98]
Nicol 2020	20	9	39	96	0.34 [0.22, 0.47]	0.91 [0.84, 0.96]
Nkereuwem 2020	31	40	15	258	0.67 [0.52, 0.80]	0.87 [0.82, 0.90]
Pooled	51	49	54	354	0.49 [0.39, 0.59]	0.88 [0.84, 0.91]



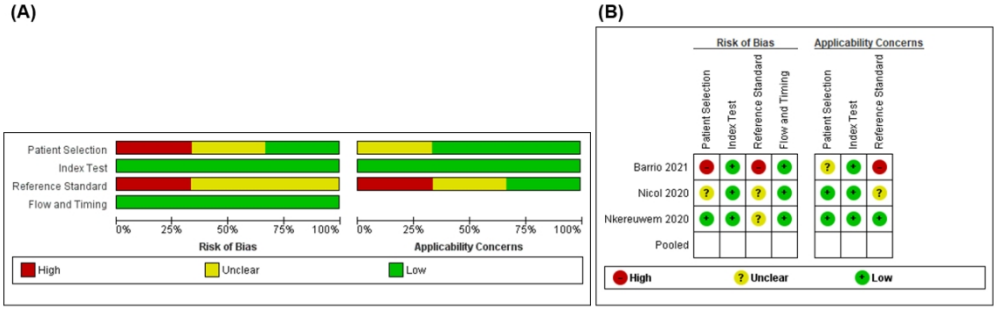
AlereLAM vs MRS in HIV-

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Nicol 2020	33	33	26	72	0.56 [0.42, 0.69]	0.69 [0.59, 0.77]
Nkereuwem 2020	14	30	32	268	0.30 [0.18, 0.46]	0.90 [0.86, 0.93]
Pooled	47	63	58	340	0.45 [0.35, 0.55]	0.84 [0.80, 0.88]



Author's contribution:

LO and NK conducted the literature search, screening of abstracts, data extraction, and analyses, supported by EMB and RS. The manuscript was written mainly by LO and NK, and reviewed and edited by EMB, MR, NH, and RS.



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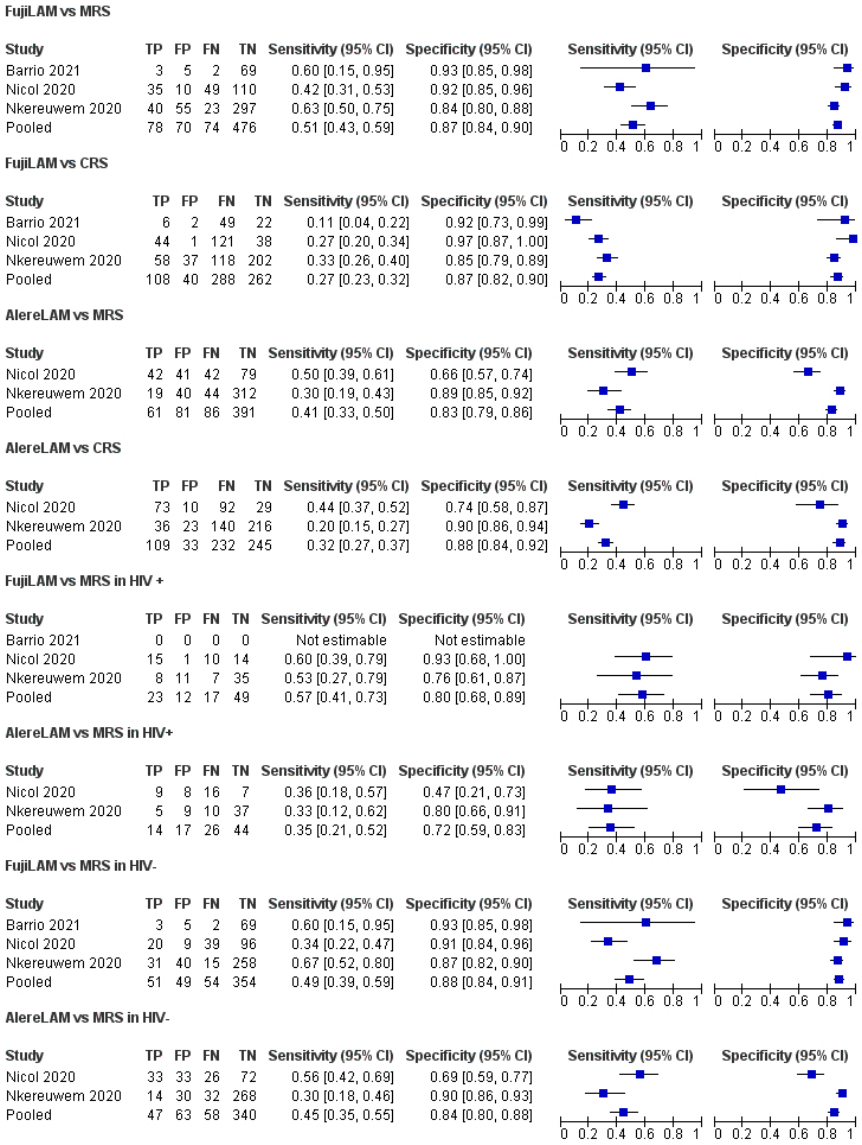

