A TRIAGE TEST FOR TUBERCULOSIS BASED ON THE SPECIFIC ANTIGENS ESAT-6, CFP10 AND RV1681 USING A NOVEL POINT OF CARE RAPID TEST

Technical Report

INTRODUCTION

Tuberculosis is an infectious disease generated by the *Mycobacterium tuberculosis* complex and one of the top 10 causes of death worldwide. In 2015 10.4 million people developed active TB and 1.8 million died as a result. However, an estimated 4.3 million were not diagnosed and did not received treatment and care (FIND, 2016).

Today the diagnostic of tuberculosis is still a major challenge in the developing world. Many regions still depend for screening and triage on long time used golden standards like microscopy or acid resistant bacteria culture which are complex, time consuming and not available at the point of care, with specificity and sensitivity closely linked to the technician performance. New technologies have been developed as gold standards, like the XPERT MTB/RIF based in molecular biology processes, but they are very expensive to be used as screening and triage tool for large populations.

Simpler and more sensitive TB screening and triage tests for the point of care are urgently needed in the developing world to avoid missing TB cases, capable of being used in health facilities with limited or null laboratory infrastructure. Due to malpractice and findings of low sensitivity in available rapid tests, in July 2011 the World Health Organization issued a warning that such blood tests should not be used for trying to diagnose active TB.

Serological tests could be suitable to be used at the point of care, as they are less intensive in lab requirements. Current rapid diagnostic immune tests based in serology use the lateral flow format and their sensitivity and specificity depend on the antigen biomarker selected the resolution of the evaluation process and readout. It must be considered that the sensitivity of a diagnostic test based on a single antigen is lower when a single biomarker is used.

ESAT6 and CFP10 have been previously recognized and used as biomarkers for diagnosing the tuberculosis disease. The ESAT6 antigen is expressed from the RD1 genome region which is deleted in all BCG strains but conserved in all members of the *Mycobacterium tuberculosis* complex and, therefore, represent a suitable biomarker for diagnostic in countries where the population has been vaccinated. Recently, CFP10 has been identified as another suitable biomarker also located in the ESAT6 operon and therefore deleted in vaccine strains (Li et al, 2016; Bekmurzayeva et al, 2013; Mohamandi et al, 2011; Slogotskaya et al, 2010).

The protein RV1681 has demonstrated also a potential as a biomarker for later active phases of the tuberculosis disease. This protein is not found in other species other than *M. tuberculosis* and *M. bovis* which would increase the sensitivity and specificity of a diagnostic test (Kashino et al, 2008).

MATERIALS AND METHODS

Ethical Approval. All protocols were approved by the Jurisdictional Ethical Committee of the State of Baja California in Mexico.

Patient samples and study design. This study was carried out with patients assigned to the Regional Hospital of Tijuana, the Regional Hospital of Ensenada and the School of Medicine of the Universidad Autonoma de Baja California (UABC), all of them located in the state of Baja California, Mexico. This area is considered to be the one with the highest incidence on human and bovine tuberculosis in the country.

The patients who took part of this study can be segmented in three groups: a) patients diagnosed with the tuberculosis disease without treatment at the moment of sampling; b) patients diagnosed with the tuberculosis disease and in treatment; and c) patients from “open population” (patients arriving to the hospital with symptoms but not previously diagnosed). Samples were stored at -80°C as sera for testing.

All patients were diagnosed for the tuberculosis disease by the reference gold standard tests used in the current diagnostic algorithms (AFB culture, microscopy and PCR) and for HIV by the ELISA test. In the first group, all patients were HIV negative. In the second group, patients already in treatment were called back to the hospital and sampled by venipuncture. In the third group, patients which reached the Hospital and clinic testing sites with symptoms which could be related to the tuberculosis disease, and without a previous treatment, were selected for sampling and also tested with the current diagnostic reference tests.

In the first group sera samples collected were processed previously to the use of the diagnostic tests by adding washed erythrocytes in a 30 µl per each 100 µl of sera sample in order to simulate the original state of the blood sample. In the second and third groups the test was used directly with the patient during their visit to the clinic.

