

Comparison of Classical Conventional Tests to XPERT MTB / RIF in the Diagnosis of Tuberculosis in People Living With HIV in Kinshasa

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ABSTRACT

Background: In countries with a high prevalence of HIV infection, diagnosis of TB / HIV co-infection with low-sensitivity Ziehl is a difficult problem because of lesions that are paucibacillary.

Objectives: To compare the Xpert® MTB / RIF test with conventional tests and to determine the level of resistance to rifampicin.

Methods: Sputum was tested at Gen-Xpert® MTB / RIF and compared to smear microscopy and culture (Standard Gold). The resistance to rifampicin was determined in comparison with results obtained by the ratio technique on Löwenstein-Jensen.

Results: Xpert®MTB/ RIF detected *M. tuberculosis* in 42 samples (60%), whereas, the cultures showed a positivity rate of 54.3%; 38 strains of *Mycobacterium tuberculosis* were isolated. The positivity rate was 44.3% at Auramine and 25.7% at Ziehl. The rate of resistance to rifampicin was 10.5% at Xpert® and 7.9% with the ratio technique. The absolute difference between Xpert®-Ziehl was 34.3%; 15.7% for Xpert®-Auramine; 5.7 % for Xpert®-Cultures; 28.6 % for Cultures-Ziehl; 10 % for Cultures-Auramine and 18.6 % for Auramine- Ziehl: Gen-XPRT is 2.3 times better than Ziehl, 1.4

times more potent than Auramine and 1.1 times better than the culture.

Conclusions: The Xpert®MTB / RIF test is more sensitive than conventional tests and at the same time give information on resistance to Rifampicin in less than 2 hours.

Key Words: Comparison; Conventional Testing; Xpert®MTB / RIF; Tuberculosis-PVV; Rifampicin Resistance.


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INTRODUCTION

Tuberculosis and HIV infection are two major public health problems in the world, particularly in sub-Saharan Africa.¹ According to the World Health Organization (WHO) report on tuberculosis, 9.6 million new cases of tuberculosis were detected in 2014, of which 12% were infected with the Human Immunodeficiency Virus (HIV).¹

HIV infection has led to an outbreak and significant increase in TB cases worldwide, particularly in sub-Saharan Africa where the incidence is very high.¹⁻⁶

Democratic Republic of the Congo (DRC) which is among the 22 countries with a heavy burden of tuberculosis and HIV infection, reported in 2014, 7206 (14%) cases of co-infection TB-HIV.¹

The problem with this form of tuberculosis is the detection of tuberculosis bacilli using the Ziehl Neelsen test recommended by the WHO. The test appears to be less effective by the presence of the paucibacillary lesions encountered in patients co-infected with HIV, in which case the detection rate is reduced by 43-51%.¹⁻⁶

Consequently, some cases escape detection because of the low sensitivity of the screening test and result in an increase in morbidity and mortality.

Hence, WHO recommend the Xpert® MTB / RIF test to overcome this difficulty in order to detect *Mycobacterium tuberculosis* and resistance to rifampicin.²

In the DRC, few tuberculosis screening and treatment (CSDT) health centers have been targeted for its implementation.

Thus, it is important to compare Xpert® MTB/RIF test with other diagnostic tests in our country in order to determine its actual contribution.

MATERIAL AND METHODS

Type and Length of Study

This is a cross-sectional and prospective study of patient samples received between July 10, 2013 and September 10, 2014, a period of 14 months.

Recruitment Sites and Sample Collections

The study examined all HIV-positive patients' samples with suspicion of pulmonary tuberculosis that occurred during the studied period. Two sputum specimens were collected on the same day from HIV positive patient after informed consent, at the Mother and Child Health Center in Ngaba (CMENgaba) and at Saint Alphonse Health Center in Matete, which are equipped with GEN-Xpert®. One sample was analyzed on the site at the CSDT's by carrying out Ziehl-Neelsen and GEN-Xpert® and a second sample was sent to the Mycobacteria Laboratory of the Faculty of Medicine of the University of Kinshasa for the cultures (LJ and 7H9) and the antibiogram on Löwenstein medium -Jensens (LJ). Any patient suspected of having pulmonary or extrapulmonary tuberculosis with negative HIV serology and any patient who refused to participate in the study were excluded.

