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Diagnostic accuracy of the Cepheid 3-gene host response fingerstick blood test in a prospective, multi-site study: interim results

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Summary

This study is the first to assess the Cepheid MTB-HR prototype cartridges in a multi-site prospective study. This is the first test shown to reach WHO target product profile for a triage test for TB regardless of geographical location and HIV status.

Abstract:

Background: The development of a fast and accurate, non-sputum-based point-of-care triage test for tuberculosis (TB) would have a major impact on combating the TB burden worldwide. A new fingerstick blood test has been developed by Cepheid (the Xpert-MTB-Host Response (HR)-Prototype), which generates a 'TB score' based on mRNA expression of 3 genes. Here we describe the first prospective findings of the MTB-HR prototype.

Methods: Fingerstick blood from adults presenting with symptoms compatible with TB in South Africa, The Gambia, Uganda and Vietnam was analysed using the Cepheid GeneXpert MTB-HR prototype. Accuracy of the Xpert MTB-HR cartridge was determined in relation to GeneXpert Ultra results and a composite microbiological score (GeneXpert Ultra and liquid culture) with patients classified as having TB or other respiratory diseases (ORD).

Results: When data from all sites (n=75 TB, 120 ORD) were analysed, the TB score discriminated between TB and ORD with an AUC of 0·94 (CI, 0·91-0·97), sensitivity of 87% (CI, 77-93%) and specificity of 94% (88-97%). When sensitivity was set at 90% for a triage test, specificity was 86% (CI, 75-97%). These results were not influenced by HIV status or geographical location. When evaluated against a composite microbiological score (n=80 TB, 111 ORD), the TB score was able to discriminate between TB and ORD with an AUC of 0·88 (CI, 0·83-0·94), 80% sensitivity (CI, 76-85%) and 94% specificity (CI, 91-96%).

Conclusions: Our interim data indicate the Cepheid MTB-HR cartridge reaches the minimal target product profile for a point of care triage test for TB using fingerstick blood, regardless of geographic area or HIV infection status.

Keywords: Tuberculosis; diagnostics; Cepheid 3-gene Host-Response prototype; multi-site; fingerstick blood; Triage test

Introduction:

Development of a non-invasive, non-sputum-based point-of care test for tuberculosis (TB) is essential for providing timely diagnosis and reducing morbidity and mortality. While there are an estimated 10 million new cases of TB each year, close to 4 million of these are missed due to lack of adequate diagnostics, further fueling *Mycobacterium tuberculosis* (Mtb) transmission [1]. In low-and-middle-income countries (LMIC), inadequate diagnostic tools not only lead to underdiagnosis but also misdiagnosis, aberrant use of antibiotics and a large delay between symptom onset and start of treatment. During the COVID-19 pandemic, this delay in diagnosis has been further exacerbated due to redirection of healthcare needs away from TB, lockdown limiting access to facilities, reduced TB testing in favor of COVD-19 testing, and increased stigma significantly affecting healthcare-seeking behaviors.

Current gold-standard tests for TB based on detection of the pathogen, are limited by cost, infrastructure requirements and lack of sensitivity particularly in people living with HIV (HIV+) and children who tend to have paucibacillary disease. Several point-of-care (POC) tests are in development, including detection of blood host transcriptomic or protein markers or bacterial antigens secreted in the urine. Recent studies have identified a urinary Lipoarabinomannan (LAM) test with up to 75% sensitivity and excellent specificity in both HIV+ and HIV- individuals [2,3].

Host transcriptomic signatures also hold great promise for development of diagnostic and/or triage tests. In 2016, Sweeney et al. identified a combinatory score (TB score) based on blood mRNA expression levels of three differentially expressed genes (Guanylate Binding Protein (GBP)5, Dual Specificity Phosphatase (DUSP)3, and Krüppel-like Factor (KLF)2), for discrimination between active TB and other diseases [4] and was found to approach the TPP for a non-sputum-based triage test across 3 independent prospective cohorts [5]. Both GBP5 and DUSP3 are involved in the pro-inflammatory response and are upregulated in TB: inducible GBP5 has been shown to mediate the antiviral interferon response during Influenza A Virus infection [6] and HIV-1 [7], while DUSP3 plays a nonredundant role as a regulator of innate immune responses by mechanisms involving the control

