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Detection of *Mycobacterium tuberculosis* Antigen in Cough Samples in Northern Ethiopia

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Abstract

Tuberculosis is a highly infectious disease that is spread from person to person by infected aerosols emitted by patients with respiratory forms of the disease. We describe a novel device, the TB "Breathalyser" (Rapid Biosensor Systems Ltd) that collects human cough samples and utilizes immuno- sensor and bio-optical technology to detect *M. tuberculosis* (MTB) Antigen (Ag85B) in them, and demonstrate its use under field conditions during a pilot study in Ethiopia. The TB Breathalyser was field tested on outpatients of three governmental Hospitals in the Tigray region, Ethiopia using a cross sectional study design. Of 427 patients tested, 192 were diagnosed as TB positive from Sputum and/or X-ray. Rapid Biosensor test results were recorded and assessed using two different thresholds for pos/neg diagnosis. Of the 192 TB positive patients 149 (at the -40 threshold) and 130 (at the -60 threshold) gave positive Breathalyser results. A further 120 (at the -40 threshold) and 106 (at the -60 threshold) were Breathalyser positive/sputum negative/X-ray negative and most were clinically positive, at an early stage of infection. The Breathalyser had sensitivities ~97-99% and specificities ~97% for early stage and actively infectious later stage patients. The Breathalyser detected 65-75% of well-established/relapsed patients. The detection of significant numbers of early stage and infectious TB patients, clearly has huge implications regarding the potential for improving the rate of detection of TB in High Burden countries where rates ~30% mostly late stage patients, are currently typical.

Keywords: AFB; Breathalyser Test; *Mycobacterium tuberculosis*

Introduction and Background

Tuberculosis is a common, and in many cases lethal, infectious disease caused by various strains of mycobacterium, usually *Mycobacterium tuberculosis,* MTB [1]. Tuberculosis usually attacks the lungs but can also affect other parts of the body. It is spread through the air when people who have an active MTB infection cough, sneeze, or otherwise transmit their saliva through the air. Most infections in humans result in an asymptomatic, latent infection, and about one in ten latent infections eventually progress to active disease, which, if left untreated, kills more than 50% of those infected in Ethiopia.

Pulmonary TB, the most important type of TB from the public health point of view, can be diagnosed by a combination of clinical examination and assessment of symptoms, chest radiography, sputum smear microscopy, and by cultivation of *M. tuberculosis* [2,3]. In Ethiopia, a significant percentage of patients, however, are not confirmed bacteriologically and are only diagnosed on the basis of high clinical suspicion and response to anti-TB drugs. Sputum smear microscopy remains the cornerstone of TB diagnosis in developing countries. The method depends upon the quality, bacterial load of the sputum specimen, the training and motivation of laboratory technicians. Although highly specific in most countries, smear microscopy is insensitive - it detects roughly 50% of all the active cases of TB. Sensitivity can be as low as 20% in children and HIV infected people. Furthermore,

smear microscopy cannot detect resistance to drugs [3,4]. Culture of TB bacteria on liquid or solid media is more sensitive and the current gold standard method for TB diagnosis rather than smear microscopy and it permits testing for drug resistance [5]. However: it requires bio safety facilities that are expensive to build and maintain and specially trained laboratory technicians to perform the procedure. Even where capacity exists, diagnosing TB with culture can take weeks because of the slow growth rate of TB bacilli. Specimens are often sent to distant laboratories, which can delay processing of specimens and lead to inaccurate results. Few developing countries have capacity for good-quality Drug-Susceptibility Testing (DST) for first-line drugs and even fewer have the capacity to test for second-line drug resistance [6].

A vital first step towards improved diagnostics is rapid and accurate screening at the point of care, but here too the existing solution - the Mantoux TB skin test - is not up to the challenge. The test involves a health worker injecting testing fluid (tuberculin) under the skin of the patient's arm, then waiting between 48 and 72 hours to see if there is a reaction - a red swelling on the injection site - indicating the presence of TB. But factors such as the patient's health and age have to be taken into account. It is also subject to interpretation bias and final results of the test can take as long as two weeks. A further disadvantage is the tendency for the test to produce both false positive and false negative results [6,7]. In the light of these issues, WHO"s strategy for controlling TB includes developing faster screening solutions that identify patients with active TB with a higher degree of accuracy than the Mantoux test. Furthermore, given that TB is most prevalent in the poorest countries, it should ideally be low-cost and capable of administration by personnel with minimal training [8] and with minimal risk of infection for the tester.