Antibody detection tests. Patient samples were tested using non-commercial versions of the Unima's TB + HIV diagnostic tests capable of detecting antibodies against ESAT6, CFP10 and RV1681 antigens from the *Mycobacterium tuberculosis* complex and p24 and gp 120 from the HIV virus. This product comprises the use of a paper diagnostic device incorporated in a plastic casing and a smartphone app which evaluates the result in the device and generates the patient diagnostic after performing on site image analysis processes and artificial intelligence algorithms.

Data analysis. For purposes of this study, samples from patients previously diagnosed with the Tuberculosis and HIV diseases by the current golden standard tests were labeled respectively as Tuberculosis + or HIV +, otherwise as Tuberculosis – or HIV -. Results obtained by the use of the Find™ TB + HIV diagnostic tests where labeled as:

Result from test	Label
Tuberculosis positive	TB +
Tuberculosis negative	TB -
Tuberculosis inconclusive	TB I
HIV positive	HIV +
HIV negative	HIV -
HIV inconclusive	HIV I

Results obtained by the Find™ TB + HIV diagnostic tests that matched the results from the reference gold standard tests were labeled as *True Positives* or *True negatives*, otherwise where labeled as *False positive or False negative*.

RESULTS

TB diagnosis on group 1. Results from the use of the TB + HIV diagnostic test on the first group of 56 patient samples were obtained by reconstituting blood samples by adding freshly washed erythrocytes from a healthy donor in order to restore the original state of the sample. All procedures were performed by trained lab technicians and under controlled conditions.

This first group of 56 patients included 49 Tuberculosis positive patients and 7 tuberculosis negative patients. All positive samples were from patients in active phase of the disease. All positive samples were taken before patient started their treatment. Patients had been vaccinated with the BCG vaccine at some point of their life. The final set of results is presented in table 1. These results showed a Sensitivity of 96% and a Specificity of 100%, with a Positive Predictive Value of 100% and a Negative predictive value of 78%.

Table 1. Diagnostic results from patient group 1

Code	Patient Source	TB Reference Diagnostic	TB Test Diagnostic	Correlation	Code	Patient Source	TB Reference Diagnostic	TB Test Diagnostic	Correlation
1HGT	Diagnosed	+	+	Correct	29HGT	Diagnosed	+	+	Correct
2HGT	Diagnosed	+	+	Correct	30HGT	Diagnosed	+	+	Correct
3HGT	Diagnosed	+	+	Correct	31HGT	Diagnosed	+	+	Correct
4HGT	Diagnosed	+	+	Correct	32HGT	Diagnosed	+	+	Correct
5HGT	Diagnosed	+	+	Correct	33HGT	Diagnosed	+	+	Correct
6HGT	Diagnosed	+	+	Correct	34HGT	Diagnosed	+	+	Correct
7HGT	Diagnosed	+	+	Correct	35HGT	Diagnosed	+	+	Correct
8HGT	Diagnosed	+	+	Correct	36HGT	Diagnosed	+	+	Correct
9HGT	Diagnosed	+	+	Correct	37HGT	Diagnosed	+	+	Correct
10HGT	Diagnosed	+	+	Correct	38HGT	Diagnosed	+	+	Correct
11HGT	Diagnosed	+	+	Correct	39HGT	Diagnosed	+	+	Correct
12HGT	Diagnosed	+	+	Correct	40HGT	Diagnosed	+	+	Correct
13HGT	Diagnosed	+	+	Correct	41HGT	Diagnosed	+	+	Correct
14HGT	Diagnosed	+	+	Correct	42HGT	Diagnosed	+	+	Correct
15HGT	Diagnosed	+	+	Correct	43HGT	Diagnosed	+	-	Error
16HGT	Diagnosed	+	+	Correct	44HGT	Diagnosed	+	+	Correct
17HGT	Diagnosed	+	+	Correct	45HGT	Diagnosed	-	-	Correct
18HGT	Diagnosed	+	+	Correct	46HGT	Diagnosed	-	-	Correct
19HGT	Diagnosed	+	+	Correct	47HGT	Diagnosed	-	-	Correct
20HGT	Diagnosed	+	+	Correct	48HGT	Diagnosed	-	-	Correct
21HGT	Diagnosed	+	+	Correct	49HGT	Diagnosed	-	-	Correct
22HGT	Diagnosed	+	+	Correct	50HGT	Diagnosed	+	+	Correct
23HGT	Diagnosed	+	+	Correct	51HGT	Diagnosed	-	-	Correct
24HGT	Diagnosed	+	+	Correct	52HGT	Diagnosed	-	-	Correct
25HGT	Diagnosed	+	+	Correct	53HGT	Diagnosed	+	+	Correct
26HGT	Diagnosed	+	+	Correct	54HGT	Diagnosed	+	+	Correct
27HGT	Diagnosed	+	+	Correct	55HGT	Diagnosed	+	+	Correct
28HGT	Diagnosed	+	-	Error	56HGT	Diagnosed	+	+	Correct

