Retrospective evaluation of the diagnostic accuracy of FluoroType® MTB assay used for detection of Mycobacterium tuberculosis in clinical samples in Greece

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**Abstract**

Tuberculosis has been and still is a serious health issue around the world, in 2013 caused 1.5 million deaths, under the same year 560 cases of tuberculosis were reported in Greece. Tuberculosis is caused by the airborne pathogen *Mycobacterium tuberculosis*.

In this study the diagnostic accuracy of the rapid molecular assay FluoroType® MTB (Hain Lifescience, Nehren, Germany) was evaluated compared to the “gold standard”, cultivation on solid media Löwenstein-Jensen slopes.

A total 962 specimens from sample with request for routine TB analysis were collected from Hospitals all over Greece between 2014 and 2015. However, 951 specimens were evaluated in this study.

The results shown that 826 specimens was FluoroType negative and 845 culture negative, 60 specimens was FluoroType positive and 41 were culture positive. The FluoroType analytical sensitivity compared to culturing was 85,4 %, the analytical specificity was 97,0 %, the PPV was 58,3% and the NPV was 99,3 %.

The result of this study indicate that the FluoroType® MTB has proven to be an accurate assay to detect *Mycobacterium tuberculosis* but should not be used as the only test to determine a Tuberculosis diagnosis.

**Keywords:** *Mycobacterium tuberculosis*, Tuberculosis, FluoroType® MTB, real-time PCR, Melt curve analysis (MCA), PCR
Introductions

Tuberculosis (TB) caused by the bacterium *Mycobacterium tuberculosis* and is a major public health issue worldwide. TB used to be a serious public health problem in Europe during the 18th, 19th and until the early 20th century but since the introduction of effective antibiotic treatment in the 1950s, the prevalence of the disease has been decreasing. However, the HIV/AIDS epidemic in the 1980s called into question the control of the disease and today TB is a major problem in developing countries (1).

TB is a huge health issue worldwide and in 2013 9 million new cases of TB were reported and 1.1 of the 9 million patients was HIV positive, 1.5 million humans died from TB, among which 360 000 was HIV positive. 56% of new TB cases occurred in Western Pacific region and South-East Asia, while Africa represent 25% but had the highest death rate relative to population (2).

In Greece under 2013, were reported 560 cases of TB, which 494 cases were new developed TB. 10% of all TB positive patients were also HIV positive. However, only 67% of all cases had a confirmed HIV status (3).

TB is one of the oldest human diseases known and findings of the disease were detected to 70.000 year old human remnants discovered in Africa. *Mycobacterium tuberculosis* is an airborne pathogen that mostly cause severe lung disease, but can also infect other parts of the body. TB infections are divided in to two major groups: pulmonary TB and extrapulmonary TB (4).

Pulmonary TB is the most common one and stands for 60-90% of all TB infections. Pulmonary TB can be divided in to two groups: primary and secondary pulmonary TB. Primary pulmonary TB occurs soon after infection and gives symptoms as fever and chest pain, this type of TB is most common among children and immunosuppressed patients. Secondary pulmonary TB (adult type TB) occurs from a recent TB infections or a previous TB infection that reappers. The symptoms of adult type TB are normally unspecific in the
beginning for example) fever, night-sweats, weight loss, feeling weakness and coughing that in time might leads to a lot of sputum and sometimes blood (4).

Extrapulmonary TB occurs in 10-40% of the cases and most HIV positive patients develop both pulmonary and extrapulmonary TB. Extrapulmonary TB includes infections in bones, joints, pleura, lymph nodes, genital tracts, pericardium, peritoneum and meninges. However, all organ system in the human body can be infected with TB (4-5). Diagnosing extrapulmonary TB by molecular testing can be difficult since analyzing technique are less sensitive to these type of samples than samples from patients with pulmonary TB (6).