LABORATORY ANALYSIS

Direct Examinations After Ziehl-Nelseen and Auramine Staining

These direct tests for resistant acid alcohol bacilli (AFB) were performed according to WHO and PATI IV guidelines.³⁻⁵ The hot method was used for Ziehl. It consisted of staining the smear with concentrated Fuchsin by heating three times at zero, 3rd and 6th minute, and then waiting 10 minutes before rinsing. The smears rinsed with water were discolored with acid alcohol for 3 minutes and recolored with methylene blue for one minute.^{3,5} Reading was done with a 100X lens. For the Auramine, the smears were stained for 15 minutes, discolored with acid alcohol for 3 minutes, and then stained with potassium permanganate for one minute. Reading was carried out with a 25X objective under fluorescence microscope.^{4,5}

Gen-Xpert®MTB / RIF

Gen-Xpert®MTB / RIF was performed at CDSTs according to WHO guidelines.⁶ One milliliter (1ml) of sputum was mixed with 2 ml of diluent and vigorously stirred for 10 to 20 times until complete liquefaction. The mixture was then incubated for 15 minutes at room temperature.⁶ Using a sterile transfer pipette supplied with the kit, 2 ml of the treated sample was collected and transferred into a cartridge and the cartridge placed into the machine for analysis. The lid of the cartridge was closed tightly. The test started within 30 minutes of transferring the sample to the cartridge and left running until all parameters were displayed. The patient's name, laboratory number, sample and sample type were entered into the machine. At the end, the results were displayed on the screen two hours later and recorded in a register as MTB- / RIF-, MTB + / RIF- or MTB + / RIF undetermined or invalid and either MTB + / RIF +.⁶

Cultures and Susceptibility

After the Ziehl and Xpert® at the CDST, some samples were placed in a refrigerated tank and carried to the Mycobacteria Laboratory of the University Of Kinshasa Faculty Of Medicine for the Auramine test, cultures (7H9 and Löwenstein-Jensens) and antibiogram. The samples were then treated with N-acetylcysteine sodium hydroxide (NALC-NaOH) for 15 minutes and neutralized with 45 ml of phosphate buffer before centrifugation at 3000 rpm for 20 minutes.⁷

A 0.5 ml inoculum was seeded in a liquid medium containing 0.5 ml of OADC (oleic acid albumin dextrose and catalase), and 0.8 ml of Panta, and while 0.2 ml was deposited on the slope of LJ medium and incubated at 37°C.⁷ After incubation, seeded 7H9 flasks were observed each day to check for signs of contamination. From the 7th to the 14th day of incubation, a smear was performed and stained with Ziehl for the detection of BAAR under a microscope.⁷ On the other hand, on LJ, used as reference (Gold standard), after 24 to 72 hours of search for contamination signs, tubes were then observed once a week.

Positive culture was determined by the presence of cauliflower colonies on the slope of the medium and verification was performed by performing the Ziehl technique.⁷ The biochemical identification of the strains was carried out on the same LJ medium using 2 µg / ml 2-thiophene carboxylic acid and Niacin test. The antibiogram was performed on LJ according to the proportion technique of Canetti and al.⁷ The following molecules at define critical concentrations were used: Rifampicin 40 µg / ml; Isoniazid 0.2µg / ml; Ethambutol 2µg / ml; Streptomycin 4µg / ml; Ofloxacin 4µ/ml; and Kanamycin 30µg / ml.⁷ Readings were made on the 28 th and 42nd day of incubation⁷. In addition, HIV serology was performed at the CSDT using the test to determine and double check.^{5,8}

Ethical Considerations

The study was approved by the Ethics Committee of the School of Public Health of the University of Kinshasa (N ° ESP / CE / 081/2010).

