EUROPEAN RESPIRATORY *journal*

FLAGSHIP SCIENTIFIC JOURNAL OF ERS

Early View

Research letter

Diagnostic sensitivity of SILVAMP TB-LAM (FujiLAM) point-of-care urine assay for extra-pulmonary tuberculosis in people living with HIV

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Please cite this article as: Kerkhoff AD, Sossen B, Schutz C, *et al*. Diagnostic sensitivity of SILVAMP TB-LAM (FujiLAM) point-of-care urine assay for extra-pulmonary tuberculosis in people living with HIV. *Eur Respir J* 2019; in press (https://doi.org/10.1183/13993003.01259-2019).

Diagnostic sensitivity of SILVAMP TB-LAM (FujiLAM) point-of-care urine assay for extra-pulmonary tuberculosis in people living with HIV

Short title: Diagnostic sensitivity of FujiLAM for EPTB

Take-home message (256 characters): FujiLAM point-of-care rapid urine test for TB detects a large proportion of pulmonary and traditionally difficult-to-diagnose extra-pulmonary forms of TB in hospitalized patients with advanced HIV.

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Article type: Research letter

To the Editor:

Diagnosing tuberculosis (TB) in people living with HIV (PLHIV) remains challenging in part, because of its diversity of clinical manifestations, including high rates of extra-pulmonary and disseminated disease [1]. In particular, disseminated TB, involving multiple organ systems, is associated with high mortality but often presents non-specifically, which may hinder prompt diagnosis [2, 3]. Xpert MTB/RIF (Xpert, Cepheid, Sunnyvale, US), is currently recommended by the World Health Organization (WHO) as the first line assay for evaluating a subset of extra-pulmonary TB disease (EPTB) manifestations[4]. To detect specific forms of EPTB such as pleural TB, TB meningitis or TB lymphadenitis, Xpert may require an invasive sample to be collected, which often limits its use for EPTB detection to hospitals where appropriate equipment is available and invasive sampling can be safely performed. Furthermore, even when concomitant pulmonary disease is present, it can be very difficult to obtain sputum in the sickest HIV patients to submit for Xpert testing [5, 6]. Therefore, an urgent priority for improving TB detection among PLHIV remains the development of rapid, point-of-care (POC) assays that use an easily obtainable clinical specimen, such as urine, and that have good diagnostic accuracy for both pulmonary and extra-pulmonary TB (EPTB), including disseminated disease [7].

The commercially-available Alere Determine TB LAM (AlereLAM, Abbott, Chicago, US) assay is a rapid, inexpensive, urinary POC TB test [8]. While its use is associated with a mortality benefit in severely ill and immunocompromised PLHIV [9, 10], it has only moderate sensitivity that is limited to patients with low CD4 counts, which has led to limited programmatic uptake [11]. We have previously reported on the Fujifilm SILVAMP TB LAM (FujiLAM) POC assay that, similar to AlereLAM, detects the presence of lipoarabinomannan (LAM) in urine [12]. It offers on average 30% improved sensitivity for detecting TB (independent of whether it is PTB or EPTB) compared to AlereLAM across subgroups stratified by CD4 strata, while maintaining high specificity. Here we report the sensitivity of FujiLAM in comparison to AlereLAM specifically for detecting EPTB in the same patient cohorts.

This post-hoc analysis utilized data from two previously published, prospective cohort studies of adults (>18 years) living with HIV who were admitted to South African district hospitals on the outskirts of Cape Town [13, 14]. Cohort A enrolled patients without a current TB diagnosis regardless of presenting signs or symptoms, and independent of CD4 count

[13]. Cohort B enrolled patients with a CD4 count <350 cells/μL in whom TB was considered the most likely diagnosis on admission [14]. A third previously published cohort was not included in the present analysis as it excluded patients with exclusively extra-pulmonary TB disease [12]. Informed consent was obtained from patients who had capacity or regained capacity and all study-related activities were approved by the Human Research Ethics Committee of the University of Cape Town.

