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Onsite Performance Verification of DETERMINE™ TB LAM Ag: A Rapid Diagnostic Test for Tuberculosis Screening in Urine

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Abstract

According to WHO, the rates of smear-negative and extra-pulmonary pulmonary tuberculosis are increasing in high prevalence HIV epidemic areas. Delays in diagnosis of tuberculosis can lead to large excess of mortality. It is extremely important to provide a strong diagnosis tool of tuberculosis if we want to reduce mortality due particularly to TB co-infection in HIV infected people in low-income countries such as Togo. This study aims to assess the performance of Determine™ TB LAM Antigen, a rapid diagnostic test (RDT) for tuberculosis. It was an evaluation study, conducted at the National Reference Laboratory for Mycobacteria located at the Sylvanus Olympio University Teaching Hospital in Lomé, Togo from 01 July to 15 November 2017. We performed the assessment onto 100 urine specimens collected from 100 subjects (HIV-infected or not). The test allows qualitative detection of the Lipo Arabinno Mannan (LAM) antigen of Mycobacteria in the urine. Bacilloscopy was chosen as gold standard. Overall, the test Determine™ TB LAM presented a sensitivity of 31.25% and a specificity of 95%. In contrast, the sensitivity and specificity of the test were respectively 82.35% and 66.67% in the group of HIV-infected subjects. In HIV non-infected subjects, the sensitivity was 17.46% and the specificity was 100%. Determine™ TB LAM Antigen test can help detect TB in HIV-infected people unable to expectorate in our settings.

Keywords

Tuberculosis, Urine, Determine™ TB LAM Ag, HIV

1. Background

Tuberculosis is a major health problem worldwide, especially in the current context of HIV infection pandemic [1] [2]. The diagnosis of tuberculosis remains difficult in highly immunocompromised subject, particularly in the case of HIV infection [3]. Tuberculosis control programs currently face some barriers related to the limitations of current diagnostic tools [4]. These include difficulties in obtaining and conditioning samples to be analysed, lack of infrastructures and materials as well as qualified human resources to carry out the test, and the delay of laboratory's response [4]. In our settings, the diagnosis of pulmonary and extrapulmonary tuberculosis relies on identification of acid fast bacilli (AFB) by the Ziehl-Neelsen (ZN) staining method and bight field microscopy. The ZN microscopy is highly specific but lacks sensitivity (20% - 80%) [5]. The ability of Sputum smear microscopy to identify patients with TB may rely on the patient's ability to produce "quality sputum". Studies have shown that HIV infected patients are not able to produce "quality sputum" or produce paucibacillary sputum [6] resulting in possibly false negative microscopy results [7]. Missing out on diagnosis of tuberculosis can lead to fatal life-threatening complications. Improving accessibility to biological diagnosis of tuberculosis remains a key factor to better manage this pathology and prevent the spread, especially in low-income countries. Thus, we need diagnostic tools that are rapid, have good diagnostic accuracy and can be used at all healthcare facilities. Given the challenges of obtaining sputum samples and limited yield in extra pulmonary TB, urine has been identified as a favourable alternative biological sample due to the ease of obtaining samples from patients, the ease of laboratory handling and processing, and the lower risk of nosocomial transmission to healthcare and laboratory workers. Several mycobacterial antigens have been identified in the urine of patients with active TB [8] [9]. This study aims to assess the performance of ALERE DETERMINE™ TB LAM Ag a rapid diagnostic test (RDT) for tuberculosis namely by determining its sensitivity, its specificity, its positive predictive value (PPV) and its negative predictive value (NPV).

2. Material and Methods

An on-site performance evaluation study of ALERE DETERMINE™ TB LAM Ag, a Tuberculosis (TB) rapid diagnostic test in urine, was conducted at the National Reference Laboratory for tuberculosis (LNR-TB) at the University Teaching Hospital (UTH) Sylvanus Olympio Hospital of Lomé in Togo from 1st July to November 15th, 2017. We used bacilloscopy as gold standard.

