Xpert Mtb / Rif Assay For Rapid Diagnosis Of Pulmonary Tuberculosis In Primary Health Care Services

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ABSTRACT: The aim of this study was to show on the example of the one peripheral laboratory, next to the sputum smear microscopy, importance of implementation new simple, rapid and more sensitive diagnostic method as Xpert Mtb/Rif test, which also can detect the drug resistance. There were retrospectively reviewed the results of direct sputum smear microscopy and XpertMTB/Rif test of 1276 patient, with suspected pulmonary TB, which were performed from April of 2015 until May of 2016 in the laboratory of Central Correctional Hospital of Minister of Corrections and Legal Assistance of Georgia. From 1276 patients, XpertMTB/Rif test has detected in 52 cases M. tuberculosis in sputum sample (MTB positive patients). From these 52 cases in 25 (48.05±6.92%) patient it was Rif/Sensitive, in 23 (44.23±6.88%) Rif/Resistant MTB. In 4 (7.69±3.69%) patients the test cannot determine the sensitivity to rifampicin. From these 52 Xpert/ MTB positive cases, only 17 (32.7±7.21%) were smear positive, other 35 (67.30±6.50%) - were smear negative. So without the XpertMTB/Rif test, in the laboratory of Central Correctional Hospital of Minister of Corrections and Legal Assistance of Georgia (primary health care service laboratory), it would be able to suspect TB only in 32.7±7.21% cases (smear positive patients) and in the same time we would not know the sensitivity to drug, which is so important for the adequate treatment. In 67.30±6.50% smear negative cases of patients without the XpertMTB/Rif test we even would not suspect laboratory positivity to TB. In these cases the laboratory diagnosis of TB would be given after sending the sputum sample in the Reference laboratory. It would need several weeks for the final results and would be the reason of delay in the beginning of the appropriate treatment and would be great chance of further spread of disease.

Key words: tuberculosis, M. tuberculosis, XpertMTB/Rif assay, smear microscopy, primary health care services. laboratory diagnostic of TB.

INTRODUCTION

One third of the world’s population is infected with Mycobacterium Tuberculosis. 9.6 million of people were infected and 1.5 million died from the disease in 2015. Over 95% of TB deaths occur in low- and middle-income countries, and it is among the top 5 causes of death for women aged 15 to 44. In 2015, an estimated 1 million children became ill with TB and 140 000 children died of TB. TB was a leading killer of HIV-positive people: in 2015, 1 in 3 HIV deaths was due to TB. Globally, 480 000 of people developed multidrug-resistant TB (MDR-TB). This illness, still today, remains to be one of the leading causes of morbidity and mortality throughout the world. Despite the advances and the fact that nearly all cases can be cured, TB remains one of the world’s biggest threats [5]. One of the most significant way to prevent the transmission of TB - is a rapid diagnosis of the pulmonary TB at the lower levels of health services. The gold standard for diagnosis of pulmonary TB is ability to detect M. tuberculosis from a sputum sample. Sputum smear microscopy is the most rapid and inexpensive test (performed in the most laboratories at the lower levels of health services), but it fails to detect the disease in around 20 to 30% of cases, especially it is not useful in HIV infected patients, besides it cannot detect drug resistance.