of ERK1/2 activation, TNF secretion, and macrophage polarization [8]. In contrast, KLF2, which regulates the inflammatory response by inhibiting the activation of monocytes [9], is downregulated in TB. This 3-gene signature has been incorporated into an automated qPCR test using the GeneXpert platform (Cepheid, USA). This Xpert-MTB-HR-Prototype quantifies the expression of the 3 transcripts in a whole-blood sample and then computes a TB score based on Ct values using an in-built algorithm. A recent publication analyzed the performance of the Xpert-MTB-HR-Prototype cartridge on stored PAXgene samples from HIV+ individuals from South Africa and Peru, and showed good classification as a triage test [10], but performance in another retrospective study in Brazil failed to reach the target product profile (TPP) criteria for a triage test [11].

The aim of this study was to perform prospective evaluation of the Xpert-MTB-HR-Prototype against GeneXpert Ultra and a composite microbiological score (Xpert Ultra and culture). Our interim results indicate the MTB-HR-prototype is the first POC test to reach WHO TPP for a triage test for TB.

Methods:

Ethics statement:

Local ethics approval was obtained through the MRC/LSHTM/Gambian government joint ethics committee (MRCG at LSHTM); the Oxford Tropical Research Ethics Committee, the institutional review board at Pham Ngoc Thach Hospital, and the ethics committee of the Ministry of Health, Vietnam (OUCRU); the Stellenbosch University Health Research Ethics Committee (SUN) and the Uganda National Council for Science and Technology (MAK). Written informed consent was obtained from all participants prior to sample collection. All participants had blood sampling performed prior to knowledge of TB status. TB status was determined using GeneXpert Ultra (Cepheid, USA) and Liquid culture (MGIT; Becton Dickinson, USA).

Patient recruitment and classification:

Patients were consecutively recruited from local health clinics at each of the sites. Inclusion criteria included cough > 2 weeks and at least one other symptom suggestive of TB (ie weight loss, hemoptysis, night sweats, fever). Sputum was taken for microbiological anlaysis and fingerstick

blood for host gene response analysis. Patients with either a positive GeneXpert Ultra result or a positive culture result were classified as having TB while those with both negative GeneXpert Ultra and culture were considered to have Other Respiratory Diseases (ORD). However, due to the possible detection of false-positive results with GeneXpert Ultra [12], patients with a reading of 'Trace' on GeneXpert Ultra who were culture positive were considered to have TB. Patients negative for both Xpert Ultra and culture were considered to have ORD. No patients with a positive GeneXpert Ultra but negative culture had prior TB.

GeneXpert MTB Host-Response analysis:

Two hundred (200) µl of fingerstick blood (FSB) was collected with a minivette containing an anticoagulant (EDTA, Becton Dickinson, USA) transferred into an EDTA-microtainer (Becton Dickinson, USA), and inverted to mix. 100µl of the sample was added to the Cepheid MTB Host Response (MTB-HR) cartridge (donated by Cepheid, USA) and loaded into the GeneXpert machine. Ct values for individual genes (DUSP, GBP5 and KLF2) were obtained together with a TB score determined by: (Ct GBP5 + Ct DUSP3)/2 - Ct KLF2.[4] If an invalid result was obtained, a second aliquot of 100µl was added to a new cartridge and the sample re-tested.

HIV testing:

All participants without known HIV infection received voluntary counselling and testing using a rapid fingerstick blood HIV-1/2 test (Alere, USA). If the rapid test was positive, the result was confirmed either with serology testing or a second rapid test. CD4 counts were determined for all HIV+ individuals at baseline.

Statistical analysis:

Data were analysed using GraphPad Prism v8.1 (Software MacKiev, USA) or R (version 4.1.0) with the pROC package (version 1.17.0.1). Mann-Whitney U-tests were performed to compare Ct values for the transcripts of interest and the TB scores between total TB and ORD patients and at individual

sites. Receiver operator characteristic (ROC) curves were used to determine the accuracy of each gene for the classification of TB, including area under the ROC curve (AUC), sensitivity and specificity. A Chi-square test was used to compare sex, prior TB, and HIV status between the groups. WHO thresholds for a triage test are minimum 90% sensitivity, 70% specificity; ideal 95% sensitivity, 80% specificity [13]. Youden's Index was used to determine optimal sensitivity and specificity for the full cohort. Values for sensitivity and specificity in terms of WHO TPP were estimated using 200 repeats of 5-fold cross-validation. For each hold-out fold, specificity was determined from the training folds' ROC curve at the lowest sensitivity ≥ 0.9 for a triage test. Mean sensitivity and the 95% confidence interval were calculated from the 1000 sensitivities thus obtained. Confidence intervals for ROC curve AUCs were obtained via 5000 bootstrap replicates.