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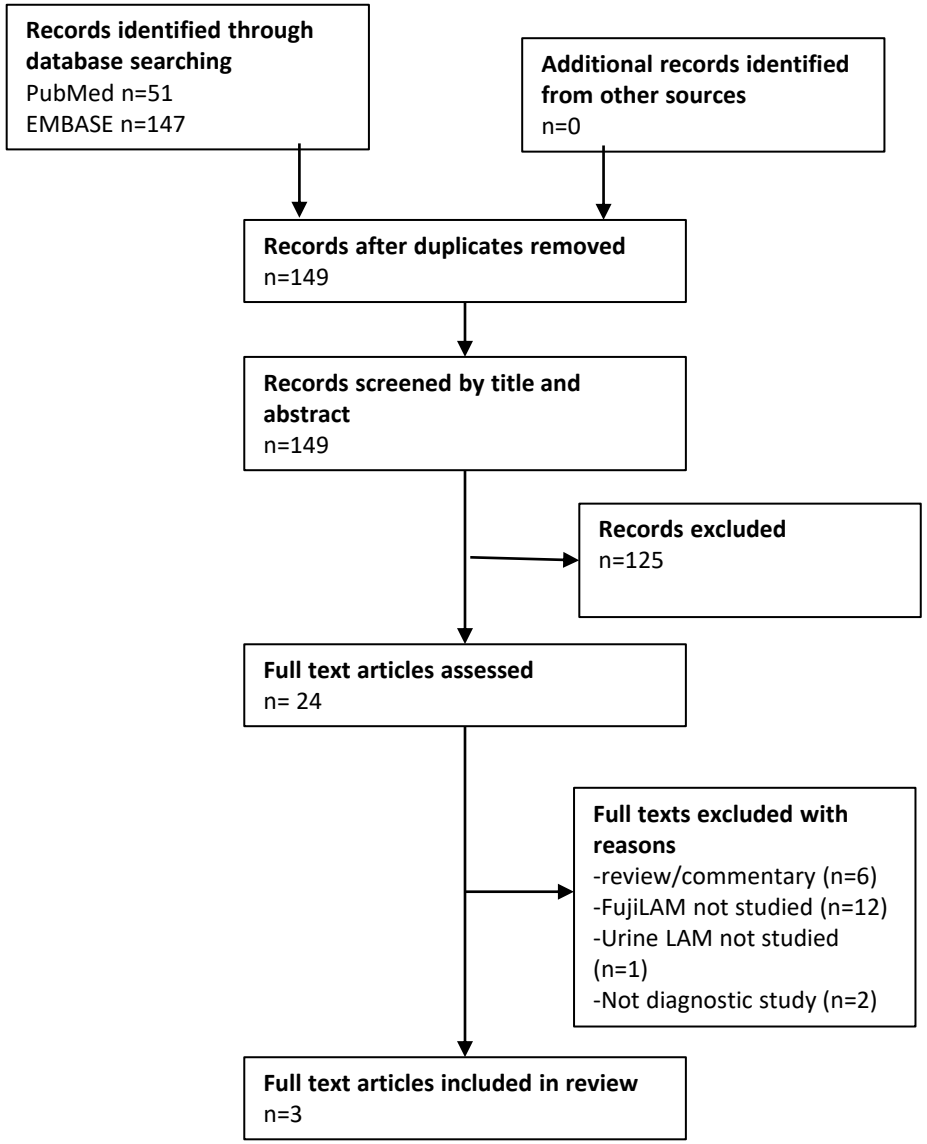
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n=149
Records excluded
n=125
Full text articles assessed
n= 24
Full texts excluded with reasons
-review/commentary (n=6)
-FujiLAM not studied (n=12)
-Urine LAM not studied (n=1)
-Not diagnostic study (n=2)
Full text articles included in review
n=3

Supplemental Table 1: Diagnostic accuracy estimates as reported by authors

	Nicol et al									Nkereuwem et al.									Barrio et al.								
	Total	TP	FP	FN	TN	Sens		Spec		Total	TP	FP	FN	TN	Sens		Spec		Total	TP	FP	FN	TN	Sens		Spec	
	(n)	(n)	(n)	(n)	(n)	%	95%CI	%	95%CI	(n)	(n)	(n)	(n)	(n)	%	95%CI	%	95%CI	(n)	(n)	(n)	(n)	(n)	%	95%CI	%	95%CI
Overall																											
MRS																											
FujiLAM	204	35	10	49	110	41.7	31.7–52.3	91.7	85,3–95,4	415	40	55	23	297	64.9	43.7–85.2	83.8	76.5–89.4	79	3	5	2	69	60	17–93	95	73-100
AlereLAM	204	42	41	42	79	50.0	39.5–60.5	65.8	57.0–73.7	415	19	40	44	312	30.7	8.6–61.6	87.8	79.0–93.7	-	-	-	-	-	-	-	-	-
CRS																											
FujiLAM	204	44	1	121	38	26.7	20.5–33.9	97.4	86.8–99.5	415	58	37	118	202	32.9	24.6–41.9	83.3	71.8–91.7	73	6	2	49	22	11	523	92	72-99
AlereLAM	204	73	10	92	29	44.2	36.9–51.9	74.4	58.9–85.4	415	36	23	140	216	20.2	12.3–29.4	90.0	81.6–95.6	-	-	-	-	-	-	-	-	-
HIVpositive																											
MRS																											
FujiLAM	40	15	1	10	14	60.0	40.7–76.6	93.3	70.2–98.8	61	8	11	7	35	54.8	28.7–81.5	75.9	61.8–86.9	-	-	-	-	-	-	-	-	-
AlereLAM	40	9	8	16	7	36.0	20.2–55.5	46.7	24.8–69.9	61	5	9	10	37	36.6	13.8–70.4	80.4	66.3–91.0	-	-	-	-	-	-	-	-	-
CRS																											
FujiLAM	-	-	-	-	-	-	-	-	-	61	14	5	30	12	31.9	18.9–47.0	71.4	46.8–91.5	-	-	-	-	-	-	-	-	-
AlereLAM	-	-	-	-	-	-	-	-	-	61	13	1	31	16	29.3	16.3–44.6	92.8	72.6–99.8	-	-	-	-	-	-	-	-	-
HIVnegative																											
MRS																											
FujiLAM	164	20	9	39	96	33.9	23.1–46.6	91.4	84.5–95.4	344	31	40	15	258	67.5	41.8–88.0	85.9	79.2–91.0	-	-	-	-	-	-	-	-	-
AlereLAM	164	33	33	26	72	55.9	43.3–67.8	68.6	59.2–76.7	344	14	30	32	268	26.6	1.2–66.4	89.1	80.7–94.7	-	-	-	-	-	-	-	-	-
CRS																											
FujiLAM	-	-	-	-	-	-	-	-	-	344	43	28	86	187	33.2	23.7–43.5	85.7	76.2–92.2	-	-	-	-	-	-	-	-	-
AlereLAM	-	-	-	-	-	-	-	-	-	344	23	21	106	194	15.3	1.7–37.5	89.3	81.0–94.7	-	-	-	-	-	-	-	-	-
Age	**<2yrs**									**<5yrs**																	
MRS																											
FujiLAM	59	9	7	13	30	40.9	23.3–61.3	81.1	65.8–90.5	194	16	35	10	133	61.8	36.6–85.5	78.5	69.1–86.0	-	-	-	-	-	-	-	-	-
AlereLAM	59	16	18	6	19	72.7	51.8–86.8	51.4	35.9–66.6	194	9	32	17	136	38.8	0.4–98.9	80.5	68.3–89.4	-	-	-	-	-	-	-	-	-
CRS																											
FujiLAM	-	-	-	-	-	-	-	-	-	194	28	23	55	88	33.3	19.8–48.3	78.4	66.5–87.2	-	-	-	-	-	-	-	-	-
AlereLAM	-	-	-	-	-	-	-	-	-	194	20	21	63	90	23.3	10.0–39.9	81.4	70.4–90.4	-	-	-	-	-	-	-	-	-
Age	**>2yrs**									**<5yrs**																	
MRS																											
FujiLAM	145	26	3	36	80	41.9	30.5–54.3	96.4	90.0–98.8	221	24	20	13	164	67.1	40.1–90.2	88.8	82.1–93.6	-	-	-	-	-	-	-	-	-
AlereLAM	145	26	23	36	60	41.9	30.5–54.3	72.3	61.8–80.8	221	10	8	27	176	26.9	7.2–54.8	95.2	89.7–98.3	-	-	-	-	-	-	-	-	-
CRS																											
FujiLAM	-	-	-	-	-	-	-	-	-	221	30	14	63	114	32.7	22.4–44.4	88.2	76.0–96.4	-	-	-	-	-	-	-	-	-
AlereLAM	-	-	-	-	-	-	-	-	-	221	16	2	77	126	17.3	9.6–27.4	98.1	93.7–99.9	-	-	-	-	-	-	-	-	-