2.1. Test Characteristics and Principle

- Bacilloscopy method

We used the Ziehl-Neelsen staining technique for bacilloscopy [10]. It is a qualitative and/or semi-quantitative examination of the smear. After coloration, to read the slides, first use the x10 objective to focus the slide. Then, add a drop of

immersion oil to the sputum smear and select the ×100 objective. Bring the sputum smear into sharp focus using the fine focus control. Adjust the illumination control to provide a comfortable level of illumination. Sputum smear slides should be read in a sweeping pattern. Do not examine the same area of the smear repeatedly. As recommended by the International Union against Tuberculosis and Lung Diseases [10], examine 100 fields before declaring a slide negative (this should take approximately 5 - 6 minutes). With a standard 1 cm × 2 cm sputum smear, being at one end of the oval and move one field horizontally after it has been examined. The 2 cm length of the smear is sufficient to examine 100 fields as required. If acid-fast bacilli (AFB) are found, use a mechanical counter to count the numbers of bacilli in each field. The observation picture about acid-fast bacilli (AFB) is shown in **Figure 1**. The reporting of sputum smear slides is done as follows:

- When no AFB are seen after examining 300 fields, report the smear as "No AFB seen".
- When very few AFB are seen *i.e.* when only 1 or 2 AFB are seen after examining 100 fields, request a further specimen to examine. Those AFB might have come from tap water (saprophytic mycobacteria), or it may be scratch of glass slide or by the use of same piece of blotting paper while drying.
- When any red bacilli are seen, report the smear as "AFB positive" and give an indication of the number of bacteria present as follows: more than 10 AFB/field at least in 20 fields, report as **+ + +**; 1 - 10 AFB/field at least in 50 fields, report as **+ +**; 10 - 99 AFB/100 fields, report as **+**; 1 - 9 AFB/100 fields, report the exact number.

- Alere Determine™ TB LAM Ag

This RDT is presented in a box of 100 strips. It is an immunochromatographic test for the qualitative detection of lipoarabinomannan (LAM) antigen of Mycobacteria in human urine. Alere Determine TB LAM Ag employs highly purified antibodies specific to the major polysaccharide antigen of the genus

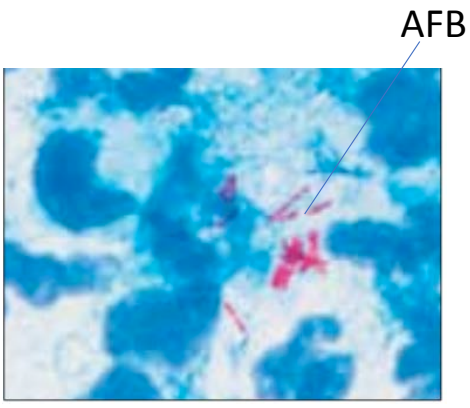

Figure 1. Image showing microscopic picture of acid fast bacilli (red sharp), Ziehl-Neelson stained smear viewed under bright field microscopy (1000× magnification) [10].

Mycobacterium: lipoarabinomannan (LAM). These antibodies are used for both the capture and the detection tracer. The capture antibodies are adsorbed onto the nitrocellulose membrane of the test strip. The detection antibody is labelled by conjugation to colloidal gold particles. After a urine specimen is added to the sample pad, the colloidal gold conjugated antibodies attach to the LAM antigen and are released by the specimen from the conjugate pad. This immunological complex is then captured by anti LAM antibodies immobilized on the nitrocellulose membrane and made visible due to the presence of the colloidal gold label. Alere Determine™ TB LAM Ag test cards must be stored at 2˚C - 30˚C until the expiration date.

As recommended, applied 60 μL of urine sample or 2 drops of urine to the sample pad (white pad marked by the arrow symbol) to perform the test. A minimum of 25 minutes is required for read result and no more than 35 minutes.

A positive result (a visible purple/gray line) indicates that LAM antigen of Mycobacteria is present in the sample at or above the detection limit of the test; whereas a negative result (no visible purple/gray line) indicates that LAM antigen of Mycobacteria is not present or below detection limit (**Figure 2**). To ensure assay validity, a procedural control bar is incorporated in the assay device.

2.2. Study Design and Patient Recruitment

First, sputum smears were collected from sick or healthy subjects, HIV-positive or not, among people referred to the national reference laboratory for TB bacilli (LNR-TB) for tuberculosis screening.