Statistical Analysis

Data recorded in the laboratory were then transferred to Excel software. They were analyzed using SPSS software version 20.0. This software allowed statistical processing of information. The mean and standard deviation were calculated for the quantitative variables. The results obtained with Gen-Xpert®MTB / RIF were compared with those obtained by the proportional LJ technique. Sensitivity, specificity, positive and negative predictive values and Kappa were calculated. The exact Fisher test as well as the Pearson chi-square were used as needed. The significance level was set at 5% and the confidence interval (CI) at 95%.

Table 1: Characteristics of population by sex according to Ziehl and Auramine results

| Sex | Number (%) | Ziehl +(%) | Ziehl -(%) | Aura +(%) | Aura -(%) |
|--------------|------------|------------|------------|-----------|-----------|
| M | 42 (60) | 10 (14.3) | 32 (45.7) | 19 (27.1) | 23 (32.9) |
| F | 28 (40) | 8 (1.4) | 20 (28.6) | 12 (17.1) | 16 (22.9) |
| Total | 70 (100) | 18 (25.7) | 52 (74.3) | 31 (44.3) | 39 (55.7) |

Table 2: Characteristics of population by sex and diagnosis by culture and Xpert®

| Sex | Number (%) | LJ/7H9+(%) | LJ/7H9-(%) | Cont* (%) | Xpert+(%) | Xpert-(%) | Invalid (%) |
|--------------|------------|------------|------------|-----------|-----------|-----------|-------------|
| M | 42(60) | 26(37.1) | 15(21.4) | 1(1.4) | 28(40) | 12(17.1) | 2(2.9) |
| F | 28(40) | 12(17.1) | 15(21.4) | 1(1.4) | 14(20) | 11(15.7) | 3(4.3) |
| Total | 70(100) | 38(54.3) | 30(42.9) | 2(2.9) | 42(60) | 23(32.9) | 5(7.1) |

* Cont: contamination

Table 3: Absolute difference between different tests (95% confidence interval)

| | Ziehl | Auramine | Xpert® | 7H9/LJ |
|------------------------|------------------|-----------------|----------------|--------|
| Ziehl | - | - | - | - |
| Auramine | 18.6 (10.6-30) | - | - | - |
| Xpert® | 34.3 (23.6-46.7) | 15.7 (8.5-26.8) | - | - |
| Cultures 7H9/LJ | 28.6 (18.7-40.8) | 10(4.5-20.1) | 5.7 (1.9-14.7) | - |

RESULTS

From July 10, 2013 to September 10, 2014; 604 suspected TB patients were enrolled. Our study sample consisted of 70 sputum smears from patients with positive HIV serology with suspicion of pulmonary tuberculosis. After seeding, 2 cultures were contaminated and 68 (97.1%) samples were analyzed. Female sex represented 40% (28) and the male 60% (42). The difference was not significant ($P = 0.12$). The median age of all patients was 33.5 years with extremes ranging from 8 to 74 years old. Twenty five percent of patients were under 26 years old and 75% (47) above.

Most strains were isolated from patients between 21 to 50 years old (67.1%). Patient distribution by site of consultation shows that 32 patients (45.7%) were from St Alphonse and 38 (54.3%) from CME Ngaba. The difference was not significant ($P = 0.5$). Table 1 indicates that detection increases using Auramine (44.3% vs 25.7%) and higher for men (27.1% vs. 17.1%). The results of the direct tests carried out are shown in Table 1.

Table 2 shows that the Xpert® MTB / RIF had a high positivity rate (60%) compared with both cultures (54.3%). Detection was higher in men than in women (40% vs 20%). The sensitivity and specificity of Ziehl were 30% (95% CI: 0.22-0.78) and 100% (95% CI: 0.89-1). The sensitivity and specificity of auramine were 70% (0.62-0.89) and 98% (0.84-0.99). The sensitivity and specificity of the culture on 7H9 were 100%. The sensitivity and specificity of Xpert® were 89.5% (95% CI: 0.76-0.96) and 93.8% (0.80-0.98). The absolute difference between the tests is shown in Table 3. Results in Table 3 shows that the highest absolute difference between different test decreases in the following order: Xpert® and Ziehl (34.3%), Cultures and Ziehl (28.6%), Auramine-Ziehl (18.6%), Xpert® -Auramine 15.7%; Cultures-Auramine 10% and Cultures-Xpert® 5.7%.