TB diagnosis on patients under treatment. Results from the use of the TB + HIV diagnostic test on the second group of 39 patient samples were obtained directly on the Hospital during the visit of the patient to the doctor to treatment follow up.

All samples were used according product instructions. All procedures were performed by a doctor or a nurse with a previous training on the use of the device.

This second group of 39 patients included 31 Tuberculosis positive patients, 8 Tuberculosis Negative, 16 HIV positive and 23 HIV Negative. Patients had been vaccinated with the BCG vaccine at some point of their life. The final set of results is presented in table 2.

Patients with HIV results labeled as -** where those who arrived with a previously inconclusive result from the current lateral flow test. According to current practice at the site this patients needed to be considered negative until a PCR test was run for confirmation to confirm or rule out the infection. All cases were later confirmed with a PCR test due to the lack of availability of the test at the moment of the initial testing.

These results showed for Tuberculosis a Sensitivity of 96.7% and a Specificity of 100% with a Positive Predictive Value of 100% and a Negative predictive value of 89%, and for HIV a Sensitivity of 100% and a Specificity of 100% with a Positive Predictive Value of 100% and a Negative predictive value of 100%.

Table 2. Diagnostic results from patient group 2

Code	Patient Source	TB Reference Diagnostic	TB Test Diagnostic	HIV Reference Diagnostic	TB Test Diagnostic	Correlation TB	Correlation HIV
Maya	Diagnosed	+	+	+	+	Correct	Correct
Morales	Diagnosed	-	-	+	+	Correct	Correct
Juarez	Diagnosed	+	+	-**	-	Correct	Correct
Magaña	Diagnosed	+	+	+	+	Correct	Correct
Patiño	Diagnosed	+	+	+	+	Correct	Correct
Osuna D	Diagnosed	+	+	+	+	Correct	Correct
Leyva	Diagnosed	+	+	-**	-	Correct	Correct
Cienfuegos	Diagnosed	+	+	+	+	Correct	Correct
Smith	Diagnosed	+	+	+	+	Correct	Correct
Sanchez	Diagnosed	+	+	+	+	Correct	Correct
Carrillo	Diagnosed	+	+	-**	-	Correct	Correct
Torres	Diagnosed	+	+	-**	-	Correct	Correct
Gorosave	Diagnosed	+	+	+	+	Correct	Correct
Sanchez	Diagnosed	+	-	+	+	Error	Correct
Ponce	Diagnosed	+	+	-**	-	Correct	Correct
Martínez	Diagnosed	+	+	+	+	Correct	Correct
Cabanillas	Diagnosed	+	+	-	-	Correct	Correct
Del Angel	Diagnosed	+	+	-**	-	Correct	Correct
Torres	Diagnosed	+	+	-**	-	Correct	Correct
Orozco	Diagnosed	+	+	+	+	Correct	Correct
Laila	Diagnosed	-	-	-	-	Correct	Correct
Morales	Diagnosed	+	+	-	-	Correct	Correct
Aguilar	Diagnosed	-	-	-	-	Correct	Correct
Rodriguez	Diagnosed	+	+	+	+	Correct	Correct
Alonso	Diagnosed	+	+	-	-	Correct	Correct
Romero	Diagnosed	-	-	-	-	Correct	Correct
Barraza	Diagnosed	+	+	-**	-	Correct	Correct
Cueza	Diagnosed	+	+	-**	-	Correct	Correct
Moreno	Diagnosed	+	+	+	+	Correct	Correct
Rodriguez	Diagnosed	+	+	-	-	Correct	Correct
Lucero	Diagnosed	+	+	-	-	Correct	Correct
Sanchez	Diagnosed	+	+	-	-	Correct	Correct
Thomas	Diagnosed	-	-	+	+	Correct	Correct
Luna	Diagnosed	-	-	-	-	Correct	Correct
Bernal	Diagnosed	+	+	-**	-	Correct	Correct
Zapata	Diagnosed	-	-	-**	-	Correct	Correct
Velazquez	Diagnosed	+	+	+	+	Correct	Correct
Ramirez	Diagnosed	+	+	-	-	Correct	Correct
Barraza	Diagnosed	-	-	-	-	Correct	Correct

