# Optimal parameters for Gene Xpert MTB/RIF and LAM Strip determine Test for Diagnosis of Tuberculosis using urine from HIV patients at Moi Teaching and Referral Hospital, Kenya

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**ABSTRACT**
*The application of Gene Xpert MTB/RIF® assay for testing non-sputum clinical samples in Western Kenya has not been reported. We are reporting on use of Zn, CD4 count, Hb, Creatinine, Proteinuria, and Hematuria as optimal parameters for Gene Xpert MTB/RIF and LAM Strip determine Test for diagnosis of tuberculosis. Urine from HIV/TB co-infected adults (n=158) with CD4+ count <200 cells/mm³ was analyzed for mycobacteria tuberculosis DNA. Acid fast-staining, culture, clinical symptoms, laboratory and radiological findings were also assessed. Acid fast staining(55%) were TB positive, however, Gene Xpert test revealed 17(11%) of the patients were TB positive with 94% and 6% being MTB detected low and medium, respectively. Rifampicin resistance was not detected in 88% of the TB Gene Xpert positive patients with the remaining (12%) being indeterminate. LAM test revealed that 28% of the patients were TB positive. Radiology revealed that 45% of the patients had infiltrates. Presence of protein or blood in urine was significantly associated with TB positivity based on LAM strip determine test (48% vs 24%; p-value=0.021). The weighted kappa coefficient was 0.48 (95% CI 0.32-0.63; exact p-value <0.0001).The findings of this study show that urine Xpert MTB/RIF® can be used for diagnosis of TB using urine and taking Zn, CD4 count, Proteinuria, and Hematuria as optimal parameters.*
**Keywords:** *Urine, mycobacterium, Proteinuria, Hematuria, HIV Infection*

## INTRODUCTION

HIV and tuberculosis combined are cause of death in patients with HIV. In 2010, 2.8 million new cases of tuberculosis were reported in Africa, the majority in the sub-Saharan area; and 37% of tuberculosis episodes were diagnosed among HIV

infected patients[1]. In 2011, some 430,000 people died of HIV– associated TB, most of them in developing countries, and 22.5 million people were estimated to be living with HIV in sub-Saharan Africa[2]. The risk of tuberculosis increases with HIV but also modifies the clinical presentation of TB[3]. HIV-infected patients experience more sputum smear–negative pulmonary tuberculosis (PTB) than HIV-uninfected patients with extra pulmonary tuberculosis (EPTB) being, contributing to delayed tuberculosis diagnosis and high mortality[4]. Alteration in the clinical and radiographic presentation of PTB among HIV-infected has long been recognized[5]. Direct smears can be are often negative and cannot differentiate mycobacterium tuberculosis from non-tuberculous mycobacterium[6]. Culture (standard) takes 2 to 8 weeks due to the slow growth rate of mycobacterium while liquid culture may take 7-10 days[7].New innovative diagnostic methods for tuberculosis with increased sensitivity and speed of diagnosis in these patient groups are needed[8,9].Urine can be cultured, tested by polymerase chain reaction (PCR) for mycobacterial transrenal DNA or for specific mycobacterial antigens (lipoarabinomannan (LAM)). Currently there is no adequate data on the diagnostic utility of urine in TB diagnosis[10]. Recently, the Gene Xpert MTB/RIF rapid molecular assay has been endorsed by the Scientific and Technical Advisory Board of the World Health Organization (WHO) as the most sensitive test for TB diagnosis in respiratory samples[11,12]. This study will determine optimal parameters for Gene Xpert MTB/RIF and LAM Strip determine test for diagnosis of Tuberculosis using HIV patients' urine.

## MATERIAL AND METHODS

**Study population:** This study evaluated 158 HIV patients attending Moi Teaching and Referral Hospital in Eldoret, Western Kenya. Urinalysis, complete blood count, biochemical analysis of urine samples was done on adults (≥18) years with CD4+<200 cells/mm^{3}. Urine samples from patients with suspected TB were analyzed for mycobacteria tuberculosis DNA. Acid fast-staining, culture, clinical symptoms, and radiological findings were also evaluated. Host factors (age, gender, patient category, site of TB, HIV status, CD+ count) was obtained from the hospital records.

**Statistical Analysis:** Statistical analysis was done using SAS version 9.3.Categorical variables were summarized as frequencies and percentages. Continuous variables were summarized as mean and standard deviation or median and the corresponding inter quartile range. Normality tests were conducted using Shapiro and Wilks normality test. The test for association between categorical variables were conducted using Pearson's Chi Square test while the test of association between continuous variables were conducted using a two sample t-test if they are normally distributed. For skewed variables, a two sample Wilcoxon rank sum test was used. Cohen's kappa statistic (k) test was used to measure the level of agreement between the Xpert MTB/RIF and LAM determine strip test in detecting TB. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) with their 95% confidence intervals (CI) were computed against the sputum microscopy method (gold standard). Logistic regression analysis was used to identify factors associated with urine MTB/RIF positivity.