Search strategy for supplementary materials

PubMED

(("child*"[Title/Abstract] OR "infant*"[Title/Abstract] OR "adolescent*"[Title/Abstract] OR "paediatric*"[Title/Abstract] OR "pediatric*"[Title/Abstract]) AND ("LAM"[Title/Abstract] OR "lipoarabinomannan"[Title/Abstract])) AND (("tuberculos*"[Title/Abstract] OR "TB"[Title/Abstract] OR MTB[Title/Abstract] OR PTB[Title/Abstract] OR mycobacterium tuberculosis[MeSH Terms] OR tuberculosis[MeSH Terms]))

EMBASE

1. mycobacterium tuberculosis/
2. tuberculosis/ or lung tuberculosis/
3. (tuberculos*).mp.
4. MTB.mp.
5. Tb.mp.
6. (child*).mp.
7. (infant*).mp.
8. (adolescent*).mp.
9. (paediatric*).mp.
10. (pediatric*).mp.
11. Lipoarabinomannan/
12. LAM.mp.
13. Lipoarabinomannan.mp.
14. 1 or 2 or 3 or 4 or 5
15. 6 or 7 or 8 or 9 or 10
16. 11 or 12 or 13
17. 14 and 15 and 16
18. (exp animals/ or nonhuman/) not human/
19. 17 not 18
20. conference*.pt
21. 19 not 20

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FujiLAM for the diagnosis of childhood tuberculosis: A systematic review

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FujiLAM for the diagnosis of childhood tuberculosis: A systematic review

Abbreviated & running title: FujiLAM for diagnosis of childhood TB

Authors and Affiliations

Laura Olbrich, MD * [1, 2, 3]; Nisreen Khambati, MD * [3]; Else Margreet Bijker, MD PhD [3], Morten Ruhwald, MD PhD [4], Norbert Heinrich, MD 1, [2], Rinn Song, MD [3, 5]

* Joint first author

1 Division of Infectious Diseases and Tropical Medicine, University Hospital, LMU Munich, Germany;

2 German Centre for Infection Research (DZIF), Partner Site Munich, Munich, Germany;

3 Oxford Vaccine Group, Department of Paediatrics, University of Oxford, Oxford, UK

4 FIND, the Global Alliance for Diagnostics. Chemin des Mines 9, 1201 Geneva, Switzerland

5 Division of Infectious Diseases, Boston Children's Hospital, Boston, Massachusetts, USA)

Key words

Childhood TB, FujiLAM, lipoarabinomannan, diagnosis

Corresponding Author

LMU Klinikum, Division of Infectious Diseases and Tropical Medicine

Leopoldstraße 5, 80802 Munich, Germany

Phone +49 89 4400-59803, Fax +49 89 336038

olbrich@lrz.uni-muenchen.de

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Conflicts

MR is employed by FIND, the Global Alliance for Diagnostics. FIND is a not-for-profit NGO that collaborates in partnerships to develop, evaluate and implement new diagnostics for LMIC. FIND has product evaluation agreements with FujiFilm and several other private sector companies that design diagnostics and related products for treatment of tuberculosis and other diseases. These agreements strictly define FIND's independence and neutrality vis-à-vis the companies whose products get evaluated and describe roles and responsibilities.

"What is already known on this topic"

- Despite recent advances, paediatric TB remains difficult to diagnose and accurate point-of-care tests that use easily obtainable non-sputum specimens are urgently needed.
- Lateral flow tests detecting urine lipoarabinomannan (LAM), including the original AlereLAM and the recently developed FujiLAM, could improve diagnosis in children in low-resource settings.
- FujiLAM's analytic sensitivity for the diagnosis of pulmonary TB has been observed to be higher compared to AlereLAM in adults.