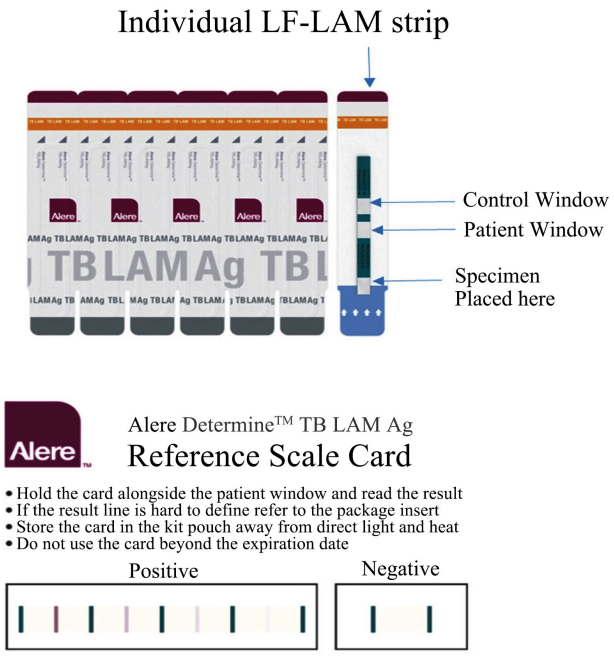

Figure 2. Picture of TB LAM Ag and reference scale for interpreting the test result [23].

For recruitment, we considered patients with obvious signs of tuberculosis, who were able to spit and urinate and who had given their informed consent to participate in the study. We excluded patients whose were receiving treatment for tuberculosis and/or diuretic therapy and/or those who presented haematuria. Thus, we included 80 positive patients in bacilloscopy namely 20 respectively with AFB3+, AFB2+, AFB1+, 20 scanty, and 20 smear negative patients confirmed by GeneXpert™ MTB/RIF. Patient inclusion chart is shown in **Figure 3**.

2.3. Urine Collection and Processing

The staff of the University Teaching Hospital (UTH) Sylvanus Olympio Hospital of Lomé in Togo has given its approval to make this assessment. We informed patients and obtained their free consent to participate into the study. In addition to sputum collection for microscopy, for each pre-inclusion patient, we collected 20 ml of urine in a sterile jar and stored it in the refrigerator (2˚ - 8˚C) for 24 hours until the patient was included. We also provided information on the status of HIV infection. A total of 100 urine samples were included (one patient-one sample of urine) and tested.