**Ethical considerations:** Ethical approval (# 0001084) was obtained from the Institution Review and Ethics Committee (IREC) of MTR.

## RESULTS

A total of 158 HIV positive patients were used as study participants. The overall mean age in years was 39 (SD, 9.1). Females formed 54% of the population. A third (31%) of the patients had CD4 count ≤ 100 cells/mm^{3}. WHO disease progressions of patients were either in their 3rd or 4th stage of HIV. The median hemoglobin level was 12.0 (IQR, 10.1-13.6) (Table 1).

**Table 1:** Demographic and clinical characteristics among HIV-infected patients in MTRH, 2011-2013

| Characteristics | N=158 n, (%) |
|---|---|
| Gender (Female) | 85 (53.8) |
| Age (years), mean (std) | 38.7 (9.1) |
| CD4 count, cell/mm$^3$ | |
| $\leq 100$ | 49 (31.0) |
| $> 100$ | 106 (67.1) |
| Missing | 3 (1.9) |
| WHO stage | |
| Stage I & II | 25 (15.8) |
| Stage III & IV | 105 (66.5) |
| Missing | 28 (17.7) |
| Hemoglobin, median (IQR) | 12.0    (10.1-13.6) |

Clinical symptoms; weight loss, TB, Pneumonia, URTI and those with a combination of TB or Pneumonia or Meningitis formed a cumulative of 73% (61/84) (Table 2).

**Table 2:** Clinical diagnoses among HIV-infected patients in MTRH, 2011-2013

| Clinical symptom | n (%) |
|---|---|
| Adenitis, TB | 1 (0.6) |
| Allergy | 1 (0.6) |
| Anemia | 3 (1.9) |
| Asymptomatic HIV | 3 (1.9) |
| Bronchospasm | 1 (0.6) |
| Conjunctivitis | 1 (0.6) |
| Dehydration | 1 (0.6) |
| Gastroenteritis | 2 (1.3) |
| Herpes zoster | 3 (1.9) |
| Hypertension | 2 (1.3) |
| Meningitis | 1 (0.6) |
| Migraine | 1 (0.6) |

| | |
|---|---|
| Milliary Tuberculosis | 5 (3.2) |
| Pneumonia | 8 (5.1) |
| Pneumonia, Tuberculosis | 7 (4.4) |
| Pneumonia, Tuberculosis | 1 (0.6) |
| Pericardial effusion | 1 (0.6) |
| RTI, Cough | 10 (6.3) |
| Tuberculosis | 15 (9.5) |
| Tuberculosis meningitis | 1 (0.6) |
| URTI | 9 (5.7) |
| Weight loss | 7 (4.4) |
| None | 74 (46.8) |

Median urine culture was 125 (IQR, 110-272). Majority (85%) had nil results on urinalysis. Blood and Protein was reported in 65% and 26% respectively among those with non-nil result. A fifth (28%) had positive acid fast and more than half (55%) of these being TB positive. Gene Xpert test showed 17 (11%) had TB positive of which 94% and 6% were MTB detected low medium, respectively. Rifampicin was not detected in 88% of the TB Gene Xpert positive patients. The remaining (12%) being indeterminate. All patients were sensitive to drugs. Using LAM test, 28% of the patients tested positive for TB. Radiological findings were; 45% had infiltrates, 19% normal, 15% milliary TB and 11% opacities. Median creatinine was 62.3 (IQR 49.1-75.9)(Table 3).

**Table 3:** Microbiological and laboratory characteristics among HIV-infected patients in MTRH, 2011-2013

| Characteristics | n, (%) |
|---|---|
| Urinalysis | |
|     + BLOOD | 15 (9.5) |
|     + BLOOD, + PROTEIN | 1 (0.6) |
|     + BLOOD, ++ PROTEIN | 1 (0.6) |
|     + PROTEIN | 6 (3.8) |
|     NIL | 135 (85.4) |
| Acid Fast (ZN) | |
|     + | 16 (10.1) |
|     ++ | 5 (3.2) |
|     +++ | 8 (5.1) |
|     NEG | 129 (81.7) |
| TB status (Gene Xpert) | |
|     MTB detected low | 16 (10.1) |
|     MTB detected medium | 1 (0.6) |
|     MTB not detected | 141 (89.2) |
| Rifampicin (RIF) resistant | |
|     RIF indeterminate | 2 (11.8) |
|     RIF not detected | 15 (88.2) |
| Drug sensitivity | |
| Streptomycin, Isoniazid, RIF, Ethambutol | 17 (100.0) |

| | |
|---|---|
| None | 0 (0.0) |
| LAM | |
| Negative | 114 (72.2) |
| Positive | 44 (27.8) |
| Urine culture | 125 (110-272) |
| Radiological findings | |
| Cavitation | 2 (1.3) |
| Adenitis | 3 (1.9) |
| Infiltrates | 71 (44.9) |
| Lung collapse | 1 (0.6) |
| Milliary TB | 23 (14.6) |
| Normal | 30 (19.0) |
| Opacities | 18 (11.4) |
| Pericarditis | 1 (0.6) |
| Pleural effusion | 3 (1.9) |
| Suggestive | 1 (0.6) |
| Tb meningitis | 4 (2.5) |
| Tb spine | 1 (0.6) |
| Creatinine, median (IQR) | 62.3 (49.1-75.9) |

Presence of protein or blood in urine was significantly associated with TB positivity based on LAM strip determine test (48% vs 24%; p-value=0.021) (Table 4).