We have evaluated here a novel device that utilizes immunosensor and bio-optical technology to detect the MTB antigen in cough samples of human subjects [9-12]. Following a period of assessment by the test developers (Rapid Biosensor Systems Ltd, Cambridge, UK) independent studies had previously been performed in India (three trials) and at the Adama Hospital, Nazareth, Ethiopia with a high prevalence of tuberculosis to assess whether the device was able detect TB antigen in the cough of patients when used in the field. The results obtained were very promising [9,11]. The study reported here was carried out on the most advanced pre-production prototype of the Rapid Biosensor "Breathalyser" which is shown in Figure 3. The location in northern Ethiopia was ideal as it was applied in range of different outpatient clinics of selected governmental Hospitals of the Tigray region.

There are no commercially available TB Screening or Diagnostic devices based on evanescent wave fluorimetric biosensors such as the TB immunosensor described here; this device directly interrogates the cough sample for the presence of the pathogen rather than markers for the disease. The Rapid Biosensor Breathalyser is generic technology which has been patented as a screening/diagnostic tool for the detection of early stage and infectious pulmonary TB [9]. It is designed to be simple, non-invasive, low cost, rapid (~5 minutes in total), safe, and designed for use in the field at point-of-care.

The system works by utilizing the principle of evanescent wave fluorimetry which is shown in Figure 1 below. Evanescent Waves are formed when light is refracted as it travels across the junction of two different media with different refractive indices. A critical angle is reached when the angle of the incident light gives rise to refraction at 90 degrees to the perpendicular. When the incident angle is greater than the critical angle, total internal reflection takes place, during which a small portion of the reflected light penetrates through the interface, creating a very thin electromagnetic field (<200-250nm) adjacent to and propagating along the interface. This is the evanescent wave.

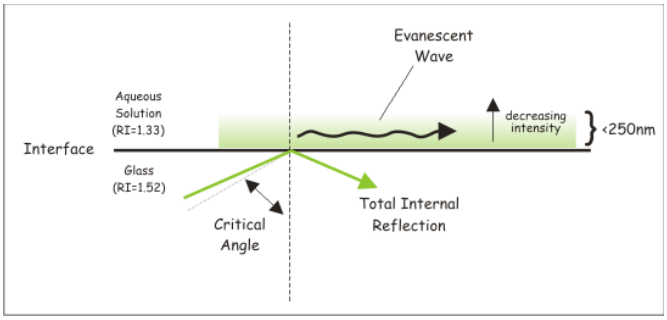

Figure 1: Evanescent Waves.

In evanescent wave immune biosensors, antibodies are attached to a glass surface and the evanescent light propagating through the near surface region excites any fluorescence that is immobilised on the surface. This is shown in Figure 2.

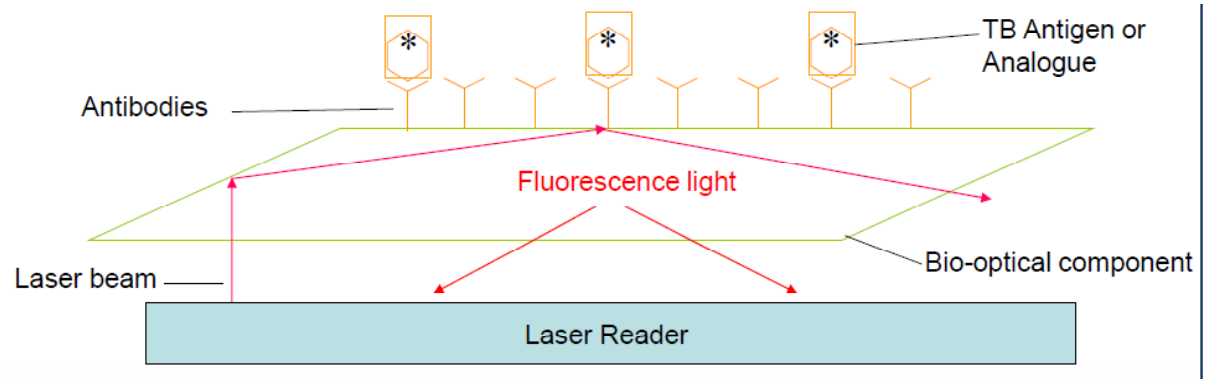

Figure 2: The RBS immunoassay system and optical arrangement.