The ratio of Auramine-Ziehl was 1.7; Xpert® MTB / RIF compared to Ziehl and Auramine with 2.3 and 1.4, respectively. On the other hand, the ratio of two crops-Ziehl was 2.1; Cultures-Auramine was 1.2 and cultures - Xpert® was 1.1.

Xpert® showed a 10.5 % (4/38) resistance of rifampicin whereas the antibiogram realized on LJ showed a resistance rate of 7.9% (3/38). Twenty-five strains (65.8%) out of 38 isolated on LJ were susceptible to all tested anti-tuberculosis drugs. In contrast, 13 strains (34.2%) were resistant to one anti-tuberculosis drug with a multiresistance (MDR-TB) of 7.9%.

DISCUSSION

The aim of this study was to compare the results of different diagnostic tests against Standard Gold (LJ) in the detection of tuberculosis and to determine the rate of resistance to rifampicin and MDR-TB. During the period of study, 70 samples of patients suspected of HIV positive pulmonary tuberculosis were analyzed. The study reported a positivity rate of 25.7, 44.3, 54.3 and 60% respectively for Ziehl, Auramine, two cultures (LJ and 7H9) and Xpert®. Sensitivity was 30, 70, 100% and 89.5%, respectively for

Ziehl, Auramine, 7H9 culture and Xpert®. The resistance to rifampicin was 10.5% for Xpert® and 7.9% for the proportional procedure on LJ. MDR-TB was 7.9% and the co-infection rate was 60%. The present study shows a slight predominance of male sex (60% vs 40%), but the difference was not significant ($p = 0.12$). This finding corroborates WHO reports and other earlier studies which show that cases are underreported among women in developing countries.^{1,9-11}

The prevalence of cases in the age group 21-50 years old (67.1%) has been demonstrated in several studies and in various WHO reports which show that tuberculosis affects the most active social strata.¹²⁻¹⁶

Of the 70 samples analyzed, 18 (25.7%) were positive for Ziehl and 31 (44.3%) for Auramine as shown in Table 1. It is noted that the positivity rate to Auramine was higher compared to that obtained by Ziehl. The absolute difference was 18.6% (95% CI: 10.6-30) as shown in Table 3 and the ratio in favor of Auramine was 1.7. The sensitivity and specificity for Ziehl were 30% and 100%, respectively. On the other hand, the positive predictive value (VPP) and the negative predictive value (VPN) were 100% and 61.5% respectively.

The 25.7% positivity rate obtained in Ziehl in this study is less than 33.6% described in Kenya by Cavanaugh and al. in a comparative study on the performance of different diagnostic tests in PVV.¹² This rate is also lower than that of 45.1% obtained in South Korea by Kim and al.¹³ However, it is higher than 9.3% reported in Ethiopia by Dereje and al. in a survey comparing conventional tests to Xpert®.¹⁴ The disparity and the low detection rate can be explained by the size of the samples, the degree of immunosuppression of the patients and the sensitivity of the tests. At Auramine, the detection rate was slightly increased to 44.3% with a sensitivity of 70% and specificity of 96.8%. By comparing these results with those of Ziehl, we note that Auramine is 1.7 times more sensitive than Ziehl. But this rate is lower than that of 63.9% obtained in India by Geraldo and al. by comparing Auramine with Xpert®.¹⁵

The difference could be explained by the sample size (70/419), the diversity of the samples used in their series and probably the immune status of the patients. This survey reports a similar rate of positive culture (54.3%) on LJ and 7H9. This observation is in line with the observation of the study carried out in Ethiopia by Dereje and al. which also reported the same rate (16.3%) for the two cultures carried out (MIGT 960 and LJ).¹⁴ By comparing the results of two cultures (LJ and 7H9) with respect to Auramine and Ziehl, the positivity rate increases to 54.3% as shown in Table 1. The absolute difference was 10% (95% CI: 4.5-20.1) with Auramine and 28.6% (95% CI: 18.7-40.8) with Ziehl as shown in Table 3. The ratio of culture to Auramine was 1.2 in favor of culture. On the other hand, the ratio between culture and Ziehl was 2.1 in favor of culture. This result shows that both cultures are 1.2 times more sensitive as Auramine and 2.1 times more than