TB diagnosis on open population. Results from the use of the TB + HIV diagnostic test on open population obtained directly on the Hospital from patients who arrived with potential tuberculosis symptoms and followed the current national algorithms for the diagnostic of the disease. During the procedure, a sample of blood was taken from the patients along with the mandatory sputum sample. The diagnostic test was done at the lab by a trained technician with whole blood no later than 2 hours after being taken and results were obtained also from the reference tests. All samples were used according product instructions.

This group was comprised by a set of 233 patients which includes 47 Tuberculosis positive patients, 186 Tuberculosis Negative, 136 HIV positive and 97 HIV Negative. Patients had been vaccinated with the BCG vaccine at some point of their life. The current set of results is presented in table 3.

Table 3. Diagnostic results from patient group 3

Code	Tuberculosis Reference	HIV Reference	Tuberculosis Test	HIV Test	Tuberculosis Correlation	HIV Correlation
101-VP	-	+	-	+	Correct	Correct
105-VP	-	+	-	+	Correct	Correct
107-VP	+	+	+	+	Correct	Correct
108-VP	-	+	-	+	Correct	Correct
109-VP	-	+	-	+	Correct	Correct
110-VP	-	+	-	+	Correct	Correct
111-VP	+	+	+	+	Correct	Correct
112-VP	+	+	+	+	Correct	Correct
113-VP	+	+	+	+	Correct	Correct
115-VP	-	+	-	+	Correct	Correct
116-VP	-	+	-	+	Correct	Correct
117-VP	-	+	-	+	Correct	Correct
118-VP	-	+	-	+	Correct	Correct
119-VP	-	+	-	+	Correct	Correct
120-VP	+	+	+	+	Correct	Correct
121-VP	+	+	+	+	Correct	Correct
122-VP	-	+	-	+	Correct	Correct
124-VP	+	+	+	+	Correct	Correct
125-VP	-	+	-	+	Correct	Correct
126-VP	+	+	+	+	Correct	Correct
127-VP	+	+	+	-	Correct	Error
130-VP	-	+	-	+	Correct	Correct
131-VP	+	+	+	+	Correct	Correct
132-VP	-	+	-	+	Correct	Correct
134-VP	-	+	-	+	Correct	Correct
136-VP	-	+	-	+	Correct	Correct
137-VP	+	+	+	+	Correct	Correct
138-VP	-	+	-	+	Correct	Correct
140-VP	-	+	-	+	Correct	Correct
141-VP	-	+	-	+	Correct	Correct
144-VP	+	+	+	+	Correct	Correct

Table 3. Diagnostic results from patient group 3 (continued)