"What this study adds"

- Using a microbiological reference standard, the sensitivity of FujiLAM for diagnosing paediatric TB ranged from 42% to 63%, whereas specificity was higher, ranging from 84% to 93%.
- Gaps in studies to be prioritised in forthcoming evaluations include prospective testing of fresh specimens, subgroup analyses for children living with HIV, and direct comparison with AlereLAM.

"How this study might affect research, practice or policy"

- Whilst more paediatric studies are needed, high specificity and use of an easy-to-obtain specimen indicates that FujiLAM could be a useful rule-in test for TB.

ABSTRACT

Background

Childhood tuberculosis (TB) remains underdiagnosed. The novel lateral flow FujiLAM assay detects lipoarabinomannan (LAM) in urine, but data on performance in children remain limited.

Methods

We conducted a systematic review assessing the diagnostic performance of FujiLAM for diagnosing paediatric TB. The last search was conducted in November 2021.

Results

We included three studies with data from 698 children for FujiLAM. For FujiLAM, sensitivity using a microbiological reference standard (MRS) were 60% (95%CI 15-95), 42% (95%CI 31–53), and 63% (95%CI 50-75), respectively. Specificity was 93% (95%CI 85-98), 92% (95%CI 85–96), and 84% (95%CI 80-88). Using a composite reference standard (CRS), sensitivity was 11% (95%CI 4-22), 27% (95%CI 20-34), and 33% (95%CI 26-40), and specificity was 92% (95%CI 73-99), 97% (95%CI 87-100), and 85% (95%CI 79-89). Subgroup analyses for sensitivity of FujiLAM in children living with HIV (CLHIV) compared to those who were negative for HIV infection were inconsistent across studies. Among CLHIV, sensitivity appeared higher in those with greater immunosuppression, although wide confidence intervals limit the interpretation of observed differences. Meta-analysis was not performed due to considerable study heterogeneity.

Conclusion

The high specificity of FujiLAM demonstrates its potential as a point-of-care (POC) rule-in test for diagnosing paediatric TB. As an instrument-free POC test that uses an easy-to-obtain specimen, FujiLAM could significantly improve TB diagnosis in children in low-resource settings, however the small number of studies available highlight that further data is needed. Key priorities to be addressed in forthcoming paediatric evaluations include prospective head-to head comparisons with AlereLAM

using fresh specimens, specific subgroup analysis in CLHIV, and extrapulmonary disease and studies in different geographical locations.

INTRODUCTION

Childhood tuberculosis (TB) is a major contributor to morbidity and mortality worldwide (1). Children below five years are disproportionally affected in case load and mortality, contributing to approximately 50% of all paediatric TB cases (2) and 80% of deaths (2, 3). The burden and mortality of paediatric TB is likely underestimated, as confirmation of disease remains challenging. There is an unmet need for accurate and easy-to-use diagnostic tests for children.

The World Health Organization (WHO) has defined target product profiles (TPP) for new non-sputum-based point-of-care (POC) diagnostics for TB and their use in children (4). Promising candidates include lateral flow assays detecting lipoarabinomannan (LAM), a glycolipid found in the mycobacterial cell-wall, secreted in urine. The first commercially available test was the Alere Determine TB LAM Ag (AlereLAM; Abbott, Palatine, IL, USA), which is the only instrument-free POC LAM test recommended by the WHO (5, 6). According to a systematic review, pooled sensitivity of the AlereLAM is 42% in adults (7), increasing to 54% in PLHIV with CD4 ≤100 cells/μL (7, 8). Recently, Fujifilm developed the Fujifilm SILVAMP TB LAM assay (FujiLAM; Fujifilm, Tokyo, Japan), a novel test detecting LAM in urine using high affinity monoclonal antibodies and silver amplification (9, 10). Initial studies in hospitalised adults with HIV showed a higher diagnostic sensitivity of 70% for TB compared to AlereLAM (9). A recent modelling study also suggested that conducting FujiLAM in adults presenting with TB symptoms averted 30% of TB deaths and 18% of incident cases between 2020 and 2035 (11).

Compared to the increasing number of publications in adults (5, 8, 12-15), few studies have explored the performance of FujiLAM in children. In children, diagnostic yield, which represents both the diagnostic accuracy of a test and feasibility of obtaining a specimen (16), is improved by the availability of a specimen such as urine, compared to sputum. FujiLAM could therefore have a positive impact to reduce the burden of childhood TB. Here, we conducted a systematic review on the diagnostic performance of FujiLAM in children for diagnosing TB.

METHODS

The protocol for this systematic review was registered at PROSPERO (CRD42021270761). Reporting was according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (17).

Search strategy and study selection

We identified studies via PubMed and EMBASE and registration of past and ongoing studies (clinicaltrials.gov, WHO trial registry). Additionally, we consulted experts in TB diagnostics to identify relevant publications. There were no restrictions on language or time of publication. The full search strategy incorporated terms (text words, keywords, and medical subject headings) related to lipoarabinomannan, tuberculosis, and children, and is presented in the Supplementary Material. The last search was conducted on 10th November 2021.

Original studies that reported diagnostic accuracy estimations on the performance of FujiLAM in children (defined as less than 18 years) for TB were included. We excluded animal studies, conference proceedings, editorials, and reviews. The eligibility assessment was performed by two investigators (LO, NK), who independently screened titles and abstracts followed by full text review. Any disagreement was resolved through discussion with a third reviewer.

Risk of bias assessment

Two independent investigators (LO, NK) assessed the quality of included studies using the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) framework (18), all standard items were applied (19). Consensus was achieved through discussion and consultation with a third reviewer if necessary. RevMan (version 5, The Cochrane Collaboration, 2020) (20) was used for visualisation.