Ziehl (Table 3). These values obtained in culture are less than 60.6% reported in South Korea by Kim and al. for both crops (LJ and 7H9)¹³ and yet more than the 16.3% obtained in Ethiopia by Dereje and al.¹⁴

The difference in rates may be due to the size of the sample, the method of treatment used and the immune status of the patients. This result illustrates that culture is more sensitive than direct techniques after staining. This statement corroborates the results of several authors in the World who have demonstrated the different detection rates using these tests.^{1,7,11-16} However, using Xpert® MTB / RIF, it was noted that the positivity rate had increased significantly by 60% (42) as shown in Table 2, with a sensitivity of 89.5% and a specificity of 93.8%. The absolute difference between Xpert® and Ziehl is 34.3% (95% CI: 23.6-46.7) with a ratio of 2.3; it is 15.7 (95% CI: 8.5-26.8) with a ratio of 1.4 between Xpert® and Auramine. This corroborates results obtained in India's by Geraldo and al. who reported an absolute difference between Xpert® and Auramine of 10.8 and a ratio of 1.17.¹⁵ This absolute difference is less than ours (15.7%). Dereje and al. also reported a rate increase using the Xpert® in their survey.¹⁴

Comparing the results of two the cultures (54.3%) with those of Xpert® MTB / RIF, the absolute difference was 5.7% (95% CI: 1.9-14.7) with a ratio of 1.1 as shown in Table 2 and 3. The 54.3% reported in this study is lower than the 74.9% obtained in India by Geraldo and al., however the ratios (1.1 / 1.2) are almost similar.¹⁵ The difference in detection rates between cultures and Xpert® could be explained by the presence of non-viable bacilli that could not grow in culture media and the difference in rates reported in surveys may be due to the size of samples analyzed (70/419). HIV / tuberculosis co-infection is a real problem that constrains the fight against tuberculosis and could prevent the achievement of the global goal. This study reported an overall detection rate of 60% among people living with HIV. This rate is higher than 47.3, 38.1 and 23.7%, respectively, described in India by Geraldo and al., in South Korea by Kim and al. and in Togo by Dagnra and al.^{13,15,16} This difference may be due to the small size of the sample, the prevalence of infection in each country, and the immune status of the patients. Lymphocyte typing was performed only in the Togo by Dagnra and al. who reported a CD4 counts below 200 μ l in 55.8% of patients.¹⁶ In our case, lymphocyte typing was not performed in order to obtain a clear explanation. Some authors, however, speak of quasi- difficulty in detection of tuberculosis if the CD4 rate collapsed.⁸

The survey showed a high proportion (10.5%) of resistance to rifampicin. This rate is far higher than that of 2.2% reported in India by Geraldo and al.¹⁵ On the other hand, it is lower than the 29.6% obtained in South Korea by Kim and al.¹³ The primary resistance to tuberculosis (34.2%) and the multidrug resistance rate (MDR-TB) around 7.9% (3 strains) show a threat to the global goal of TB elimination if sufficient attention is not done. The rate of contamination (2.9%) obtained in this survey is similar to that recommended by the WHO and the rate of invalid results with Xpert® is, however, higher than those obtained by other authors.¹³⁻¹⁶

Although this study showed the contribution of each test, it also has limitations due to small sample size and the non-typing of CD4 from patients in order to know the degree of impairment of immunity It is well known from literature that when the CD4 count is less or equal to 200 / mm, opportunistic infections take place.¹²

CONCLUSION

Xpert® is better suited for rapid and early detection of tuberculosis and for determination of resistance to rifampicin, which is one of the key molecules used in the management of patients. The culture test is also better, but not used routinely, microscopy although less sensitive will remain an indispensable tool for the detection of the bacilliferous cases because of its simplicity, speed and low cost.

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