Code	Tuberculosis Reference	HIV Reference	Tuberculosis Test	HIV Test	Tuberculosis Correlation	HIV Correlation
2017-10-VP	-	+	-	+	Correct	Correct
2017-11-VP	-	+	-	+	Correct	Correct
2017-12-VP	-	+	-	+	Correct	Correct
2017-13-VP	-	+	-	+	Correct	Correct
2017-14-VP	+	+	+	+	Correct	Correct
2017-15-VP	-	+	-	+	Correct	Correct
2017-1-VP	+	+	+	+	Correct	Correct
2017-2-VP	+	+	+	+	Correct	Correct
2017-5-VP	-	+	-	+	Correct	Correct
2017-6-VP	+	+	+	+	Correct	Correct
2017-7-VP	+	+	+	+	Correct	Correct
2017-8-VP	-	+	-	+	Correct	Correct
2017-9-VP	+	+	+	+	Correct	Correct
APJ41M	-	-	-	-	Correct	Correct
JJEN65M	-	-	-	-	Correct	Correct
JLNC76M	-	-	-	-	Correct	Correct
MLMV40F	-	-	-	-	Correct	Correct
MMRN70F	-	-	-	-	Correct	Correct
RGR35M	-	-	-	-	Correct	Correct
VAGD24M	-	-	-	-	Correct	Correct
100VP23M	-	+	-	+	Correct	Correct
103VP38F	-	+	-	+	Correct	Correct
147VP30M	-	+	-	+	Correct	Correct
148VP51M	-	+	-	+	Correct	Correct
154VP32M	+	+	-	+	Error	Correct
155VP19F	+	+	+	+	Correct	Correct
156VP28M	+	+	+	+	Correct	Correct
157VP48M	-	+	-	+	Correct	Correct
158VP43F	-	+	-	+	Correct	Correct
159VP44M	-	+	-	+	Correct	Correct
160VP33M	-	+	-	+	Correct	Correct
161VP29M	-	+	-	+	Correct	Correct
162VP52M	-	+	+	+	Error	Correct
163VP18M	+	+	+	+	Correct	Correct
164VP30M	-	+	-	+	Correct	Correct
165VP32M	+	+	-	+	Error	Correct
166VP28M	-	+	-	+	Correct	Correct
167VP39M	-	+	-	-	Correct	Error
168VP32M	+	+	+	+	Correct	Correct
169VP24M	-	+	-	+	Correct	Correct
170VP41M	-	+	-	+	Correct	Correct
171VP49M	-	+	-	+	Correct	Correct
172VP47M	-	+	-	+	Correct	Correct
26VP43F	+	+	+	+	Correct	Correct
27VP44M	-	+	-	+	Correct	Correct
28VP32M	-	+	-	+	Correct	Correct
29VP33M	-	+	-	+	Correct	Correct
30VP36F	-	+	+	+	Error	Correct
31VP42F	+	+	+	+	Correct	Correct
32VP64M	-	+	-	+	Correct	Correct
33VP26M	-	+	-	+	Correct	Correct
34VP37M	-	+	-	+	Correct	Correct
35VP53M	+	+	+	+	Correct	Correct
40VP44M	-	+	-	+	Correct	Correct
41VP44M	-	+	-	+	Correct	Correct

Table 3. Diagnostic results from patient group 3 (continued)

Code	Tuberculosis Reference	HIV Reference	Tuberculosis Test	HIV Test	Tuberculosis Correlation	HIV Correlation
42VP35F	-	+	-	+	Correct	Correct
43VP62M	-	+	-	+	Correct	Correct
44VP49F	-	+	-	+	Correct	Correct
45VP29F	-	+	-	+	Correct	Correct
48VP38M	-	+	-	+	Correct	Correct
49VP44M	-	+	-	+	Correct	Correct
50VP45M	-	+	-	+	Correct	Correct
52VP51M	-	+	-	+	Correct	Correct
53VP25M	-	+	-	+	Correct	Correct
55VP30M	+	+	+	+	Correct	Correct
56VP27M	-	+	-	+	Correct	Correct
57VP26F	-	+	-	+	Correct	Correct
58VP45M	-	+	-	+	Correct	Correct
59VP33F	-	+	-	+	Correct	Correct
63VP27M	-	+	-	+	Correct	Correct
64VP33F	-	+	-	+	Correct	Correct
66VP33M	-	+	-	+	Correct	Correct
67VP29M	-	+	-	+	Correct	Correct
68VP52M	-	+	+	+	Error	Correct
69VP27F	-	+	-	+	Correct	Correct
70VP44M	-	+	-	+	Correct	Correct
72VP37F	-	+	-	+	Correct	Correct
75VP45M	-	+	+	+	Error	Correct
76VP45M	-	+	-	+	Correct	Correct
78VP45M	-	+	-	+	Correct	Correct
79VP43M	+	+	+	+	Correct	Correct
80VP35M	+	+	+	+	Correct	Correct
81VP26F	+	+	+	+	Correct	Correct
82VP24M	-	+	-	+	Correct	Correct
83VP26M	-	+	-	+	Correct	Correct
84VP48M	-	+	-	+	Correct	Correct
86VP41F	-	+	-	+	Correct	Correct
87VP30F	-	+	-	+	Correct	Correct
88VP46F	+	+	+	+	Correct	Correct
89VP35F	-	+	-	+	Correct	Correct
90VP47M	-	+	-	+	Correct	Correct
91VP52M	-	+	-	+	Correct	Correct
93VP19M	-	+	-	+	Correct	Correct
95VP44M	-	+	-	+	Correct	Correct
96VP35F	-	+	-	+	Correct	Correct
97VP61M	-	+	-	+	Correct	Correct
98VP66F	-	+	-	+	Correct	Correct
102VP27M	+	+	+	+	Correct	Correct
145VP33M	+	+	+	+	Correct	Correct
146VP30M	+	+	+	+	Correct	Correct
149VP32M	+	+	+	+	Correct	Correct
16VP30F	+	+	+	+	Correct	Correct
36VP37M	+	+	+	+	Correct	Correct
38VP37M	+	+	+	+	Correct	Correct
39VP44F	+	+	+	+	Correct	Correct
46VP40F	+	+	+	+	Correct	Correct