Results:

Participant information:

We recruited 224 participants (from a total of 1200 expected participants for all sites) between December 2020 and May 2021. Of these, 28 participants did not have results for the Cepheid MTB-HR cartridges as recruitment began prior to obtaining the cartridges and 1 participant did not have a GeneXpert Ultra result. Data from the remaining 195 participants are presented.

We analysed the MTB-HR-prototype using fingerstick blood samples from a total of 75 patients with Xpert ultra positive results (TB) and 120 with Xpert ultra negative results (ORD) (Table 1). 20% of the TB group were females compared to 43% of the ORD group (p=0.019; Table 1). Additionally, 5.3% of the TB group were HIV+ compared to 17% of the ORD group (not significant) with a median [interquartile range (IQR)] of 379 [90-793] CD4 cells/µl for TB and 344 [179-689] CD4+ T cells/µl for ORD (ns). No significant difference in age was seen between the groups with median [IQR] of 30 [26-38] years for the TB group and 36 [28-45] years for the ORD group (Table 1). 2 patients (3%) in the TB group and 8 (7%) in the ORD group had a history of prior TB (ns; Table 1). For the GeneXpert Ultra positive (TB) group, 51% had a reading of 'high', 20% of 'medium', 19% of 'low' and 10% of 'trace or very low' (Table 1).

Analysis of data from all sites:

Analysis of individual genes for all participants, showed significantly lower Ct values (higher input mRNA) for both DUSP3 and GBP5 genes in TB compared to ORD patients ($p<0.0001$ for both; Figs. 1A, B). No difference in KLF2 was seen between the groups (Fig. 1C) thus when the TB score was automatically calculated, it was significantly lower in TB compared to ORD patients ($p<0.0001$; Fig. 1D).

When Xpert-MTB-HR was evaluated against Xpert Ultra for the full cohort, the TB score discriminated between TB and ORD with an AUC of 0·94 (CI, 0·91-0·97), sensitivity of 87% (CI, 77-93%) and specificity of 94% (88-97%) (Fig. 2A, black line). Considering MTB-HR as a triage test, at a sensitivity of 90%, specificity was 86% (CI, 75-97%) which meets the minimal TPP criteria for a triage test. Further analysis revealed that GBP5 alone could discriminate between TB and ORD with an AUC of 0·93 (0·88-0·97) with a sensitivity of 90% and specificity of 86% (CI, 83-90%), thus also meeting the minimum TPP criteria for a triage test (Fig. 2B, black line). Analysis of DUSP3 alone resulted in an AUC of 0·86 with a sensitivity of 89% (CI, 80-95%) but reduced specificity of 61% (CI, 52-69%) (data not shown). Thus, only results for TB score and GBP5 are shown for the remaining analyses.

When GeneXpert Ultra trace results were excluded (n=68 TB, 120 ORD), we found a slightly higher AUC for the TB score of 0·95 (CI, 0·92-0·98) and for GBP5 of 0·94 (CI, 0·90-0·98) (data not shown). When HIV+ participants were excluded (n= 67 TB, 97 ORD), there was no change in the AUC for the TB score (Fig. 2A, green line) or GBP5 alone (Fig. 2B, green line), with a sensitivity of 91% for both and specificity of 83% (CI, 70-96%) for the TB score and 86% (CI, 80-92%) for GBP5.

When the Xpert-MTB-HR was evaluated against a composite microbiological score (n=80 TB, 98 ORD), the TB score was able to discriminate between TB and ORD with an AUC of 0·88 (CI, 0·83-0·94), a sensitivity of 80% (CI, 76-85%) and a specificity of 94% (91-96%). Setting sensitivity to 90% for a triage test, specificity reduced to 58% (CI, 48-68%). GBP5 alone was able to discriminate

between TB and ORD with an AUC of 0·87 (CI, 0·81-0·93), a sensitivity of 86% (CI, 77-92%) and specificity of 76% (CI, 66-83%) (Figs. 2C and D).