The cough collection tube contains a prism coated using a standard biotin/avidin sandwich, with anti-TB antibodies primed with fluorescently labelled analogues which are comprised artificially modified peptide sub-sequences of the T-cell epitope from MTB Ag85B [10,13].

When native Ag85B antigens are present they displace the analogue which is more weakly bound to the antibody, causing a decrease in fluorescent signal at the surface. The displaced analogues diffuse into the bulk of the collected sample and since they are no longer within the evanescent region of the prism, they no longer contribute to the detected fluorescent signal. The change in fluorescence is measured by inserting the cough collection tube into a portable, hand- held, battery operated, and evanescent wave fluorimeter called hereafter the "Reader". A digital readout over 2 minutes is sufficient to determine whether or not a displacement is taking place and the size of the change is an indicator of the quantity of bacilli present in the sample. The cough collection tube is one use only and is incinerated after use. The Reader is multi-use. These are shown in Figure 3.

Figure 3: The Reader and collection Tube.

Materials and Methods

The study was conducted in three governmental hospitals of the Tigray region, north Ethiopia; the Mekelle hospital, the Ayder referral hospital (Mekelle University College of Health Science) and the Wukro hospital. The region has an estimated population exceeding 4.3 million with rural residents representing 86% of the total population. With an estimated area of 50,078.64 square kilometers, this region has an estimated density of 86.15 people per square kilometer. There are 36 districts each with a population range of 40,000 to 120,000. Health care is provided primarily through government health institutions. Health services access, as defined by residence within 10 kilometers of a health facility, is approximately 62%. The region is predominantly mountainous and access to transport is limited in rural areas. In the order of referral from lower to higher institutions, there are 278 health posts, 26 health centers and 12 hospitals. The region also has a well-organized structure of about 76% of the region has trained Health Extension Workers (HEWs) and trained Developmental Arms (DAs) widely distributed in 2715 small villages [14,15].

The cross sectional study was performed from April 2012 to January 2013 with the aim of evaluating the efficiency and efficacy of the breathalyser test in the diagnosis of MTB among outpatient clinics of selected governmental Hospitals. All TB suspected patients visiting the OPD with an age of more than 12 years and no previous tuberculosis treatment and requested for laboratory diagnoses in selected governmental hospitals during the study period were included in the study. Since it is a method evaluation we have included 427 TB suspected patients to evaluate the screening test. These patients were selected by symptoms such that they fall into one of the following categories, encompassing and ensuring that the full range of patient sub-groups is sampled: Early stage infectious, Long term established TB, Relapsed TB, Possible MDR or XDR TB, Undiagnosed and with no known history and Persons who either have had, or are currently exposed to an environment where TB is prevalent. Ethical clearance was obtained from the

Ethical Committee of the College of Health Sciences and support letter from the Tigray Regional Government Health Bureau. Informed consent was obtained from all participants prior to data collection. Anonymity and confidentiality were also ensured. The Treatment regime was based on clinical exam, microscopy and X-ray results not on the result of the breathalyser.

The TB Breathalyser Test was administered as follows: the subject was asked to cough into a collection tube in the sequence, cough, breath, cough, breath, cough, following inhalation of nebulised droplets of a 0.9% saline solution to aid the cough response and harvest any bacteria. Sputum sample collections were avoided in these tests.

For each subject, clinical examination, including clinical history were recorded; Chest X-ray; and 3 Sputum samples were collected (where possible) and were examined for MTB AFB by staining with the standard Zeihl-Nelsen technique. The results interpreted as per the guidelines in Revised National Tuberculosis Control Programme (RNTCP.) It should be noted that at the time of the trial, the TB bacilli were difficult to differentiate because the staining material provided by the Ethiopian Government does not correlate with the frequency region of the fluorescence microscopes in use. NB Single sputum samples from approx. 50% of subjects were sent to Addis Ababa for culture examination (facilities are not available on site); however, transportation over the 700 km distance and sample handling issues rendered many of the samples unusable. Data were analyzed using STATA software version 11. These results were analyzed and correlated in terms of: Sensitivity, Specificity, predictive values and confirmed diagnosis - correlation with sputum smear, X-ray and clinical examination.