**Table 4:** Association between Urinalysis result and TB results based on Gene Xpert result and LAM determine strip techniques

| | TB Testing Technique | | | | | |
|---|---|---|---|---|---|---|
| | Gene Xpert result | | | LAM determine result | | |
| Urinalysis result | Negative n (%) | Positive n (%) | P-value | Negative n (%) | Positive n (%) | P-value |
| Negative | 122 (90) | 13 (10) | 0.277 | 102 (76) | 33 (24) | 0.021* |
| Positive | 19 (83) | 4 (17) | | 12 (52) | 11 (48) | |

*significant at α=0.05

Weighted kappa coefficient was 0.48 (95% CI 0.32-0.63; exact p-value <0.0001). Hence, the amount of agreement between the Gene Xpert and LAM was modest (Table 5).

**Table 5:** Cohen's kappa statistic (k) test for the agreement between the Gene Xpert MTB/RIF and LAM determine techniques in detecting TB

| LAM determine technique | Gene Xpert technique | | Total | Kappa (k) (95% CI) | P-value |
|---|---|---|---|---|---|
| | Positive | Negative | | | |
| Positive | 17 | 27 | 44 | | |
| Negative | 0 | 114 | 114 | 0.48 (0.32-0.63) | <0.001 |
| Total | 17 | 141 | 158 | | |

## DISCUSSION

Tuberculosis in urine was found to be high in this study suggesting a major challenge in the diagnosis of TB in patients who are TB/HIV co-infected. This may be associated with poor diagnosis and delayed treatment. These findings are in agreement with other studies recently reported from South Africa, which found high TB prevalence using urine from immunosuppressed persons and recommended a further investigation of creatinine[13]. The hemoglobin level (12.0) was within the normal ranges implying that participants were not anemic.

This study established that clinical symptoms; weight loss, TB, Pneumonia, URTI and those with a combination of TB or Pneumonia or Meningitis diagnosed. Also infiltrate, milliary TB and opacities. This is because the clinical presentation of MTB in AIDS can resemble that of non-AIDS patients and MTB can often be the first AIDS-defining illness, particularly in regions where the incidence of MTB is high in the general population. Chest x-ray (CXR) is mostly used as the diagnostic tool for TB but HIV/TB co-infected patients have normal CXR results despite the fact that their culture results are positive [14, 15]. Tuberculosis should be suspected in patients with

fever, cough, night sweats and weight loss, regardless of chest roentgenogram findings[16,17].

Gene Xpert test, patients were identified as TB positive of which 94% were MTB detected low and 6% were MTB detected medium. The study has proven that urine Xpert is a useful tool in detecting TB/HIV patients living in western Kenya and offer incremental yield when it comes to diagnosis of tuberculosis using urine. Rifampicin resistance was not detected and all patients were sensitive to drugs. This designates no drug resistance and the diagnosed cases are treatable.

This current study showed that patients tested positive for TB using LAM. LAM in urine indicates the presence of disseminated tuberculosis. i.e. tuberculosis other than pulmonary tuberculosis, mostly affecting the kidney. The organ distribution of Mycobacteria tuberculosis (MTB) in AIDS is widespread.

It was also noted that presence of protein (proteinuria) or blood (hematuria) in urine was significantly associated with TB positivity based on LAM test.HIV patients who have severe immunosuppression have high bacillary load leading to shedding of LAM in urine and also due to HIV associated nephropathy (HIVAN), a

nephrotic syndrome signaling renal abnormalities and a failure in glomerular filtration mechanism. HIVAN is characterized by marked proteinuria and a rapid progression to renal failure and end stage renal disease (ERSD) [18].Immune complex mediated glomerulonephritis (proliferative glomerulonephritis) may also occur in HIV-infected patients presenting with proteinuria and renal failure[19].

## CONCLUSSION AND RECOMMENDATION

In conclusion, our study shows that urine Xpert MTB/RIF® is a highly sensitive and specific tool for diagnosis of TB using urine in severely immunosuppressed individual Western Kenya population where sputum diagnosis is not possible. On the other hand urinary LAM strip test is highly sensitive in diagnosis of tuberculosis in HIV patients particularly in immunosuppressed individual.

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