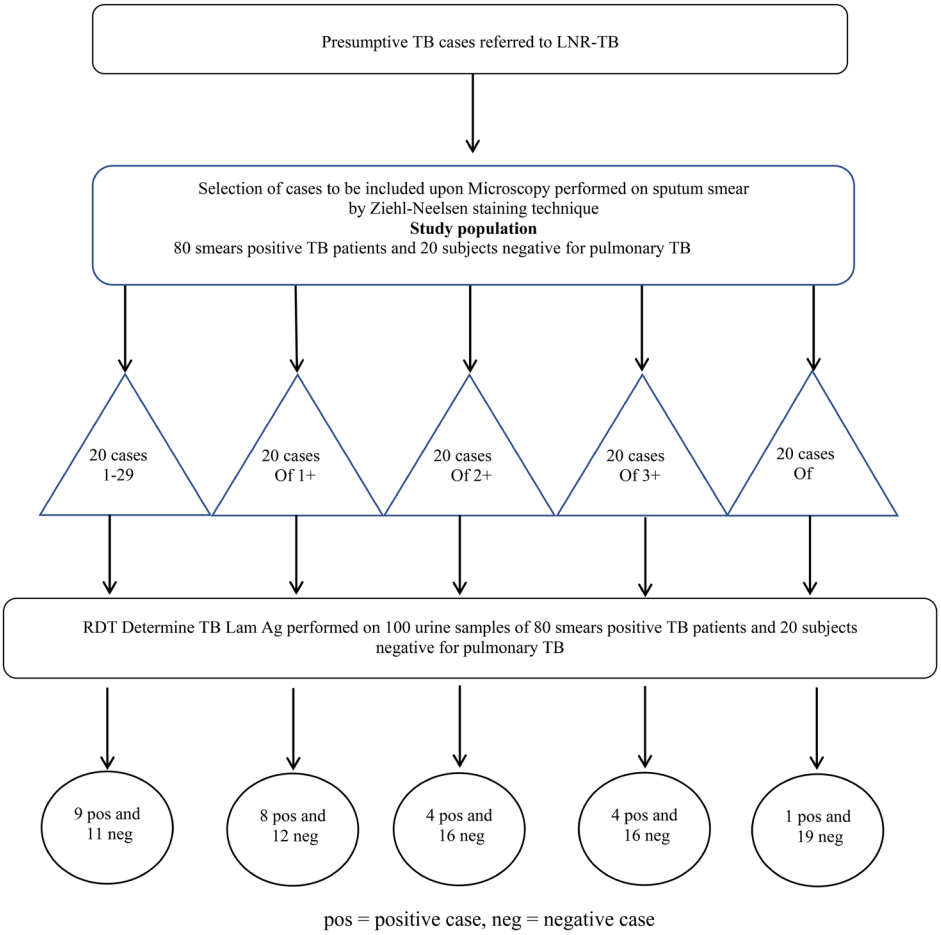

Figure 3. Patient recruitment diagram.

2.4. Data Analysis

Data have been entered by the Microsoft Excel software version 2016 and processed by the software Epi-info version 7.2. We determined sensitivity (True Positive/True Positive + false negative), specificity (True negative/True negative + False positive), positive predictive value (PPV) (True Positive/true positive + False positive) and negative predictive value (NPV) (True negative/True negative + False negative).

3. Results

We included 100 subjects. Of them, 20 were HIV infected (Table 1) including 17 out of 80 TB patients with smears positive and 3 patients out of 20 in subjects tested negative for pulmonary TB upon bacilloscopy and GeneXpert MTB/RIF.

3.1. Alere Determine™ TB LAM Ag Test Testing Results

We tested 100 urines samples of study population for onsite evaluation of Alere Determine™ TB LAM Ag test. Based on this alternative sample, we found 26 subjects positive to TB LAM Ag versus 74 negative (Table 2).

Of the 80 HIV-negative subjects, 11 were found positive for TB Lam Ag by the RDT out of 63 who had smear-positive (Table 3). Using the Alere Determine™ TB LAM Ag test, amongst 17 HIV infected subjects with smear positive, 14 were detected positive. One false positive case was found among 3 HIV infected patient free from pulmonary tuberculosis (Table 2)

Table 1. Bacilloscopy results regarding to HIV status.

HIV status	Bacilloscopy					
	3+	2+	1+	Scanty	Negative	Total
HIV (+)	1	4	5	7	3	20
HIV (−)	19	16	15	13	17	80
Total	20	20	20	20	20	100

Table 2. Results from urine testing for TB Lam Ag.

Patient status	Alere Determine™ TB LAM Ag		Total
	positive	negative	
HIV positive and bacilloscopy Positive	14	3	17
HIV positive and bacilloscopy negative	1	2	3
HIV negative and bacilloscopy Positive	11	52	63
HIV negative and Bacilloscopy negative	0	17	17
Total	26	74	100

Table 3. Performance of Alere Determine™ TB LAM Ag test.

	Sensitivity	Specificity	PPV*	PNV*
HIV infected patients	82.4%	66.7%	93.3%	40.0%
Non HIV Patients	17.5%	100.0%	100.0%	24.6%
All patients	31.2%	95.0%	96.1%	25.7%

*PPV = predictive positive value, *PNV = predictive negative value.

3.2. Performance of Alere Determine™ TB LAM Ag Test

Overall, the RDT TB LAM presented, a sensitivity of 31.2% (25/80) and a specificity of 95.0% (19/20). In the group of HIV-infected people, the test showed a sensitivity of 82.4% (14/17) and a specificity of 66.7% (2/3), versus a sensitivity of 17.5% (11/63) and a specificity of 100.0% (17/17) in the group of non-infected HIV patients (**Table 3**).