Classical laboratory diagnosis of tuberculosis
The classical way and still the gold standard to microbiological diagnose tuberculosis is by cultivation on solid media, such as Löwenstein-Jensen (LJ) slopes. LJ slopes is a medium made of coagulated eggs and glycerol (1,7) . The LJ medium is then incubated for up to 8 weeks at 37°C, the cultures are then controlled for growth of Mycobacterium once a week for all the 8 weeks. However, a positive result can be given out after 3-5 weeks (8).

Another classical way to diagnose tuberculosis is by using microscopy (9). The patient samples is stained with the acid-fast stain Ziehl-Neelson and then investigated under light microscopy (7) and were Mycobacterium is stained red and the background is stained blue (6). However, the method is not as sensitive as the cultivation but has always been used as a compliment to cultures since a primary result can be giving out right the way. The smears require at least 10^4 bacteria’s/mL to be detected compared to cultivation that only require 10^1-10^2 bacteria’s/mL for detection. There also been developing liquid-based culturing systems that support faster growth of Mycobacterium but it also enhance the chances for contaminations (1, 9).

When analyzing tuberculosis all samples first need to get decontaminated to eliminate the normal flora. Since the normal flora also grows at the cultivation media used for culturing Mycobacterium and the normal flora can easily inhibit the growth of Mycobacterium by overgrowing. With the decontaminated method there is a risk of killing Mycobacterium
tuberculosis bacilli so therefore some specimens should not undergo decontamination, for example spinal- and synovial fluid, bone marrow, biopsies and specimens from lymph nodes and abscesses. Modified petroff is a common method used for decontamination (10).

Actual rapid molecular methods
To enhance the survival and immediate treatment rapid molecular techniques, such as Real-time PCR, has been recently developed. The result can be given out usually after only 1-2 days, and even on the same day (9).

The first rapid nuclei tests for M. tuberculosis were developed during the 1990s. Since then, different types of assays has been developed, targeting different genomic regions (16s rRNA, IS6110 etc.). Using specific probes to hybridize with RNA or DNA products generated following amplifications procedures, for example PCR.

Today real-time PCR with melt curve analysis (MCA) are used as rapid test for M. tuberculosis. Real-time PCR is a PCR based method were both amplification and detection takes place in the PCR tube and the amplification can be followed trough from the beginning until the end of the analysis. The detection is possible since the use of different fluorochromes. The chromogenic substance can be a free molecule that binds to double stranded DNA, for example SYBR-green or bound to a probe, for example TaqMan-probe or Molecular beacon probes (9).

FluoroType® MTB assay
FluoroType® MTB (Hain Lifescience, Nehren, Germany) is a rapid molecular diagnostic commercial system, using real-time PCR to detect M. tuberculosis complex (MTB-complex) based on HyBeacon technology. The method gives results for multiple specimens within only 3 hours (9, 11). The amplification takes place in the FluoroCycler® machine (Hain Lifescience, Nehren, Germany), while the detection is performed by MCA with single-stranded fluorescence labeled probes that are complementary to the amplified DNA (Figure 1) (12-13).
The first step is the extraction of DNA from patient samples by using FluoroLyse® (Hain Lifescience, Nehren, Germany) kit that includes all the necessary reagents. The extraction is divided into three steps, first the patient sample is centrifuged and the pellet containing the cells is kept. Afterwards the cells are lysed with the Lysis buffer that create an alkaline condition and by an incubation in water bath. The third step is neutralization with the Neutralization buffer. After the neutralization step the DNA may directly be used for amplification or stored at -20°C until amplification (14).

The amplification kit FluoroType® MTB (Hain Lifescience, Nehren, Germany) is used for amplification, the kit includes two buffers containing Hybeacon probes, amplification control and other regents that are optimized for the assay. The buffers from the FluoroType kit are mixed with the DNA that been extracted with the FluoroLyse® kit according the kit instructions and afterwards the PCR and MCA will take place in the FluoroCycler®.