Analysis of individual sites:

When the TB score was evaluated against GeneXpert Ultra for Gambian samples (n=22 TB, 35 ORD), we found an AUC of 0·94 (CI, 0·87-1·00) (Fig. 3A, black line). Considering Xpert-MTB-HR as a triage test, at a sensitivity of 94%, specificity was 91% (CI, 86-96%). When GBP5 was analyzed alone, we found an AUC of 0·96 (CI, 0·91-1·00) (Fig. 3B, black line) with a sensitivity of 91% (CI, 72-98%) and specificity of 91% (CI, 78-97%). When the TB score was evaluated against a composite microbiological score (n=25 TB, 31 ORD), we found an AUC of 0·83 (CI, 0·70-0·96) with a sensitivity of 88% (CI, 70-96%) and a specificity of 84% (CI, 67-93%).

When the TB score was evaluated against GeneXpert Ultra for South African samples (n=6 TB, 32 ORD), we found an AUC of 0·89 (CI, 0·69-1·00) (Fig. 3A, green line). Considering Xpert-MTB-HR as a triage test, at a sensitivity of 95%, specificity was 69% (CI, 31-100%). When GBP5 was analyzed alone, we found an AUC of 0·88 (CI, 0·73-1·00) (Fig. 3B, green line) with a sensitivity of 83% (CI, 44-99%) and specificity of 91% (CI, 76-97%). When the TB score was evaluated against a composite microbiological score (n=6 TB, 32 ORD), we found an AUC of 0·89 (CI, 0·73-1·00) with a sensitivity of 83% (CI, 44-99%) and specificity of 94% (CI, 80-99%).

When the TB score was evaluated against GeneXpert Ultra for Ugandan samples (n=17 TB, 33 ORD), we found an AUC of 0·94 (CI, 0·85-0·99) (Fig. 3A, red line). Considering Xpert-MTB-HR as a triage test, at a sensitivity of 93%, specificity was 78% (CI, 67-89%). When GBP5 was analyzed alone, we found an AUC of 0·89 (CI, 0·78-1·00) (Fig. 3B, red line) with a sensitivity of 94% (CI, 73-100%) and specificity of 82% (CI, 66-91%). When the TB score was evaluated against a composite microbiological score (n=18 TB, 32 ORD), we found an AUC of 0·89 (CI, 0·79-1·00) with a sensitivity of 89% (CI, 67-98%) and specificity of 75% (CI, 58-87%).

When the TB score was evaluated against GeneXpert Ultra for Vietnamese samples (n=30 TB, 20 ORD), we found an AUC of 0·95 (CI, 0·89-1·00) (Fig. 3A, blue line). Considering Xpert-MTB-HR as a triage test, with a sensitivity of 92%, specificity was 83% (CI, 57-100%). When GBP5 was analyzed alone, we found an AUC of 0·96 (CI, 0·90-1·00) (Fig. 3B, blue line) with a sensitivity of 93% (CI, 79-99%) and specificity of 90% (CI, 70-98%). When the TB score was evaluated against a composite microbiological score (n=31 TB, 17 ORD), we found an AUC of 0·90 (CI, 0·82-0·99) with a sensitivity of 84% (CI, 67-92) and specificity of 88% (CI, 66-98%).

Discussion:

This is the first study to perform prospective point-of-care analysis of the Xpert-MTB-HR-Prototype using fingerstick blood from patients presenting with symptoms suggestive of TB but prior to microbiological confirmation. Our interim results show that the Xpert-MTB-HR prototype test meets the minimal criteria set out in the TPP for a triage test when evaluated against GeneXpert Ultra. This was a multi-site study within Africa and South-East Asia, and despite different host genetics, circulating Mtb strains (*M. Beijing* in Vietnam, *M. tuberculosis sensu stricto* and *M. africanum* in sub-Saharan Africa) and co-morbidities, all sites reached the TPP for a triage test. We also found that a single gene, GBP5 had the same sensitivity and specificity for TB amongst total respiratory infections compared to TB score, allowing for potential further simplification of the analytical process.