Patients were systematically evaluated for the presence of TB. Whenever possible, patients provided two sputum samples, a blood sample and a urine sample for mycobacteriology; those unable to produce sputum or urine samples were not excluded from study enrollment. Sputum specimens were tested using smear fluorescence microscopy, MGIT liquid culture (Becton Dickinson, Franklin Lakes, USA), and Xpert MTB/RIF Version G4. Blood specimens were tested using BACTEC™ Myco/F Lytic culture (Becton Dickinson, Franklin Lakes, USA). Sediments from urine specimens were tested using Xpert after centrifugation of 30-40ml. The routine clinical team obtained additional specimens (sputum and non-sputum) as clinically indicated. FujiLAM and AlereLAM were performed on biobanked urine samples according to manufacturers' instructions and read by two investigators blinded to patient status and all other test results [12]. Microbiologically-confirmed TB was defined by the detection of *M. tuberculosis (MTB)* on any clinical specimen using either culture or Xpert. All patients with microbiologically-confirmed TB were classified into one of three mutually-exclusive groups: pulmonary TB (PTB) (TB detected in sputum only), EPTB (TB detected in extra-pulmonary specimen(s) only), or PTB+EPTB (TB detected in both sputum and at least one extra-pulmonary specimen). The sensitivity (and corresponding 95% confidence intervals) of FujiLAM and AlereLAM was calculated for each form of TB as well as for individual forms of EPTB.

Of 1,079 eligible patients, 111 had a TB status that could not be classified, 90 did not have urine samples, and 6 had missing urine results; therefore, 872 patients (420 from cohort A and 659 from cohort B) had complete results and were included in this analysis. The median age was 36 (IQR 30-43) years, 54% were female, the median CD4 count was 84 (IQR 32-188) cells/μL, and 45% had previously been treated for TB. Among 872 patients, 553 (138 from cohort A and 415 from cohort B) had microbiologically-confirmed TB (prevalence 56%) on at least one specimen, 88 (37 from cohort A and 51 from cohort B) had possible TB and 231 (189 from cohort A

and 42 from cohort B) had no evidence of TB. Of those with confirmed TB, 126/553 (23%) had PTB, 156/553 (28%) had EPTB, and 271/553 (49%) had both PTB+EPTB. The urine LAM assays performed best in those with PTB+EPTB, with FujiLAM detecting 91% (95%CI: 87-94; 246/271) of cases compared to 61% (95%CI: 55-67; 165/271) using AlereLAM (**Figure 1a**). In patients with PTB or EPTB only, FujiLAM detected 60% (95%CI: 51-69; 76/126) and 67% (95%CI: 59-75; 105/156) of cases, respectively, which was compared to 19% (95%CI: 12-27; 24/126) and 41% (95%CI:33-49; 64/156), respectively for AlereLAM (**Figure 1a**).

The sensitivity for FujiLAM across different extra-pulmonary forms of TB disease ranged from 47-94% as shown in **Figure 1b**. Notably, FujiLAM detected TB in 94% (95%CI: 90-97) of patients with TB mycobacteremia and 88% (95%CI: 84-92) of those with TB confirmed by urine Xpert or culture. It also demonstrated moderate sensitivity in patients with microbiologically-confirmed pleural TB (68%, 95%CI: 55-80) and with TB meningitis (47%, 95%CI: 24-71). AlereLAM's sensitivity ranged from 16-70% and performed best in those with TB mycobacteremia (70%, 95%CI: 64-76) and TB confirmed by urine Xpert or culture (61%, 95%CI: 55-67).

Overall, FujiLAM showed substantially higher sensitivity over the commercially available AlereLAM, for detecting both pulmonary and extra-pulmonary TB in HIV-inpatients. This suggests that FujiLAM may have clinical utility as a first-line test for the rapid detection of TB in HIV-patients, independent of disease location. Given that a large proportion of patients with HIV-associated TB have EPTB and a diagnosis may only be possible using a non-sputum sample that may be challenging to obtain, an up-front FujiLAM test could substantially reduce the time to diagnosis. FujiLAM was able to detect TB in 67% (n=105/156) of patients who could not produce a sputum sample or did not have evidence of pulmonary disease; such patients comprised 28% of the study cohort.

FujiLAM performed best in those with TB mycobacteremia as well as those with concomitantly positive sputum and non-respiratory cultures, detecting >90% of cases. *Mycobacterium tuberculosis* bacteremia is one of the most common blood stream infections among PLHIV in sub-Saharan Africa [3] and such patients have an extremely high mortality risk. FujiLAM's excellent performance in those with mycobacteremia suggests a mechanistic association between disease dissemination and urinary LAM. This finding is supported by our recent preliminary study that showed a good association between detection of LAM in urine and serum of TB

patients, independent of HIV-status [15]. However, even for patients with forms of disease such as pleural TB and TB meningitis that may be compartmentalized, FujiLAM had moderate sensitivity, which could add substantial benefit in these cases. Taken together, these findings suggest that LAM antigenuria is likely indicative of glomerular filtration of circulating LAM (or LAM fragments) in addition to renal TB [16]. Further research, that aims to detect LAM with ultra-sensitive platforms, as well as characterization of LAM structure in urine is needed to better understand the mechanisms by which LAM enters the bloodstream and urine. This may help to further refine urine-based diagnostics and catalyze the development of blood-based assays. As the overall load of mycobacteria is expected to be higher in HIV-positive patients, our findings should not be generalized to HIV-negative individuals with EPTB.