The procedure in FluoroCycler® is divided into different steps. First an amplification takes place and afterward hybridization with the HyBeacon probes that are in complex with a fluorophore. The HyBeacon probe is complementary to MTB-complex specific sequence. After these steps the fluorophore is removed from the probe and the fluorescence is detected while the stringency is increasing. If DNA from MTB-complex is present a specific melting point (T_m) is detected during the MCA which later on is showed as a characteristic melting curve. The MTB-complex has a T_m of 70.5°C and the T_m of the amplification control (AC) is 60.0°C. The result is showed as melting curves. If the sample is negative for M. tuberculosis the amplification control is showed but if the characteristic melting curve MTB-complex exist
the sample is positive for MTB-complex and patient that produced the sample is probably infected with *M. tuberculosis* (Figure 2) (13).

Evaluation studies of the FluoroType have previously been made by Eigner et al., 2013 and Hoffmann et al., 2014. Both studies evaluated the diagnostic performance of the Fluorotype® MTB in routine condition by comparing the result with result from culturing patient samples on LJ media (15-16).

**Figure 2.** Different alternatives of melting curves after a run of the FluoroCycler®. Were AC stands for amplification control and MTB complex stands for *Mycobacterium tuberculosis*-complex (12).

**Aim**

To evaluate the diagnostic accuracy of the FluoroType® MTB (Hain Lifescience, Nehren, Germany) compared to the reference method culture on LJ medium (and/or on semi-automated liquid culture system MGIT Bactec 960) and clinical data when available.
Material and method

A total 962 specimens from with request for routine TB analysis were used in this study. All samples were routine pulmonary (sputum, bronchial secretions, alveolar washing, brushing, bronchoalveolar lavage and gastric fluid) and extrapulmonary (cerebrospinal fluid, lymph nodes, acetic fluid, synovial fluid, pleural fluid, pericardial fluid, bone, tissue and urine) specimens from patients with a clinic suspicion of TB or with previous TB findings. All samples were collected between 2014 and 2015, from hospitals over Greece.

Specimens were processed by the N-acetyl-Lcysteine and sodium hydroxide method (Modified Pedroff method) before any laboratory diagnosing. All samples were analyzed with Ziehl-Neelsen AFB direct microscopic examination and cultivated on either solid Löwenstein-Jensen (LJ) slopes or on liquid Bactec MIGT 960 (Becton Dickinson, Sparks, US) culture media according to manufactures instruction.

For the FluoroType® MTB assay all samples underwent DNA extraction and PCR amplification according to the respective kit instructions, FluoroLyse® and FluoroType® MTB. The amplification and the detection took place in FluoroCycler® according the manufactures instruction.

Analysis and evaluation of result

The cumulative lab data was processed using the Statistic data editor SPSS 17.0. The analytical sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the method for the diagnosis of tuberculosis was calculated as followed:

\[
\text{Sensitivity} = \frac{\text{FluoroType and culture positive}}{\text{FluoroType and culture positive} + \text{FluoroType negative and culture positive}} \times 100
\]

\[
\text{Specificity} = \frac{\text{FluoroType positive and culture negative}}{\text{FluoroType positive and culture negative} + \text{FluoroType and culture negative}} \times 100
\]

\[
\text{PPV} = \frac{\text{FluoroType and culture positive}}{\text{FluoroType and culture positive} + \text{FluoroType positive and culture negative}} \times 100
\]

\[
\text{NPV} = \frac{\text{FluoroType positive and culture negative}}{\text{FluoroType positive and culture negative} + \text{FluoroType negative and positive}} \times 100
\]
Ethical consideration

Ethical approval was not needed for this type of study by all results was approved by National Reference Laboratory for Mycobacteria, “Sotiria” Chest Diseases Hospital, Athens, Greece.
**Result**

In total 962 specimens from samples with request for routine TB analysis was investigaded in this study. However, only 951 samples that had a confirmed diagnoses from responsible clinicians was evaluated and included in the final result.