Surprisingly, there were very few differences in the performance of the cartridges seen between the sites. South Africa had the lowest AUC but this is likely due to the lower number of participants (only 6 in the TB arm) and is expected to change following full recruitment of 1200 participants. The Vietnam site performed similarly to sites in Africa suggesting the cartridge is applicable for global use, regardless of ethnicity, and underlying epidemiology of the region. This is of importance since the RNA signatures that provided the basis for the test, were mainly derived from sub-Saharan African cohorts. Both Gambia and Vietnam reached ideal specifications for a triage test when evaluated against GeneXpert Ultra. The accuracy of the prototype was reduced when evaluated against sputum culture as seen previously [10], with specificity lower than minimal criteria

for a triage test when sensitivity was set at 90%. This is likely due to the smaller number of participants with available culture results. Our interim results suggest the Xpert-MTB-HR prototype could be used as a screening tool for individuals who cannot produce sputum or who have paucibacillary disease (ie extrapulmonary TB, children), and would drastically reduce the biohazards associated with sputum production and culturing in the first instance. Whilst the performance of the catridges may change following evaluation of our full cohort (including children), we felt it was important to publish these interim results in order to publicise one of the most promising triage tests for TB and the first one to reach the WHO TPP in the world. Given the very narrow 95% CI for all sites, and the similarity of performance between diverse study populations (including PLHIV), it is likely our performance will be retained due to our robust study design. However, we did not include cut-off values as these are likely to change following full evaluation of our cohort and evaluation by Cepheid of all data generated by the multiple consortia analyzing the prototype prior to commercialization.

The major limitation of this study is the relatively small sample size, however our strengths were the use of a multi-site study, inclusion of both HIV+ and HIV- participants, prospective analysis and an appropriate control group of patients presenting with respiratory symptoms suggestive of TB but who were determined to have another respiratory infection. This allowed us to control for non-specific effects of inflammation and is particularly important in analysis of the 3-gene signature which will be affected by host inflammation. Indeed, the elevation of the GBP5 and DUSP3 pro-inflammatory genes with no difference in the anti-inflammatory KLF2 gene in the TB group shows that despite all patients presenting with a respiratory infection, the pro-inflammatory response associated with these biomarkers is greater in TB than other infections. We are currently determining which pathogens are present in nasopharyngeal swabs from the non-TB group as this may help to determine if we are able to achieve high discrimination between infections versus non-infectious disease diagnoses (i.e. COPD).

The use of fingerstick blood for the Xpert-MTB-HR prototype was a major strength as previous studies have used stored Paxgene RNA samples. We transferred the blood to an EDTA minivette which precluded any issues with clotting prior to addition to the cartridge. It is likely that prior COVID infection may increase risk of progression to active TB disease as has been seen for prior influenza A virus infection [14] and will also be evaluated as part of our study. Importantly, HIV co-infection did not affect the accuracy of the test despite CD4 counts <500 in both groups. However, there was a borderline significant difference in TB score with lower median score in the HIV+ versus the HIV- individuals within the ORD group.

In summary, our preliminary data show great promise for the use of the Xpert-MTB-HR-Prototype fingerstick blood test for screening of TB patients at the point-of-care. The ability to accurately discriminate based on a single gene (GBP5) suggests the potential for further simplification of the test. While these findings are based on small participant numbers and small subgroups, they justify further evaluation.

NOTES

Author contributions: JSS & GW conceived and designed the study; JSS, GW, TNTT, HMK, HMD, JW, TJS, AG (9), PC obtained funding; AG (1), OO, AN, MN, TR, JS, STM developed protocols, recruited participants and collected data; GVDS & KS designed the database; BS & JW provided project support; JSS wrote the original draft and JSS, GW, TNTT, HMK, HMD, TJS, AG (9), JW, STM, JS & TR reviewed & edited manuscript drafts. JSS & GVDS verified the underlying data.