Patients were not systematically evaluated for the presence of EPTB beyond mycobacterial blood cultures and urine Xpert; additional systematic sampling (e.g., pleural fluid or cerebrospinal fluid) could not be justified as it would require invasive sampling where this was not clinically indicated. Coupled with challenges in universally obtaining clinically-indicated samples (e.g., sputum), some misclassification of TB category (PTB, EPTB or PTB+EPTB) is likely present. Furthermore, because the overall cohort was severely immunocompromised (increasing the likelihood of disease dissemination), and because those with lower, site-specific mycobacterial burdens (CSF or pleural fluid) may not have been diagnosed by Xpert or culture, the true sensitivity of urinary FujiLAM (and AlereLAM) for localized extra-pulmonary disease is possibly an overestimate and may not be generalizable to all patients with these forms of disease. However, the additional specimens collected by the routine clinical team mirrored common practice in settings with a high burden HIV-associated TB and followed clinical symptomatology. Finally, we did not evaluate the specificity of FujiLAM for specific disease forms given a lack of systematic EPTB sampling. We have previously reported the cohort-specific specificity as well as the estimated specificity using a Bayesian bivariate random-effects model in three cohorts: using a composite reference standard, the specificity of FujiLAM was 95.7% (95%CI: 92.0-98.0) compared to 98.2% (95%CI: 95.7%-99.6) for AlereLAM [12].

In conclusion, our results suggest that the POC FujiLAM has good sensitivity for detecting both pulmonary and extra-pulmonary forms of TB in patients with advanced HIV, in which conventional diagnostics may be slow, require infrastructure and equipment, or rely on samples that are difficult to obtain. While appropriate sampling should still be undertaken to allow for drug susceptibility testing, FujiLAM

may be an appropriate first microbiological TB investigation for all hospitalized PLHIV, allowing for more rapid initiation of anti-TB therapy.

Author Contributions: TB, BS, ADK, CS, EIR, MPN, GM and CMD designed the parent study and it was overseen by TB, BS, ET, AT, MPN, GM and CM. BS, ADK, CS, RB, AW, and GM coordinated the individual study sites. ADK, TB, CD designed the statistical analysis and ADK undertook the statistical analyses. ADK, TB and BS developed the first manuscript draft. All authors contributed to interpretation of data and editing of the article and approved the final version of the manuscript before submission.

Conflicts of interests: TB and CD were previously employed by FIND and EIR, AT, EM and SS are currently employed by FIND. FIND is a not-for-profit foundation that supports the evaluation of publicly prioritized tuberculosis assays and the implementation of WHO-approved (guidance and prequalification) assays using donor grants. FIND has product evaluation agreements with several private sector companies that design diagnostics for tuberculosis and other diseases. These agreements strictly define FIND's independence and neutrality vis-a-vis the companies whose products get evaluated and describe roles and responsibilities. TB reports a patent in the field of lipoarabinomannan detection. All other authors declare no competing interests.

Acknowledgments: The investigators are grateful to the clinical and administrative staff of the Western Cape Department of Health as well as the patients who contributed to this data. The authors also wish to thank the late Stephen D. Lawn who designed and led the Cohort1 study. This work was funded by Global Health Innovative Technology (GHIT) Fund grant number G2015-201, UK Department for International Development (DFID) grant number 300341-102, Dutch Ministry of Foreign Affairs grant number PDP15CH14, Bill and Melinda Gates Foundation grant number OPP1105925, Australian Department of Foreign Affairs and Trade (DFAT) grant number 70957 and the German Federal Ministry of Education and Research (BMBF) through KfW. The Cohort1 study was funded by the Wellcome Trust (088590 and 085251). BS received salary support from the Wellcome Trust (grant number 088316). GM was supported by Wellcome Trust (098316 and 203135/Z/16/Z), the South African Research Chairs Initiative of the Department of Science and Technology and National Research Foundation (NRF) of South Africa (Grant No 64787), NRF incentive funding (UID: 85858) and the South African Medical Research Council through its TB and HIV Collaborating Centres

Programme, with funds received from the National Department of Health (RFA#SAMRC-RFA-CC:TB/HIV/AIDS-01-2014). CS received funding from the South African Medical Research Council through the National Health Scholarship Programme. ADK was supported by the National Institute of Allergy and Infectious Diseases (Grant No T32 AI060530). The funders had no role in the study design, data collection, data analysis, data interpretation, or writing of this report. The opinions, findings and conclusions expressed in this manuscript reflect those of the authors alone.