Table 3. Diagnostic results from patient group 3 (continued)

Code	Tuberculosis Reference	HIV Reference	Tuberculosis Test	HIV Test	Tuberculosis Correlation	HIV Correlation
47VP37F	+	+	+	+	Correct	Correct
54VP30M	+	+	+	+	Correct	Correct
71VP35F	+	+	+	+	Correct	Correct
81VP26F	+	+	+	+	Correct	Correct
94VP29M	+	+	+	+	Correct	Correct
99VP41M	+	+	+	+	Correct	Correct
1FCB	-	-	-	-	Correct	Correct
2FCB	-	-	-	-	Correct	Correct
3FCB	-	-	-	-	Correct	Correct
4FCB	-	-	-	-	Correct	Correct
5FCB	-	-	-	-	Correct	Correct
6FCB	-	-	-	-	Correct	Correct
7FCB	-	-	-	-	Correct	Correct
8FCB	-	-	-	-	Correct	Correct
9FCB	-	-	-	-	Correct	Correct
10FCB	-	-	-	-	Correct	Correct
11FCB	-	-	-	-	Correct	Correct
12FCB	-	-	-	-	Correct	Correct
13FCB	-	-	-	-	Correct	Correct
14FCB	-	-	-	-	Correct	Correct
15FCB	-	-	-	-	Correct	Correct
16FCB	-	-	-	-	Correct	Correct
17FCB	-	-	-	-	Correct	Correct
18FCB	-	-	-	-	Correct	Correct
19FCB	-	-	-	-	Correct	Correct
20FCB	-	-	-	-	Correct	Correct
21FCB	-	-	-	-	Correct	Correct
22FCB	-	-	-	-	Correct	Correct
23FCB	-	-	-	-	Correct	Correct
24FCB	-	-	-	-	Correct	Correct
25FCB	-	-	-	-	Correct	Correct
26FCB	-	-	-	-	Correct	Correct
27FCB	-	-	-	-	Correct	Correct
28FCB	-	-	-	-	Correct	Correct
29FCB	-	-	-	-	Correct	Correct
30FCB	-	-	-	-	Correct	Correct
31FCB	-	-	-	-	Correct	Correct
32FCB	-	-	-	-	Correct	Correct
33FCB	-	-	-	-	Correct	Correct
34FCB	-	-	-	-	Correct	Correct
35FCB	-	-	-	-	Correct	Correct
36FCB	-	-	-	-	Correct	Correct
37FCB	-	-	-	-	Correct	Correct
38FCB	-	-	-	-	Correct	Correct
39FCB	-	-	-	-	Correct	Correct
40FCB	-	-	-	-	Correct	Correct
41FCB	-	-	-	-	Correct	Correct
42FCB	-	-	-	-	Correct	Correct
43FCB	-	-	-	-	Correct	Correct