Data collection

The following information was extracted from the original publications by LO and NK independently with any discrepancies discussed with a third reviewer:

i. Characteristics of cohort (including age, clinical presentation, country of origin, HIV-status)

ii. In- and exclusion criteria

iii. Reference standards

iv. Diagnostic accuracy measures

Summary measures and data analysis

The outcome measures were sensitivity and specificity of FujiLAM to diagnose active TB in children, using a microbiological reference standard (MRS; culture and/or WHO-endorsed nucleic acid amplification tests - NAAT) or a composite reference standard (CRS). Sensitivity was defined as probability of a positive test in diseased children. Specificity represented the probability of a negative test result when the disease was absent. Point estimates and confidence intervals were calculated using the raw data provided by the original publication with the statistical software of RevMan (version 5, The Cochrane Collaboration, 2020) (20).

Patient and Public Involvement

Being a systematic review, this research was done without patient or public involvement. As a secondary analysis, no ethical approval was sought.

RESULTS

Study results

149 unique records were identified from which 24 full texts were reviewed for eligibility and 3 studies met inclusion criteria (**Supplemental figure 1**). No further registered trials or publications on preprint servers were identified**. Table 1** shows the study characteristics. The clinical settings differed; two studies were conducted in sub-Saharan Africa and one in Haiti. Studies also varied in the healthcare level for recruitment and proportion of children with microbiologically confirmed TB. In all studies,

enrolment was prospective, but FujiLAM was evaluated on biobanked samples. Due to study heterogeneity and the small number of studies, a meta-analysis was not done.

Quality

Figure 1 summarises the risk of bias and applicability of included studies, there were no disagreements between reviewers. Regarding patient selection, one study enriched their cohort by specifically including known microbiologically confirmed CLHIV; therefore risk of bias was deemed unclear (21). Another study had a high risk of bias because the authors did not explicitly state whether samples were taken consecutively and also recruited healthy controls, which can over-estimate diagnostic performance (22). The index test domain was at low risk of bias, with all studies reporting blinded interpretation by two readers. Due to the inherent challenges of microbiological investigations in confirming TB disease in children (23-25), two studies had an unclear risk of bias for correctly classifying the target condition, despite including culture (21, 26). One study was judged as having a high risk of bias as the MRS only included Xpert MTB/RIF and not culture (22). Risk of bias was low for most studies regarding patient flow except for one study in which only certain patients received the MRS. Full details of the QUADAS-2 assessment are included in **Supplementary Table 1**.

Test accuracy

Table 2 outlines the in- and exclusion criteria, microbiological investigations (specimen collected and tests performed), reference standards, and case definitions of studies. All studies applied an MRS, being roughly equivalent to the National Institutes of Health (NIH) case definition of "confirmed TB" (25), requiring microbiological confirmation of MTB, although underlying tests and testing algorithms varied between the studies. CRS were also used, with both microbiologically confirmed and clinically diagnosed TB defined as CRS positive, however, underlying clinical information varied between studies.

In total, 698 children were included in this analysis. The sensitivity and specificity of index tests across the studies are shown in **Table 3**, **Table 4,** and **Figure 2**. Two studies reported on invalid

results, which were excluded from final analyses. One study reported n=1 invalid result (26), another stated n=22 invalid results (21), which reduced to n=4 after re-testing.

Microbiological reference standard (MRS)

When applying the MRS, sensitivity of FujiLAM was estimated at 60% (95%CI 15-95) (22), 42% (95%CI 31–53) (21), and 63% (95%CI 50–75) (26) for the three studies respectively. Confidence intervals were wide and overlapped for all three studies. In contrast, specificity estimations were more consistent across studies, with 93% (95%CI 85-98) (22), 92% (95%CI 85–96) (21), and 84% (95%CI 80–88) (26) (**Figure 2**). Two studies performed head-to-head comparisons with AlereLAM (21, 26). Nkereuwem et al., found that FujiLAM had a sensitivity more than double of AlereLAM (31%, 95%CI 9-62), whilst maintaining a similarly high specificity (88% 95%CI 79-94) (26). In the study by Nicol et al, sensitivity of AlereLAM was slightly higher (50%, 95%CI 40-61), although specificity was much lower (66%, 95% CI 57-74) (21).

Composite reference standard (CRS)

When applying the CRS, sensitivity of FujiLAM was 11% (95%CI -22) (22), 27% (95%CI 20-34) (21), and 33% (95%CI 26-40) (26). Sensitivity was pronouncedly lower than for MRS, with differences of 49% (22), 15% (21), and 30% (26). Specificity estimates were closer to MRS with 92% (95%CI 73-99) (22), 97% (95%CI 87-100) (21), and 85% (95%CI 79-89) (26).

Results stratified by HIV

One study excluded CLHIV, therefore only two studies assessed performance in this subgroup. Data only allowed for comparison to the MRS (**Figure 2**). For CLHIV, sensitivity of FujiLAM was 60% (95%CI 39-79) (21) and 53% (95%CI 27-79) (26). Specificity in CLHIV was 93% (95%CI 68-100) (21) and 76% (95%CI 61-87) (26). One study demonstrated test performance stratified by CD4-count, suggesting a higher sensitivity of 80% (95%CI 38-96) in children with CD4-counts <200/uL, compared to 55% (95%CI 34-74) in children with CD4-counts >200/uL (21). In contrast, specificity was higher in children with CD4-counts >200/uL (100%, 95%CI 74-100; compared to 75%, 95%CI 30-95) (21) For both performance estimates, confidence intervals were very wide and overlapped.

In HIV-negative children, estimates on FujiLAM performance differed considerably. While one study reported a lower sensitivity, 34% (95%CI 22-47) versus 60% (95%CI 39-79) in CLHIV (21), another stated a sensitivity of 67% (95%CI 52-80) compared to 53% (95%CI 27-79) in CLHIV (26), but again confidence intervals overlapped. Specificity estimates for HIV negative children (91% (95%CI 8-96) (21), and 87% (95%CI 82-90) (26) were overall comparable to those in CLHIV.