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Conflict of interest statement:

All authors declare they have no conflicts of interest

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Table 1: patient information

	Mtb detected	Mtb not detected	P-value
n=	75	120	
Age (median [IQR])	30 [26-38]	36 [28-45]	ns
Females (n (%))	20 (27)	52 (43)	0.019
HIV positive (n (%))	4 (5.3)	17 (14)	ns
CD4 (median [IQR])	379 [90-793]	344 [179-689]	ns
Prior TB (n (%))	2 (3)	8 (7)	ns
Xpert ultra reading			
Trace (n (%))	7 (9)	NA	
Very low (n (%))	1 (1)	NA	
Low (n (%))	14 (19)	NA	
Medium (n (%))	15 (20)	NA	
High (n (%))	38 (51)	NA	
Liquid culture			
Positive	68	8	
Negative	2	110	
Contaminated	3	1	
Not available	2	1	

IQR, interquartile range; TB, tuberculosis; ORD, other respiratory diseases;
HIV, human immunodeficiency virus; CD4 count, cells/µl blood; NA, not applicable

Figure legends:

Figure 1: Xpert MTB-HR prototype test analysis in tuberculosis (TB) and other respiratory diseases (ORD) patients detected by GeneXpert Ultra. A): DUSP3, B): GBP5, C): KLF2, D): TB score. Data were analysed using Mann-Whitney U-test. Bars indicate 10-90% percentile, line indicates median, and dots indicate outliers. Ct value (Cycle Threshold) derived from the GeneXpert machine.

Figure 2: Xpert MTB-HR prototype analysis of all participants combined against Xpert Ultra. A) ROC curve for TB score all participants (black line) and HIV- participants only (green line). B) ROC curve for GBP5 all participants (black line) and HIV negative participants only (green line); C) All participants evaluated against a composite microbiological score for TB score and D) all participants evaluated against a composite microbiological score for GBP5.

Figure 3: Evaluation at individual sites against Xpert Ultra. ROC curves are shown for Gambia (black line), South Africa (green line), Uganda (red line) and Vietnam (blue line).