Advantages of sputum smear microscopy are, that it is cheap, rapid with relatively short turnaround time (24 hours) easy to perform, can detect most (90%) of infectious patients [2,4], and do not require high level biosafety measures. Disadvantages of sputum smear microscopy are that it has low sensitivity (30-40 %), especially in HIV patient, cannot detect drug resistance - reason of delay starting appropriate treatment, needs two samples (tables N1,2) and after sputum smear microscopy patient needs further investigations for the final diagnoses of TB [2, 4]. Based on the above reasons, there was a need to create new rapid and more sensitive technologies, which could be studied in many regions. These technologies are sensitive and specific for diagnosis of TB. The new technologies include XpertMTB/Rif test. Culture of sputum yields a correct diagnosis in up to 90% of cases [2,4], and it also can detect types of drug resistance, but results are only available in 4-8 weeks. XpertMTB/Rif test is not recommended for direct testing of smear-negative clinical specimens due to limited sensitivity. Both methods require advanced laboratory infrastructure, qualified staff, special safety precautions and thus cannot be implemented at the lower levels of health services. In December 2010 World Health Organization (WHO) endorsed Xpert Mtb/Rif technology, as a new molecular test for rapid diagnosis of TB, as well as testing for Resistance to the Rifampicin, in less than two hours. In a multi-center study sensitivity of XpertMTB/Rif was 90.3 % among culture confirmed TB cases, 76.9% among smear negative, culture positive TB patients and the specificity was 99%. [1, 7]. Xpert Mtb/Rif use as the initial diagnostic test was recommended by WHO for all individuals with suspected TB [1, 7]. XpertMTB/Rif is an automated polymerase chain reaction (PCR) test - following sample loading, all steps in the assay are completely automated and self-contained. XpertMTB/Rif is a single test that can both detect M. tuberculosis complex
and Rifampicin resistance within two hours after starting the
test, with minimal hands-on time. In addition, the assay’s
sample reagent, used to liquefy sputum, has potent
tuberculocidal properties and largely eliminates biosafety
concerns during the test procedure. These features allow
the technology to be taken out of a reference laboratory and
used at first point of care.

**Objectives:** The aim of this study was to show on the
example of the one peripheral laboratory (in the era of
increasing drug resistant TB in the world and in the
country), next to the sputum smear microscopy (only available method for diagnosis TB in the lower level
laboratories until 2015) , importance of implementation new
simple, rapid and more sensitive diagnostic method as
Xpert MTB/Rif test, which also can detect the drug
resistance.

**Methods.** We retrospectively reviewed the results of
direct sputum smear microscopy and XpertMTB/Rif test of
1276 patient, with suspected pulmonary TB, which were
performed from April of 2015 until May of 2016 in the
laboratory of Central Correctional Hospital of Minister of
Corrections and Legal Assistance of Georgia. For the
smear microscopy the sputum samples were stained by the
Ziehl-Neelsen (ZN) method The XpertMTB/Rif test was done
on GeneXpert platform (Cepheid), this test was
implemented in the laboratory in the April of 2015 [2,3].
The installation of the XpertMTB/Rif was performed by the
special trained staff from the Reference Laboratory. The
installation was complete in 1 day. Prior to installation the
following logistical requirements were fulfilled : 1. Air
conditioning - XpertMTB/Rif is designed for indoor use only.
Operating temperature should be between 15-30 degrees.
The machine should be placed under an air vent 2. Stable
electricity supply - power interruptions can lead to
instrument damage and incomplete testing. It is therefore
mandatory to have a uninterruptable power supply (UPS)
for operation of Xpert MTB/Rif test. 3. Storage of cartridges
- the single use cartridges require substantial storage
space. 4. Waste management - disposal of cartridges is
similar to that of other biological waste- sterilization and
appropriate chemical waste incineration. Laboratory staff
was trained by the special team from the Reference
Laboratory. The quality of work in the laboratory is under
the control by the Central Reference Laboratory of Georgia.

**Results.** From April of 2015 all patients with suspected
pulmonary TB have undergone testing with both smear
microscopy and XpertMTB/Rif testing (table 3). From 1276
patients which were investigated from the April 2015 to May
of 2016 with suspected pulmonary TB, in 52 patients
XpertMTB/Rif test has detected *M. tuberculosis* in sputum
sample (MTB positive patients). From these 52 cases in 25
(48.0±6.92%) patient it was Rif/Sensitive, in 23
(44.23±6.88%) Rif/Resistant MTB. In 4 (7.69±3.69%)
patients the test cannot determine the sensitivity to
rifampicin. From 25 MTB (+) Rif/S cases 18 (51.42±8.44%)
were smear negative and 7 (41.17±11.93%) smear-positive,
from 23 MTB (+)Rif/R cases 14 (40.0±8.28%) were smear-
negative and 9 (52.94±12.10%) smear-positive and from
the 4 MTB (+) cases, where the resistance was not
determine 3 (8.57±4.7%) were smear-negative patients and
only 1 (5.88±5.66%) was smear positive. These results
show that from these 52 Xpert/ MTB positive cases, only 17
(32.7±7.21%) were smear positive, other 35 (67.30±6.50%)
patients were smear negative. Results of cultural investigations of all 52 patients were positive for TB.