Table 3. Diagnostic results from patient group 3 (continued)

Code	Tuberculosis Reference	HIV Reference	Tuberculosis Test	HIV Test	Tuberculosis Correlation	HIV Correlation
44FCB	-	-	-	-	Correct	Correct
45FCB	-	-	-	-	Correct	Correct
46FCB	-	-	-	-	Correct	Correct
47FCB	-	-	-	-	Correct	Correct
48FCB	-	-	-	-	Correct	Correct
49FCB	-	-	-	-	Correct	Correct
50FCB	-	-	-	-	Correct	Correct
51FCB	-	-	-	-	Correct	Correct
52FCB	-	-	-	-	Correct	Correct
53FCB	-	-	-	-	Correct	Correct
54FCB	-	-	-	-	Correct	Correct
55FCB	-	-	-	-	Correct	Correct
56FCB	-	-	-	-	Correct	Correct
57FCB	-	-	-	-	Correct	Correct
58FCB	-	-	-	-	Correct	Correct
59FCB	-	-	-	-	Correct	Correct
60FCB	-	-	-	-	Correct	Correct
61FCB	-	-	-	-	Correct	Correct
62FCB	-	-	-	-	Correct	Correct
63FCB	-	-	-	-	Correct	Correct
64FCB	-	-	-	-	Correct	Correct
65FCB	-	-	-	-	Correct	Correct
66FCB	-	-	-	-	Correct	Correct
67FCB	-	-	-	-	Correct	Correct
68FCB	-	-	-	-	Correct	Correct
69FCB	-	-	-	-	Correct	Correct
70FCB	-	-	-	-	Correct	Correct
71FCB	-	-	-	-	Correct	Correct
72FCB	-	-	-	-	Correct	Correct
73FCB	-	-	-	-	Correct	Correct
74FCB	-	-	-	-	Correct	Correct
75FCB	-	-	-	-	Correct	Correct
76FCB	-	-	-	-	Correct	Correct
77FCB	-	-	-	-	Correct	Correct
78FCB	-	-	-	-	Correct	Correct
79FCB	-	-	-	-	Correct	Correct
80FCB	-	-	-	-	Correct	Correct
81FCB	-	-	-	-	Correct	Correct
82FCB	-	-	-	-	Correct	Correct
83FCB	-	-	-	-	Correct	Correct
84FCB	-	-	-	-	Correct	Correct
85FCB	-	-	-	-	Correct	Correct
86FCB	-	-	-	-	Correct	Correct
87FCB	-	-	-	-	Correct	Correct
88FCB	-	-	-	-	Correct	Correct
89FCB	-	-	-	-	Correct	Correct
90FCB	-	-	-	-	Correct	Correct

These results showed for Tuberculosis a Sensitivity of 95.7% and a Specificity of 97.8%, with a Positive Predictive Value of 91.8% and a Negative predictive value of 98.9%. For HIV the Sensitivity is 98.5% and a Specificity of 100%, with a Positive Predictive Value of 100% and a Negative predictive value of 97.9%.

DISCUSSION

Tuberculosis is currently one of the top priorities for eradication in the developing world. In 2015 it was considered one of the top 10 causes of mortality in the world, reaching 11 million new infections diagnosed just in that year. Six countries account for 60% of the total tuberculosis cases: India, Indonesia, China, Nigeria, Pakistan and South Africa (WHO, 2016).

While in South and North America tuberculosis has not been one of the top problems in Public Health, the alarming emergence of multi drug resistant strains (MDR) of the *Mycobacterium tuberculosis* complex, along with low budgets for public health in all Latin America and the lack of reliable low cost point of care tuberculosis tests to achieve mass coverage in TB diagnostics, increases the risk for this disease to become a major health threat in the region.

Currently, in Latin America and most of the developing world, patients are diagnosed by a series of *gold standard* tests which depend on the availability of specialized infrastructure and highly trained personnel which normally are extremely scarce in these regions. These tests, including culture, microscopy, immune and molecular biology tests, are cost, labor and technology intensive and therefore not suited for large scale screening, triage or first contact test to address the massive need of population-wide eradication programs. A technology which allows a low cost and large scale screening and triage of potential infected patients would increase access to on time medication and also would decrease costs for National Health Systems related to lab scale diagnostic and confirmation tests by focusing on those patients which are pre-screened to really have the disease.