DISCUSSION

We examined the accuracy of the recently developed FujiLAM to diagnose paediatric TB across the available literature. While there are numerous studies evaluating FujiLAM for diagnosing TB in adults (5, 8, 12-15), there are only three paediatric publications (21, 22, 26). The estimated sensitivities ranged from 42% to 63%, whereas specificity was higher, ranging from 84% to 93%, when applying an MRS. Although sensitivity targets for the WHO TPP for a diagnostic (>66%) or triage (>90%) test were not met, the high specificity of FujiLAM across all studies is promising, especially given the rapidity and ease of use. Urine can mostly be obtained within the first 24h of admission, compared to sputum where collection is difficult, and benefits for diagnostic yield are likely (27, 28). FujiLAM could have particular utility when used in combination within a diagnostic algorithm to rule-in TB in children with a high pre-test probability, like CLHIV or malnourished children in high endemic settings (29).

The estimated sensitivity of FujiLAM here is comparable to results from a multicenter diagnostic accuracy study in HIV-negative adults (53%) (5). In this study, a strong association of sensitivity with bacterial load was observed, which likely impacts performance in children, as they generally have paucibacillary disease. Diagnostic evaluations for TB in children remain difficult, as available reference standards are imperfect. While the MRS might miss TB cases, a CRS potentially includes children not ill with TB, both hampering the interpretability of sensitivity estimates. Using an MRS will underestimate the number of children with TB and therefore overestimate the number of true negatives, as MRS can misclassify paediatric TB positive cases as negative cases. How studies define their reference standards may also contribute to hetereogeneity in accuracy estimates. While

all studies applied the NIH clinical case definitions for intrathoracic tuberculosis (25), underlying clinical and microbiological investigations varied. For example, one study solely performed NAAT but not culture, and only in cases with positive smear microscopy or abnormal chest Xray, potentially underestimating sensitivity (22). This heterogeneity of classifications outlines the necessity of applying standardised diagnostic classifications rigorously to enable cross-comparisons and meta-analyses (23-25).

Patient cohorts (and therefore pre-test probabilities) also differed considerably between studies. Participants were recruited from different levels of health care, reflecting real-life variation, which is favorable for the generalisability of results (30). However, all tests were performed on biobanked specimen in research laboratory settings. Broger and colleagues compared FujiLAM read-outs of fresh vs. biobanked samples from adult patients, and while categorical agreement was high, a reduction of positive percentage agreement was observed (31). Studies using FujiLAM on fresh specimens, prospectively, and in real-life settings will need to be conducted in children.

Important subgroups for diagnostics tests include CLHIV and the very young, who are at high risk of dying from TB (2). We found that the two studies reporting FujiLAM's accuracy in CLHIV had contrasting results, with reliable conclusions difficult to draw due to small numbers and overlapping confidence intervals. Analyses stratified by age were only performed in the African studies, but different age cut-offs were used (21, 26), hence direct comparison was not possible. Estimates in the original publications suggest a similar sensitivity, but a decrease in specificity in younger children. An explanation could be contamination in nappy-wearing children, with specificity potentially compromised due to corynebacteria, dust, soil, and stool (29, 32, 33). Future studies should follow strict collection criteria to prevent contamination and describe them in detail. Finally, data on LAM-assays in extrapulmonary cases (EPTB) remain scarce and reported sensitivities of FujiLAM range from 47-94% in adults (34). Extrapulmonary manifestations are more common in children, but only one study recruited those cases and did not show subgroup analysis (22).

The FujiLAM assay was developed to improve the sensitivity of AlereLAM, therefore comparison between the two is scientifically and clinically relevant. Only two studies performed paired head-to-

head comparisons; whereas FujiLAM sensitivity was significantly higher compared to AlereLAM in one study using an MRS, (26) AlereLAM was more sensitive in another (21). Moreover, within each study, most confidence intervals between the two tests overlapped, suggesting a lack of evidence for test superiority. The range of estimates for sensitivity and specificity for FujiLAM in CLHIV in this systematic review against a MRS (53-60% and 76-93%, respectively) was also similar to estimates for AlereLAM from a Cochrane review of HIV positive children (42-56% and 80-95%) (7), the group in whom AlereLAM is currently recommended by the WHO (35). Since these indirect comparisons between different studies can be biased by differences in population and setting, more studies that directly compare AlereLAM and FujiLAM in paired analyses are needed to understand whether FujiLAM could replace AlereLAM as a POC test in children.

All included studies, and thus this review, have limitations and data gaps. The geographical distribution of cohorts included sub-Saharan Africa and Haiti and results may not be generalisable to other regions. Important subgroup analyses could not be performed due to unavailability of data and variable application of definitions, such as test performance in EPTB, CD4-count in CLHIV (except for one study), and specific age-groups. Finally, no comment could be made on the impact of FujiLAM on clinical outcomes such as mortality reduction, as has been shown for AlereLAM in hospitalised HIV-infected adults (36).

This review summarises the current evidence of FujiLAM, with the high specificity demonstrating its potential as a POC rule-in test for diagnosing paediatric TB. It reflects the current state of knowledge, highlighting that more data on FujiLAM in children are needed to understand the diagnostic value of this test in different groups at scale and suggests the priorities to be addressed in forthcoming evaluations. In particular, the need for prospective assessments that directly compare FujiLAM to AlereLAM in real-life settings, recruitment from several geographical regions, and subgroup analyses focusing on CLHIV and EPTB.