**Conclusion.** XpertMTB/Rif test helped to diagnose TB,
involving the detection of sensitivity to rifampicin in smear-
positive (17 cases - 32.7±7.21%) patients. The patients with
the positive sputum smear microscopy, without
XpertMTB/Rif test, needed further investigation for the
estimation of drug resistance, it needed time and was
reason of delay starting appropriate treatment. XpertMTB/Rif test detected *M. tuberculosis* and determined the
sensitivity to rifampicin even, in the smear- negative
patients (35 cases - 67.30±6.50%). In these 35 smear
negative patients, without Xpert/ MTB/Rif test, there was
possibility of delay diagnosis of TB, accordingly delay of
treatment. According to above mentioned, without the
XpertMTB/Rif test, in the laboratory of Central Correctional
Hospital of Minister of Corrections and Legal Assistance of
Georgia (primary health care service laboratory), it would
be able to suspect TB only in 32.7±7.21% cases (smear-
positive patients). In the same time laboratory personnel not
know the sensitivity to drug, which is so important for the
adequate treatment. In 67.30±6.50% (smear-negative cases) of patients without the XpertMTB/Rif test there were
even would not suspect positivity to TB. In these cases the
laboratory diagnosis of TB would be given after sending the
sputum sample in the Reference laboratory. It would need
several weeks for the final results and would be the reason
of delay in the beginning of the appropriate treatment and
would be great chance of further spread of disease.
XpertMTB/ Rif test had increased dramatically by 34.6% of
TB laboratory detection compared to smear microscopy.
In the laboratory of Central Correctional Hospital - at the lower
level of health services - close to the patient - it was first
time possible to give definitive laboratory diagnosis of TB in
two hours. Before the implementation of XpertMTB/Rif test
(2000-2015 April), for the final laboratory diagnosing of TB,
after the sputum smear microscopy, were sending the
samples of sputum to Reference Laboratory. It needed
several weeks for the final results and was the reason of
delay in the beginning of the appropriate treatment.

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http://www.who.int/tb/publications/implementing_TB_di-
agnotics/en/.


**Table 1: Sputum Smear Microscopy for Diagnosis of TB**

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
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<tbody>
<tr>
<td>- Detect most infectious patients</td>
<td>- Cannot detect drug resistance – reason of delay starting appropriate treatment</td>
</tr>
<tr>
<td>- Cheap method</td>
<td>- Low sensitivity (30-40%), especially in HIV patients</td>
</tr>
<tr>
<td>- Rapid method, easy to perform (in 24 hours)</td>
<td>- Needs two samples</td>
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<td>- Need no special safety precaution</td>
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</tbody>
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**Table 2: Results of sputum smear microscopy of patients with TB**

<table>
<thead>
<tr>
<th>Smear Negative (-)</th>
<th>Smear Positive (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient remain untreated (if there is no signs of TB on chest XR) until the final result of culture and after some time he is a source of infection for other members of the community</td>
<td>Patients may be inappropriately treated, without DST (drug susceptibility test) drug resistant strains may continue to spread</td>
</tr>
</tbody>
</table>

**Table 3: Comparison of smear microscopy and XpertMTB/Rif testing results**

<table>
<thead>
<tr>
<th>MTB</th>
<th>Total</th>
<th>N=52</th>
<th>Smear(-) 67.30±6.50%</th>
<th>N=35</th>
<th>Smear(+) 32.7±7.21%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Abs.</td>
<td>%</td>
<td>Abs.</td>
<td>%</td>
<td>Abs.</td>
</tr>
<tr>
<td>MTB(+)RIFS</td>
<td>25</td>
<td>18</td>
<td>48.0±6.92</td>
<td>51.42±8.44</td>
<td>41.7±11.93</td>
</tr>
<tr>
<td>MTB(+)RIFR</td>
<td>23</td>
<td>14</td>
<td>44.23±6.88</td>
<td>40.0±8.28</td>
<td>52.94±12.10</td>
</tr>
<tr>
<td>MTB(+)RIF not determined</td>
<td>4</td>
<td>3</td>
<td>7.69±3.69</td>
<td>8.57±4.71</td>
<td>5.88±5.66</td>
</tr>
</tbody>
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