But in order for a new diagnostic test to achieve this goal, it has to comply the guidelines and recommendations of international health authorities, like the WHO, in terms of sensitivity, specificity and predictive values.

The object of this study is a Tuberculosis and HIV test which aims to increase accuracy of the triage of patients suspected of having an active Tuberculosis infection by detecting a set of biomarkers which have shown in previous published works high levels of correspondence to the presence of the disease (Li et al, 2016; Bekmurzayeva et al, 2013; Mohamandi et al, 2011; Slogotskaya et al, 2010; Kashino et al, 2008).

According to the software algorithm to identify positive versus negative cases, each of the individual results from each biomarker (ESAT6, CFP10, RV1681, p24 and p26) and their combinations define the final overall result of the test.

The levels of sensitivity, and predictive values from the tests performed in the three groups can be summarized in tables 5 and 6 along with overall metrics including results from all tests.

Table 5. Accuracy metrics for the Tuberculosis test

Metric	Group 1	Group 2	Group 3	Overall
Sensitivity	95.9%	96.8%	95.7%	96.1%
Specificity	100.0%	100.0%	95.7%	98.0%
Positive Predictive Value	100.0%	100.0%	97.8%	96.9%
Negative Predictive Value	77.8%	88.9%	98.9%	97.5%

Table 6. Accuracy metrics for the HIV test

Metric	Group 1	Group 2	Group 3	Overall
Sensitivity	NA	100.0%	98.5%	98.7%
Specificity	NA	100.0%	100.0%	100.0%
Positive Predictive Value	NA	100.0%	100.0%	100.0%
Negative Predictive Value	NA	100.0%	98.0%	98.4%

Another significant observation was the finding of four cases of extrapulmonary tuberculosis that were correctly diagnosed as positive to Tuberculosis by using the Diagnostic test confirmed by biopsy and AFB test at the Hospital lab. While in pulmonary cases getting a sputum test is not significantly invasive, for extrapulmonary cases it requires having a biopsy of the patient. Having a device capable of detecting extrapulmonary cases would significantly simplify this process at the point of care and could increase the possibility of finding these cases.

Another goal in this study was to evaluate the usability of the test in terms of time required to have results versus current processes and the opinion of users about the user experience. In Mexico, for example, the current national diagnostic algorithm requires a screening AFB test to all patients suspected to have the tuberculosis disease. A negative AFB test is considered to be Tuberculosis negative and all positive AFB cases are considered for treatment and sputum samples are taken for a culture/microscopy test for confirmation and to rule out DR and MDR cases. The process for having AFB results take from 12 to 24 hours and require taking, manipulating and sending a sample to the lab. The culture/microscopy test also requires manipulating and sending samples to the lab and would require several weeks to have conclusive results.

On the other hand, using a triage test as the one used in this study would require the doctor or nurse only to take a sample of blood from a finger prick, put it on the paper device and take a picture of the device with a smartphone app, a process that would take only 10 to 15 minutes. Users with just basic knowledge on clinical procedures, like doctors and nurses, required only a previous training on its use which took no longer than 30 minutes.

In conclusion, the TB + HIV diagnostic test showed a high level of accuracy in an initial set of 328 patients who participated in this study. These results showed similar or higher Sensitivity, Specificity and Predictive Values than current screening, triage and referral tests used in diagnostic algorithms. Also, it presents significantly higher Sensitivity and Specificity than the set of Rapid Tests analyzed by the World Health Organization in its evaluation of commercially available rapid TB tests to assess their performance, reproducibility and operational characteristics and to identify promising candidates (WHO, 2008).

Along with this, the usability and data management capabilities can simplify procedures at the clinic and decrease the need of invasive procedures on the patients. To validate these results, new sets of data must be generated with larger cohorts, including patients in different status of the disease like extrapulmonary infections, HIV coinfection, patients with diabetes, patients from high burden regions, etc.

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