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TABLE AND FIGURES

Table 1: Cohort characteristics

		Nicol et al	Nkereuwem et al.	Barrio et al.
Cohort size		241	415	79
Study design		Prospective enrolment Plus enrichment of CLHIV	Prospective enrolment	Prospective enrolment Plus control cohort
Index test	**Comparator**	AlereLAM	AlereLAM	none
	Sample storage	Yes, -80°C	Yes, -80°C	Yes, -20°C
Country		South Africa	Gambia, Mali, Nigeria, Tanzania	Haiti
Health care level of recruitment of study participants		Tertiary hospital	Mixed (community, Tertiary hospital, urban comprehensive health care)	Reference hospital
Age in months (median) Median (IQR)		45.2 (21.2 – 88.8)	67.2 (27.6-111.6)	76 (58–121)
Age categories	**< 5 yrs.**	118 (58%)	194 (47%)	24 (30%)
	≥ 5 yrs.	86 (42%)	221 (53%)	55 (70%)
Male sex		111 (54%)	225 (54%)	51 (65%)
TB status	**Confirmed TB**	84 (41%)	63 (15%)	5
	Unconfirmed TB	81 (40%)	113 (27%)	50
	Unlikely TB	39 (19%)	239 (58%)	24
HIV status	**HIV infected**	40 (20%)	61 (15%)	excluded
	CD4 cells/uL median (IQR)	552 (206–849)	-	-
Malnutrition	**Stunted**	73 (40%)	134 (32%)	12 (21%)

Table 2: Definitions of reference standards and diagnostic classifications

		Nicol et al	Nkereuwem et al.	Barrio et al.
Enrolment criteria	**Inclusion criteria**	- Symptoms suggestive of TB (pulmonary)	- Symptoms suggestive of TB (pulmonary)	- Symptoms suggestive of TB (pulmonary and extrapulmonary) - Controls: negative TST and QFT-GIT, and no signs or symptoms of TB
	Exclusion criteria	- More than 72 hours of TB treatment or prophylaxis - Not a resident in Cape Town	- Not specified - Presense of mediastinal lymphadenopathy alone	- Anti-TB treatment for two or more weeks before enrolment - HIV positivity or other known immunodeficiencies or immunosuppressive treatment
Symptoms of TB		- Cough of any duration and at least one of the following: - Household contact with an infectious tuberculosis source case within the preceding 3 months - Loss of weight or failure to gain weight in the preceding 3 months - Positive tuberculin skin test (TST) - Suggestive CXR	Symptoms suggestive of pulmonary tuberculosis: - Persistent or unremitting cough for more than 2 weeks - and either weight loss, failure to thrive, or persistent unexplained fever	Not specified
TB sampling & microbiological investigations		At least one induced sputum Xpert MTB/RIF or Xpert MTB/RIF Ultra ® & MGIT	At least one induced sputum Xpert MTB/Rif Ultra® (all sites) & MGIT/LJ (not Nigerian site)	Three consecutive respiratory samples (induced or nasopharyngeal/nasogastric aspiration) Smear microscopy, Xpert MTB/RIF if positive smear microscopy OR abnormal Xray
TB case classification	**Confirmed TB**	Any induced sputum culture or Xpert MTB/RIF positive for M. tuberculosis	Bacteriological confirmation of Mycobacterium tuberculosis (culture, Xpert MTB/RIF assay, or both) from at least one respiratory specimen	Any sputum Xpert MTB/RIF positive for M. tuberculosis
	Unconfirmed TB	All children not defined as confirmed or unlikely TB	Bacteriological confirmation not obtained, and at least one (if TST/QFT-GIT pos) OR two (if TST/QFT-GIT neg) of the following: - Symptoms or signs suggestive of tuberculosis - Chest radiograph consistent with tuberculosis - Close tuberculosis exposure - Positive response to tuberculosis treatment (requires documented positive clinical response to tuberculosis	Bacteriological confirmation not obtained And positive TST/QFT-GIT and at least one of the following - X-rays consistent with TB - signs and symptoms of TB - close TB exposure, or - positive response to TB treatment OR if TST/QFT-GIT negative at least - two clinical criteria - X-rays consistent with TB - signs and symptoms of TB - close TB exposure, or - positive response to TB treatment
	Unlikely TB	All of the following: - TB culture negative - No tuberculosis treatment given - Documented improvement of symptoms and signs at follow up visit	Bacteriological confirmation not obtained and criteria for unconfirmed tuberculosis not met	Only evidence of M. tuberculosis infection OR presented only one clinical criterion compatible with TB Controls: negative TST and QFT-GIT, and no signs or symptoms of TB
Definition of reference standards	**Microbiological reference standard (MRS)**	Positive = confirmed TB Negative = unconfirmed & unlikely TB	Positive = confirmed TB Negative = unconfirmed & unlikely TB	Positive = confirmed TB Negative = unconfirmed & unlikely TB + controls
	Composite reference standard (CRS)	Positive = confirmed & unconfirmed TB Negative = unlikely TB	Positive = confirmed & unconfirmed TB Negative = unlikely TB	Positive = confirmed & unconfirmed TB Negative = unlikely TB + controls

Table 3: Diagnostic accuracy estimates as reported by the original publications, with 95% confidence intervals (CI)

	Barrio et al.									Nicol et al									Nkereuwem et al.								
	Total	TP	FP	FN	TN	Sens		Spec		Total	TP	FP	FN	TN	Sens		Spec		Total	TP	FP	FN	TN	Sens		Spec	
	(n)	(n)	(n)	(n)	(n)	%	95%CI	%	95%CI	(n)	(n)	(n)	(n)	(n)	%	95%CI	%	95%CI	(n)	(n)	(n)	(n)	(n)	%	95%CI	%	95%CI
Overall																											
MRS	79	3	5	2	69	60	17–93	95	73-100	204	35	10	49	110	41	32–52	92	85–95	415	40	55	23	297	65	44–85	84	77–89
CRS	73	6	2	49	22	11	5-23	92	72-99	204	44	1	121	38	26	21–34	97	87–100	415	58	37	118	202	33	25–42	83	72-92
HIV positive																											
MRS	-	-	-	-	-	-	-	-	-	40	15	1	10	14	60	41–77	93	70–99	61	8	11	7	35	55	29–82	76	62–87
CRS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	61	14	5	30	12	32	18.9–47.0	71	47–92
HIV negative																											
MRS	-	-	-	-	-	-	-	-	-	164	20	9	39	96	34	23–47	91	85–95	344	31	40	15	258	68	42–88	86	79–91
CRS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	344	43	28	86	187	33	24–44	86	76–92
Age										**<2yrs**									**<5yrs**								
MRS	-	-	-	-	-	-	-	-	-	59	9	7	13	30	41	23–61	81	66–91	194	16	35	10	133	62	37–86	79	69–86
CRS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	194	28	23	55	88	33	20–48	78	67–87
Age										**>2yrs**									**<5yrs**								
MRS	-	-	-	-	-	-	-	-	-	145	26	3	36	80	42	30–54	96	90–99	221	24	20	13	164	67	40–90	89	82–94
CRS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	221	30	14	63	114	33	22–44	88	76–96