Results

A single breathalyser test was performed on 427 consenting adults presenting with respiratory problems. Samples for analysis were collected in disposable collection tubes. The mean age was 40 yrs. (range 16-84) and 198 female and 228 male participants were included in the study. There was no medical complaint other than respiratory illness reported by the patients during the triage. All individuals tolerated the breathalyser procedure well and a result was obtained for each patient tested. The time required for the combined nebulization, sample collection and breathalyser test did not exceed 7 min, including the time taken to instruct the patient. As a new improved optical design of Reader, compared to the previous iterations, was used in this study, the threshold for positive/negative diagnosis had to be determined in the first few tests, by reference to laboratory measurements previously conducted correlated with previous trial results. Two pos/neg thresholds (these being -40 and -60) were used for the study in order to assess the impact on the specificity and sensitivity obtained. Table 1 shows the results and correlation of the diagnostic tests for each of the hospitals; together with the stage of the condition.

Our best estimate of the sensitivity and specificity of the Breathalyser test has been made on the basis of correlation with sputum smear, and/or X-ray and/or the clinical diagnosis on the data provided by the Ethiopian medics as evaluated in relation to standard clinical diagnostic parameters. Of the 427 subjects, 192 were diagnosed as TB positive from sputum and/or X-ray results. Of these 149 (using the -40 threshold) and 130 (using the -60 threshold) gave positive Breathalyser results. A further 120 (at -40), 106 (at -60) gave positive breathalyser results. The clinical evidence strongly suggests that most of these Breathalyser pos/ Sputum neg/X-ray neg results also had MTB (Clinical assessment indicated that 5 at the -40 threshold (4 at the -60 threshold) were false positives; 4 Breathalyser positives at Wukro could not be conclusively confirmed because of lack of sufficient clinical data.) The sensitivity and specificity for the two thresholds with respect to different test expectations are given in Table 2 and Table 3 showing the Sensitivities, Specificities, Positive and Negative Predictive values for the Breathalyser test as compared to the values obtained for the sputum and X-ray tests.It should be noted that Wukro is a field hospital with very limited diagnostic resources, so their normal detection rate for TB is usually significantly lower than in the other two hospitals; the calculations of sensitivity and specificity have therefore been included in Table 2 both including and not including the results from Wukro. If the assumption that all test subjects should give a positive Breathalyser result regardless of stage and progression of the disease is applied, the breathalyser test gave 87.62% sensitivity and 95.83% specificity with 98.18% positive predictive value and 75.16% negative predictive value at the -40 threshold. While at the -60 threshold, it had 80.55% sensitivity and 97.01% specificity with 98.33% positive predictive value and 69.52% negative predictive value. On the other hand, sputum microscopy had 100% specificity and 16.94% (17.75%) sensitivity with 100% positive predictive values and 31.08% (35.04%) negative predictive values when compared to the breathalyser test using -40 and (-60) Thresholds.

Test results		Ayder -40	total	Ayder 60	total	Mekelle 40	total	Mekelle -60	total	Wukro 40	total	Wukro 60	total	All hospitals total -40	All hospitals total -60
Spu neg/X-ray neg/RBS neg		37	**37**	38	**38**	51	**51**	57	**57**	27	**27**	35	**35**	115	130
Spu pos/X-ray neg/RBS neg	well est	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	0	0
	relapsed	0		0		0		0		0		0			
	early stage	0		0		0		0		0		0			
Spu neg/X-ray pos/RBS neg	well est	6	**7**	7	**9**	5	**12**	8	**20**	8	**11**	9	**15**	30	**44**
	relapsed	1		1		7		9		1		3			
	early stage	0		1		0		3		2		3			
Spu neg/X-ray neg/RBS pos	well est	5	**62**	5	**61**	5	**26**	4	**21**	0	**32**	0	**24**	120	106
	relapsed	7		7		4		4		0		0			
	early stage	50		49		17		13		28		20			
	Insuff data for diag	0		0		0		0		4		4			
Spu neg/X-ray neg/RBS pos	definite false pos		1		1		4		3		**0**		**0**	5	4
Spu pos/X-ray pos/RBS neg	well est	3	**3**	4	**4**	4	**5**	8	**9**	0	**0**	0	**0**	8	13
	relapsed	0		0		1		1		0		0			
	early stage	0		0		0		0		0		0			
Spu pos/X-ray neg/RBS pos	well est	0	**0**	0	**0**	0	**1**	0	**1**	0	**0**	0	**0**	1	1
	relapsed	0		0		0		0		0		0			
	early stage	0		0		1		1		0		0			
Spu neg/X-ray pos/RBS pos	well est	38	**61**	37	**59**	9	**35**	7	**27**	4	**9**	3	**5**	105	91
	relapsed	5		5		8		6		3		1			
	early stage	18		17		18		14		2		1			
Spu pos/X-ray pos/RBS pos	well est	17	**24**	16	**23**	9	**15**	5	**11**	0	**4**	0	**4**	43	38
	relapsed	4		4		1		1		0		0			
	early stage	3		3		5		5		4		4			
Total			**195**		**195**		**149**		**149**		**83**		**83**	**427**	**427**