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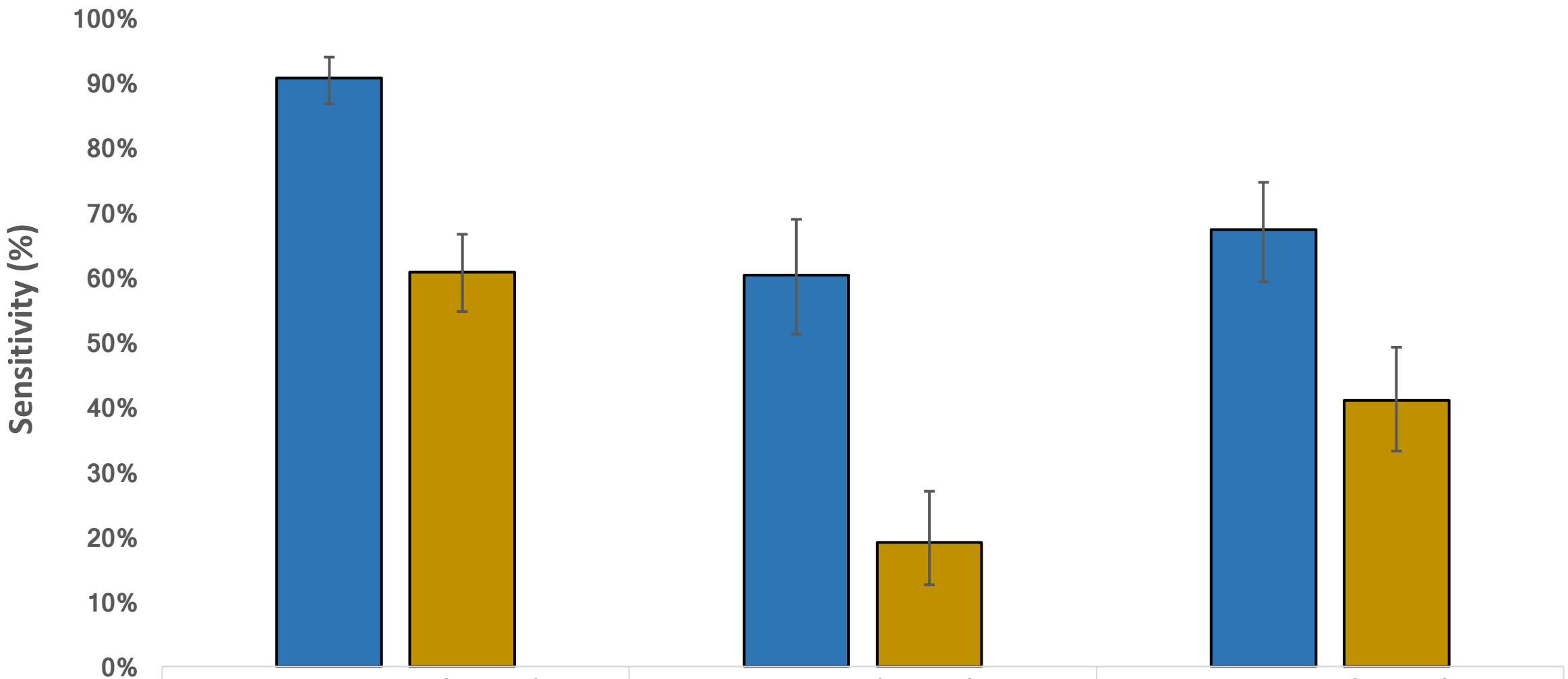
Figure legend:

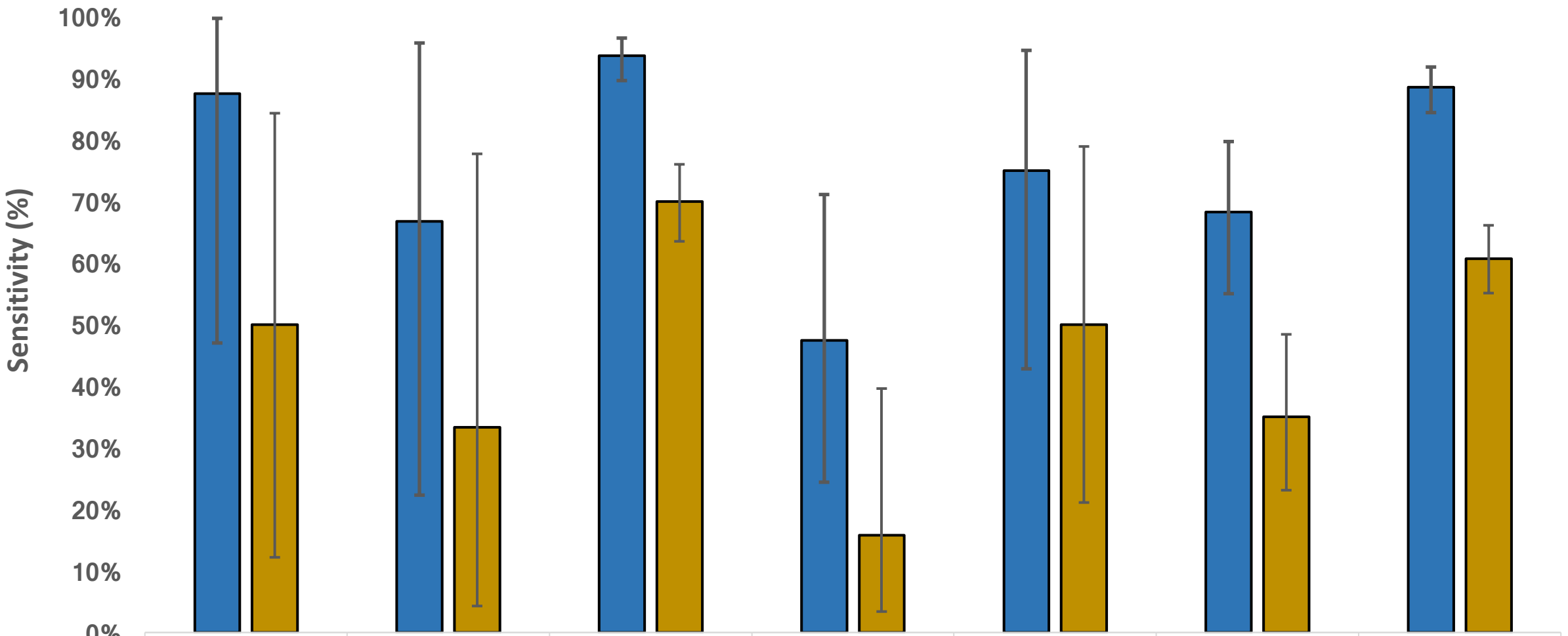
Figure 1. The diagnostic sensitivity of FujiLAM and AlereLAM by **a)** type of TB disease (pulmonary, extra pulmonary or both; n=553), **b)** site of disease involvement in patients with confirmed EPTB. Bars represent 95% confidence intervals. The numbers in parenthesis denote 95% confidence intervals. *Of note, the same patient may have multiple sites of confirmed disease (e.g., pulmonary, blood, urine, etc). Sputum, blood and urine were obtained from all patients whenever possible and additional specimens were obtained at this discretion of routine medical team, however the ability to produce sputum was not a requirement for study entry. This analysis was limited to among those with both FujiLAM and AlereLAM results available.

Figure 1a.



	PTB and EPTB (n=271)	PTB only (n=126)	EPTB only (n=156)
FujiLAM	91% (87-94; 246/271)	60% (51-69; 76/126)	67% (59-75; 105/156)
AlereLAM	61% (55-67; 165/271)	19% (12-27; 24/126)	41% (33-49; 64/156)

Figure 1b.



	Abscess culture (n=8)	Ascites culture (n=6)	Blood culture (n=220)	CSF Xpert/ culture (n=19)	Lymph node culture (n=12)	Pleural Fluid Xpert/ culture (n=60)	Urine Xpert/ culture (n=313)
FujiLAM	88% (47-100)	67% (22-96)	94% (90-97)	47% (24-71)	75% (43-95)	68% (55-80)	88% (84-92)
AlereLAM	50% (16-84)	33% (4-78)	70% (64-76)	16% (3-40)	50% (21-79)	35% (23-48)	61% (55-66)