4. Discussion

To our knowledge, this is the first study of performance assessment in field conditions of a rapid diagnosis test for tuberculosis in Togo through consecutively enrolled subjects referred to LNR-TB for tuberculosis screening. Even the development of the point-of-care urine-based lateral flow lipoarabinomannan test has been hailed as a significant advance [11], Alere Determine™ TB LAM Ag in our context, showed at glance poor sensitivity and specificity compared to bacilloscopy which is the mainstay in the diagnosis of pulmonary and extra pulmonary tuberculosis in the resource poor setting [5]. Sensitivity and specificity values, we found, respectively 31.2% and 95.0% are somewhat comparable to 39.0% - 66.7% for sensitivity and 98.6% for specificity indicated by manufacturers especially. Other studies have been conducted in Africa on the evaluation of the performance of this test. Indeed, in 2016, in Swaziland, Munyaradzi *et al.* reported a sensitivity of 20% and a specificity of 96.2% [12]. In addition, Drain *et al.* in South Africa have in their study found a sensitivity of 42.1% and specificity of 84.9% [13]. The difference found between the performances recovered could be explained by the difference of study scheme, we can appoint the gold standard or reference test and the percentage of PLHIV subject included in these studies. This could also be proven by the fact that the prevalence of tuberculosis and HIV is higher in South Africa (where the manufacturers study was conducted) and in Swaziland than in our country.

In the course of these evaluations, we noticed that Alere Determine™ TB LAM Ag does not in any case present sensitivity values that reach 50% which means that more than once in two, this test does not identify a TB patient. As for the specificity, although lower than the 98.6% given by the manufacturer, our study and those of Swaziland and South Africa showed values not higher than 85.0%. This means that once in six, the RDT identifies a healthy subject as having tuberculosis (false positive subject).

This evaluation, we conducted revealed that DETERMINE™ TB LAM Ag is better to identify TB in People Living with HIV (PLWHIV), as reported by manufacturers. Indeed, we got a 17.5% as sensitivity value in subjects not HIV-infected versus 82.4% in PLWHIV. These results are supported by the findings of Munyaradzi *et al.* in Swaziland [12], which found an increase in the sensitivity of RDT correlated with a depression of immunity, in particular by a decrease in the level of TCD4 lymphocytes, and an association between the rate increase of LTCD4 and an increase in the values of specificity. Others evaluation studies showed greatest utility of DETERMINE™ TB LAM Ag in facilitating the diagnosis of TB in HIV-positive hospital in patients with advanced immunodeficiency and symptoms of TB [3] [14] [15] [16]. Our opinion is not supported by Thit *et al.* in Myanmar [17]. For these authors, the TB-LAM Ag test had limited clinical utility in the management of HIV-positive patients in this Asian referral hospital setting. In sub-Saharan Africa, the use of Lateral Flow-LAM testing to diagnose TB proved cost-effective [18] [19] and reduced 8-week mortality in HIV-positive hospital inpatients [20]. The positive RDT result obtained on the urine of a PLWHIV who had a negative bacilloscopy in our assessment could be related to extra-pulmonary tuberculosis. In fact, bacilloscopy performed on sputum in cases of extra-pulmonary tuberculosis is not helpful, with a sensitivity ranging from 10% - 20% [21]. Similarly, the sensitivity of bacilloscopy is reduced in immunocompromised individuals. A study conducted by Peter *et al.* In South Africa in 2012 as part of the evaluation of the same test had revealed in a group of patients with extra-pulmonary tuberculosis, a significant sensitivity of 41% by Determine TB LAM Ag against 0% by bacilloscopy [22]. These findings around area where Tuberculosis cases are wide spread proved an added value of Determine TB LAM. Furthermore, this RDT is easy to implement. The test requires no laboratory infrastructure, it is cheaper and simple to perform than the Gene Xpert MTB/RIF assay, and lacks the infection risks associated with sputum collection [23]. The Determine TB LAM test is easy to use. The conditions of conservation of this test (2˚C - 30˚C) and the shortness of the execution time (25 minutes) make it suitable for use at the service points of the care facilities. The ease of obtaining urine samples is an appropriate alternative for people unable to expectorate or who are suspected of having extra-pulmonary tuberculosis requiring invasive diagnostic sampling.

The relatively small number of strips available for the evaluation, as well as the use of bacilloscopy as a reference test, may be limitations for this study. In addition, the sometimes less easy reading of the result of this RDT can lead to interpretation bias. Indeed, four positivity grades of this test have been described in the user guide instituted by WHO in 2015 [23].

5. Conclusion

The diagnosis of tuberculosis is not always easy, especially in extra-pulmonary forms, in pulmonary forms where the patient cannot expectorate, or in pauciba-

cillary patients. The Determine™ TB LAM Ag test, while having poor performance in non-HIV infected subjects, may be an alternative tool for TB screening in HIV patient and to detect extra pulmonary tuberculosis cases.

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Conflicts of Interest

The authors declare no conflict of interest.

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