NB Mekelle patients on line 40 and 56 N says are not TB pos so I have counted them as negs (Mekelle claimed pos Xrays)

We have discovered definite RBS false pos at Mekelle (4(3 at -60)) and one at Ayder so have a row for these.

Patients who are RBSpos with spu and Xray neg and have been judged by the hosp physicians to be pos on basis of clinical - are indicates with the hosp definition in red.

these are at Ayder: 7 + 3 on TB meds (where they say neg). At Mekelle: 10.

Mekelle lines 124 and 135 judged by them to be well est + relapsed respectively on clinical alone - not expected to be RBS pos anyway.

There are five datasets where pos sputum cultures were produced but the other results and clinical symptoms do not support the diagnosis. We have left these as all negatives in the above table (background of grey in t

There is considerable doubt about the validity of the sputum culture results because of the sample and data handling (as well as the nature of the samples themselves.)

Table 1: Results and correlation of diagnostic tests for each of the hospitals with stage of condition.

Assumptions/Hospitals	-40 Reader	Threshold	-60 Reader	Threshold
TB diagnosed on the basis of clinical examination, sputum smear and/or culture and X-ray.	Sensitivity	Specificity	Sensitivity	Specificity
Assuming all TB positive patients should be breathalyser positive (including well-established and relapsed): Ayder +Mekelle	89.24%	94.62%	82.86%	95.96%
Assuming breathalyser negative patients that are well established and/or relapsed are true negatives: Ayder + Mekelle.	100%	95.83%	98.07%	97.08%
Assuming all TB positive patients should be breathalyser positive (including well-established and relapsed): Ayder +Mekelle + Wukro	87.62%	95.83%	80.55%	97.01%
Assuming breathalyser negative patients that are well established and/or relapsed are true negatives: Ayder + Mekelle.	99.25%*	96.79%*	97.07%*	97.82%*
Assuming that all breathalyser positive results that are not correlated with sputum positive and/or X-ray positive, are false positives; Breathalyser negatives that are well established and/or relapsed are true negatives; Ayder +Mekelle + Wukro	98.64%*	54.71%*	94.74%	62.07%*
*Excludes 4 patients from Wukro who were Breathalyser positive but there was insufficient clinical data for diagnosis.				

Table 2: Sensitivity and specificity at -40/-60 thresholds for the Breathalyser using different assumptions regarding which patient groupings are detectable.

Diagnostics	-40 Threshold				-60 Threshold			
	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)
Breathalyser expected positive for	87.62 (83.41 -91.09)	95.83(90.54 -98.62)	98.18(95.79 -99.40)	75.16(67.54 -81.78)	80.55(75.54 -84.92)	97.01(92.53 -99.16	98.33(95.78 -99.53)	69.52(62.38 -76.03)
Breathalyser only expected positive for Early stage & infectious patients *	99.25(97.02-99.87)	96.79(92.29-98.81)	98.15(95.47-99.32)	98.69(94.87-99.77)	97.07(93.80-98.71)	97.83(94.17-99.30)	98.31(95.43-99.46)	96.26(92.13-98.35)
Sputum microscopy	16.94(12.92 -21.61)	100(96.81 -100.00)	100(93.08 -100.00)	31.08(26.4 -36.07)	17.75(13.55 -22.61)	100(97.17-100.00)	100(93.08 -100.00)	35.04(30.19 -40.13)
X-ray	60.59(54.88 -66.09)	100(96.81 -100.00)	100(98.02 -100.00)	48.73(42.19 -55.30)	63.48(57.68 -69.00)	100(97.17 -100.00)	100(98.02 -100.00)	54.85(48.28 -61.30)
*Excludes 4 patients from Wukro who were Breathalyser positive but there was insufficient clinical data for diagnosis.								