Table 4: Diagnostic test performance stratified by clinical case definition

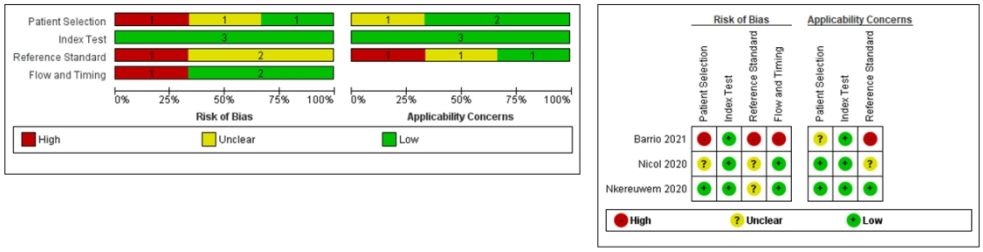
	All	Confirmed TB n/N (%)				Unconfirmed TB				Unlikely TB				Controls			
FujiLAM result		pos		neg		pos		neg		pos		neg		pos		neg	
Barrio	79	3/5	60%	2/5	40%	3/50	6%	47/50	94%	1/4	25%	3/4	75%	1/20	5%	19/20	95%
Nicol	204	35/84	42%	49/84	58%	9/81	11%	72/81	89%	1/39	3%	38/39	97%	-	-	-	-
Nkeurewen	415	40/63	63%	23/63	37%	18/113	16%	95/113	84%	37/239	15%	202/239	85%	-	-	-	-

Figure 1: Assessment of study quality of FujiLAM paediatric studies using the QUADAS-2 framework. Risk of bias and applicability concerns graph (A) and summary (B): review authors' judgements about each domain presented as percentages across included studies

Figure 2: Forest Plots of performance of lateral flow LAM assays against MRS and CRS. Performance estimates were calculated using the raw numbers provided in the studies and visualised using RevMan (20).

Author's contribution:

LO and NK conducted the literature search, screening of abstracts, data extraction, and analyses, supported by EMB and RS. The manuscript was written mainly by LO and NK, and reviewed and edited by EMB, MR, NH, and RS.



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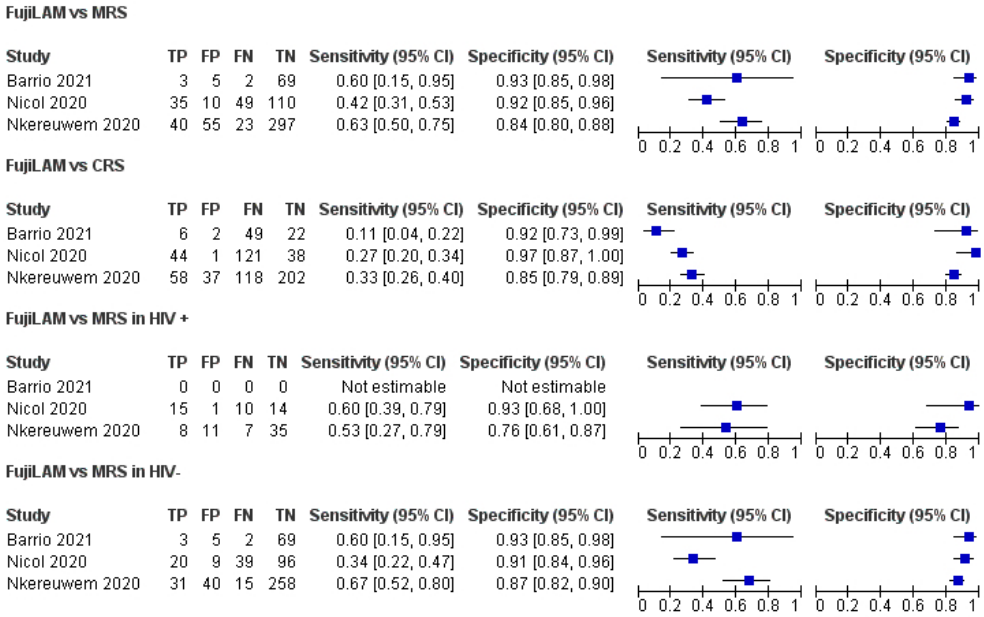

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Search strategy for supplementary materials

PubMED

(("child*"[Title/Abstract] OR "infant*"[Title/Abstract] OR "adolescent*"[Title/Abstract] OR "paediatric*"[Title/Abstract] OR "pediatric*"[Title/Abstract]) AND ("LAM"[Title/Abstract] OR "lipoarabinomannan"[Title/Abstract])) AND (("tuberculos*"[Title/Abstract] OR "TB"[Title/Abstract] OR MTB[Title/Abstract] OR PTB[Title/Abstract] OR mycobacterium tuberculosis[MeSH Terms] OR tuberculosis[MeSH Terms]))

EMBASE

1. mycobacterium tuberculosis/
2. tuberculosis/ or lung tuberculosis/
3. (tuberculos*).mp.
4. MTB.mp.
5. Tb.mp.
6. (child*).mp.
7. (infant*).mp.
8. (adolescent*).mp.
9. (paediatric*).mp.
10. (pediatric*).mp.
11. Lipoarabinomannan/
12. LAM.mp.
13. Lipoarabinomannan.mp.
14. 1 or 2 or 3 or 4 or 5
15. 6 or 7 or 8 or 9 or 10
16. 11 or 12 or 13
17. 14 and 15 and 16
18. (exp animals/ or nonhuman/) not human/
19. 17 not 18
20. conference*.pt
21. 19 not 20