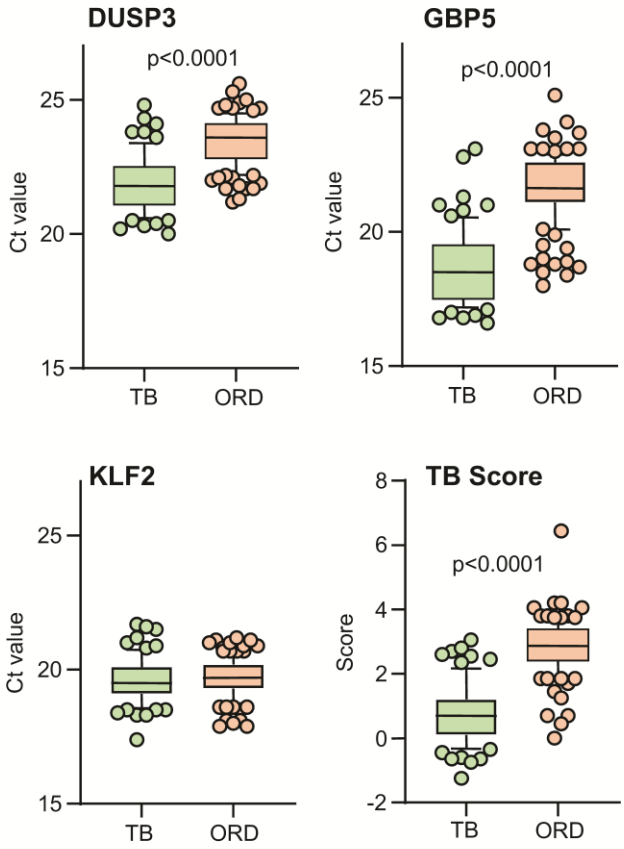

Figure 1

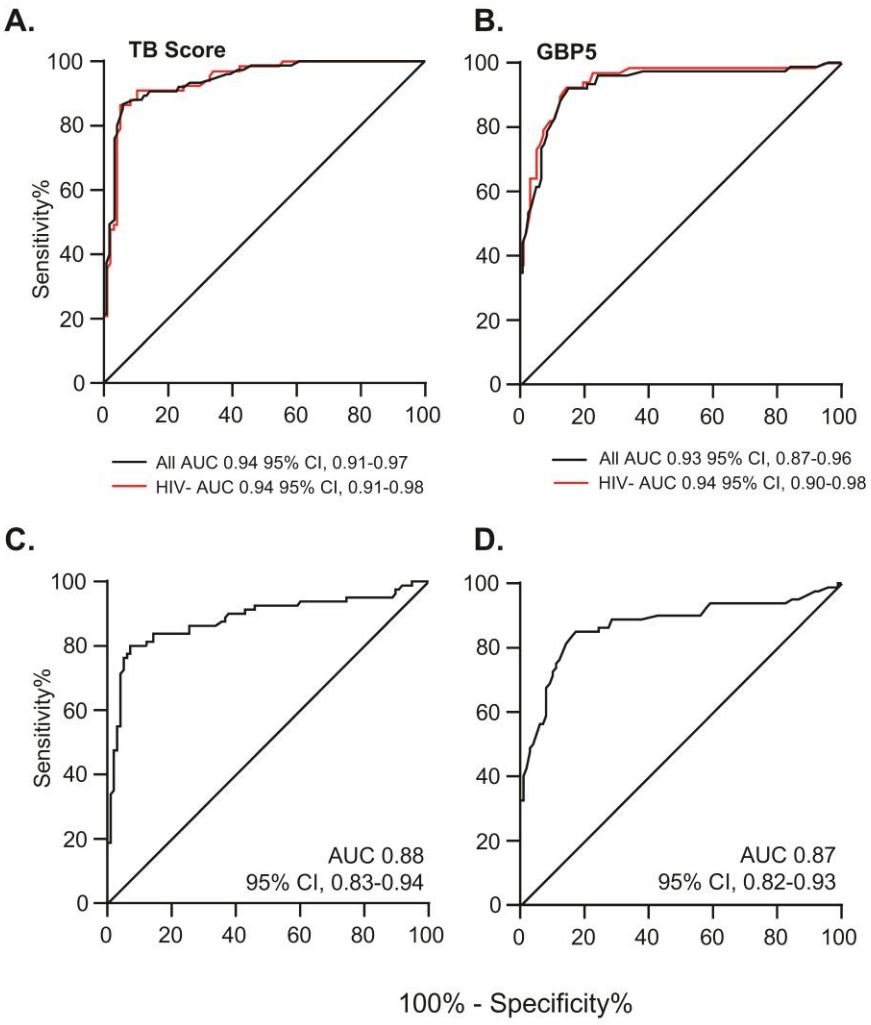

Figure 2

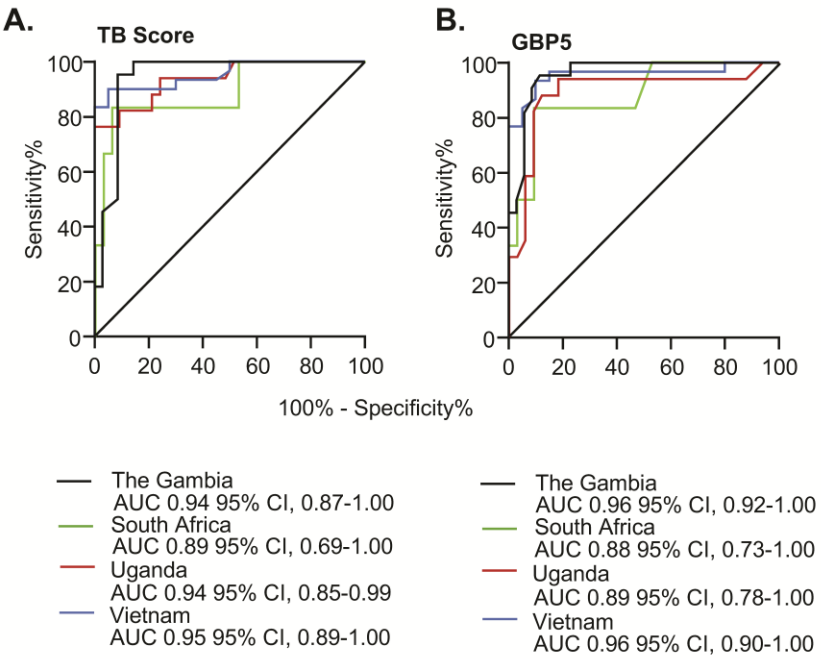

Figure 3