Table 3: Showing sensitivity, specificity and predictive values with 95% confidence Interval, at -40 and -60 threshold of the Breathalyser, Sputum microscopy and X-ray, assuming standard clinical diagnosis as positives.

However, on the basis that we do not expect to detect well established or relapsed patients, we suggest that the rows shaded in pale grey on Table 1 should be applied to the data. Previous trials had indicated that the Breathalyser test should be positive only for patients for whom the stage and progress of the disease means that they are actively infectious, i.e. are coughing out "free" bacilli. Previous results [11,12] have shown that when the disease is well established, or occurs in relapsed patients, the bacilli quickly become "locked up" in the sputum and the harvest of free bacilli is relatively low. Taking this into account, the expectation is that many well established and relapsed patients, are expected to give negative Breathalyser results and so these results should be included as true negatives (rather than false negatives) in calculations of sensitivity and specificity. On that basis, the sensitivity for early stage and infectious later stage patients (i.e. those still actively coughing out bacilli) was found to be 99.25% (-40 threshold) and 97.07% (-60 threshold), with the specificity of 96.79% (at -40) and 97.83% (at -60.) with positive predictive values of 98.15% at -40 (98.31% at -60) and negative predictive values of 98.69% at -40 (96.26% at -60.).

Until the advent of the Rapid Biosensor Breathalyser system there was very little information available regarding the aerosolisation, collection and analysis of bacilli by individual patients because there were no available, appropriate methods for collecting and testing the samples. Previous work on the transmission of TB suggests there is considerable variation in infectiousness; some patients infect a large number of contacts whereas others do not appear to pass on the disease [16].

Fennelly and colleagues [17] developed a device for capturing cough generated aerosols from individual patients. Samples of cough were subjected to culture to assess the numbers of colony forming units of *M. tuberculosis*. Results were obtained in 6 to 10 weeks and showed variation in the quantities of cultured aerosols produced by individual patients; however, the Rapid Biosensor Breathalyser appears to be the only workable method available for "real time" detection of TB in real patients. It should be noted that despite the fact that we do not expect to detect well established or relapsed patients, the improvements that we have made to the design have allowed a higher number of these patients to be detected than were observed in previous tests as shown in Table 4.

Stage of MTB	Breathalyser positive (% detected by Breathalyser test)	Breathalyser negative (% not detected by Breathalyser test)	Total	Breathalyser positive (% detected by Breathalyser test)	Breathalyser negative (% not detected by Breathalyser test)	Total
Well established	87 (77%)	26 (23%)	113	77 (68%)	36 (32%)	113
Relapsed	32 (76%)	10 (24%)	42	28 (67%)	14 (33%)	42
Early stage	146 (99%)	2 (1%)	148	127 (95%)	7 (5%)	134
Total	265	38	303	232	57	289
Excludes 4 patients from Wukro who were Breathalyser positive but there was insufficient clinical data for diagnosis.						

Table 4: Breathalyser test results at -40 and -60 threshold correlated with stage of MTB for TB Positive patients.

Discussion

We have demonstrated the use and applicability of a novel portable device for assessing TB antigen in breath/cough. It is a 'point of care' test that would allow assessment of individuals in the community whilst they undertake their normal daily routines. Having demonstrated that the test can be performed in a clinic with minimal training and as screening in the community, it has paramount importance for Ethiopian health care system and other similar settings. Compared to the current diagnostic regime employed in Ethiopia, the breathalyser has significantly higher specificity and sensitivity. The Breathalyser test has higher Negative Predictive values compared to culture and X-ray results. The Positive predictive value is also high but lower than for the culture and X-ray results (where it is 100% as is the specificity), however this must be viewed in the context of the sensitivity found for these tests, especially for sputum, which is incredibly low. This might be due to poor sputum production, viability of the bacteria, mis-interpretation, the lab conditions & instrumentation [3, 5 - 6.] Rather lower numbers of sputum positive samples (both smear and culture) than might be expected were obtained in this trial because of the problems inherent there.

The Breathalyser had a higher detection rate compared to sputum and X-ray results. On one hand; the Rapid Biosensor test detects the very early stages of the disease when it is forming in the Larynx (our findings correlate with the findings of an earlier Indian trial [11] where 9 patients were found in the very early stages of the disease before any other results became confirmed positive.) Additionally, the current iteration of the prototype device, significant numbers of those well established and relapsed patients are being detected; i.e. those who are coughing bacilli out.

By contrast; Approx. 50% of TB patients do not produce sputum and just over 50% of those that do are smear negative [3,4,18]. This severely hampers the use of sputum testing. Testing on sputum is very susceptible to contamination and is dependent on the disease being sufficiently established that it has started to colonize in the sputum. It also requires high skill levels of individuals and good quality instrumentation.

X-rays are non-specific for MTB and so their accuracy is reliant on the skills of the radiographer interpreting the X-ray [3]. Once again the presence of the disease can only be detected once it has started to damage the lungs with small nodes appearing as the condition establishes and larger lesions forming once it becomes well established. Both detection thresholds for the Breathalyser test gave sensitivities and specificities that are at a very desirable level for a TB screening/diagnostic device. The -40 threshold demonstrates higher sensitivity than the -60 threshold, but the resulting specificity is lower. It is envisaged that the final commercial product will be further de-skilled, hopefully resulting in even higher accuracy, and the threshold will be carefully chosen to optimise sensitivity with respect to specificity.

Compared to the current "Normal" diagnostic procedure used in Ethiopia, the Breathalyser test has good specificity and sensitivity. Ethiopia currently has a TB epidemic, which could be a consequence of the fact that diagnosis is based on a protocol, which according to Ethiopian Medics, tends to favor the diagnosis of Pneumonia for similar respiratory symptoms. The protocol is based on an algorithm proposed by the WHO some years ago. Ethiopian medics are trained/instructed to treat respiratory symptoms as Pneumonia before considering TB. If the antibiotic treatment fails, then TB is considered; however, the antibiotics often mask the underlying TB which is then able to establish and presents as well established TB later on. This means that there are very few cases of early stage TB diagnosed in Ethiopia, with obvious economic, personal and transmissibility implications [18,19].

The detection of the additional patients (over and above those detected by sputum and/or X-ray) who were mostly at an early stage of infection, using the Breathalyser, is highly significant as this constitutes an additional 38% (35%) of the total positive MTB cases. We know that only 30% of cases are usually diagnosed in Ethiopia and these are usually at a late stage of the disease. Detection of this additional patient grouping takes the rate up closer to the 70% desired by the WHO and more importantly, the ability to detect early stage patients, at which time they are highly infectious, could be beneficial in two ways; by improving the prognosis and treatment outcome because of earlier stage intervention, and by reducing the potential for spreading the infection to other individuals.

Finally, although the Rapid Biosensor Breathalyser was originally designed as a screening test, in these trials it has shown higher specificity and sensitivity among relapsed and well-established TB patients compared to earlier results. Despite the fact that the system was not originally conceived to detect any well-established or relapsed patients, percentages for detection of well-established and relapsed patients ~65-75% are extremely promising with respect to use of the system as a diagnostic tool.

Conclusion and Recommendation

We have demonstrated that The Rapid Biosensor Breathalyser can be used as screening tool for early stage TB in tuberculosis high burden countries with sensitivity and specificity far greater than that for sputum smear and X-ray tests. The Rapid Biosensor test detects early stage and actively infectious TB in minutes - and can do this at a stage before any of the usual methods can be applied. The portability and ease of use lends itself to adoption for use in rural or third world communities. The method also shows considerable promise as a diagnostic tool.

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Authors' contributions

Conceived and designed the research project: Kiros Tedla, Afewerk Mulugeta

Performed the experiments: Kiros Tedla, Afewerk Mulugeta, Megbaru Alemu, G. Kidan G. Her and Areya G. yesus

Analyzed the data: Kiros Tedla, Afewerk Mulugeta, Elaine M. McCash and Nicol J. Murray

Wrote the paper: Kiros Tedla.

Elaine M. McCash and Nicol J. Murray provided technical knowledge, protocols and training for the breathalyser test.

Elaine M. McCash and Nicol J. Murray have a financial interest in the project.

All authors read and approved the final report.

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