60 specimens was FluoroType positive and 41 was culture positive. In total 826 specimens were FluoroType negative and 845 culture negative. 35 specimens were contaminated during culturing, 30 specimens was invalid on FluoroType and one sample was not cultured, these 66 sample was not included in any statistical calculations. Regarding the result from the Microscopy 53 samples was positive and 856 total negative (Table 1).

However, four of the specimens that were FluoroType positive and culture negative was specimens from patients with previous TB and one sample that were FluoroType positive and culture negative was later confirmed positive with Xpert® MTB/RIF (Cepheid, USA), with a new sample from the same patient. 13 specimens that were FluoroType positive and culture negative was confirmed negative with either FluoroType or Xpert® MTB/RIF with new samples from the same patients. Three specimens that were culture positive and FluoroType negative had a growth less than five colonies on the LJ slopes and was confirmed positive by responsible clinician. 90 % of the specimens analyzed by the FluoroType was negative compared with 92% negative results from culturing of the same samples. 4 % of the culturing results was positive compared with the 7% FluoroType positive samples (Table 1).

**Table 1.** The data that was collected in the study from culturing, FluoroType analysis and microscopy results.

<table>
<thead>
<tr>
<th></th>
<th><strong>Contaminated, n</strong></th>
<th><strong>Not done, n</strong></th>
<th><strong>Culture negative, n</strong></th>
<th><strong>Culture positive, n</strong></th>
<th><em><em>Total</em>, n (%)</em>*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Invalid</strong></td>
<td>0</td>
<td>1</td>
<td>28</td>
<td>2</td>
<td>30</td>
</tr>
<tr>
<td>FluoroType negative, n</td>
<td>33</td>
<td>0</td>
<td>820</td>
<td>11</td>
<td>826 (90)</td>
</tr>
<tr>
<td>FluoroType positive, n</td>
<td>2</td>
<td>0</td>
<td>25</td>
<td>35</td>
<td>60 (7)</td>
</tr>
<tr>
<td><em><em>Total</em>, n (%)</em>*</td>
<td>35</td>
<td>1</td>
<td><strong>845 (92)</strong></td>
<td><strong>41 (4)</strong></td>
<td><strong>951</strong></td>
</tr>
<tr>
<td>Microscopy negative, n</td>
<td>33</td>
<td>0</td>
<td>817</td>
<td>6</td>
<td>856 (94)</td>
</tr>
<tr>
<td>Microscopy positive, n</td>
<td>2</td>
<td>0</td>
<td>24</td>
<td>27</td>
<td>53 (6)</td>
</tr>
<tr>
<td><em><em>Total</em>, n (%)</em>*</td>
<td>35</td>
<td>1</td>
<td><strong>841 (93)</strong></td>
<td><strong>33 (4)</strong></td>
<td><strong>909</strong></td>
</tr>
</tbody>
</table>
n= number of samples, % = how may percent in the specific category. *The total specimens in that category that was included in the final result and in the calculations.

In total 804 of the investigated specimens were pulmonary, 141 specimens were extrapulmonary and six specimens with unknown origin. 691 of the pulmonary samples was FluoroType negative and 54 FluoroType positive. However, 709 of the pulmonary samples was culture negative and 36 culture positive. Among the extrapulmonary specimens six were FluoroType positive and five culture positive, 130 were FluoroType negative and 131 culture negative (Table 2).

Table 2. Describing the data that was collected from both culturing and FluoroType analysis and also showing the incidents between pulmonary, extra pulmonary samples and samples with unknown origin.

<table>
<thead>
<tr>
<th></th>
<th>Contaminated, n</th>
<th>Not done, n</th>
<th>Culture negative, n</th>
<th>Culture positive, n</th>
<th>Total*, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Invalid, n</td>
<td>0</td>
<td>1</td>
<td>23</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>FluoroType negative, n</td>
<td>32</td>
<td>0</td>
<td>688</td>
<td>3</td>
<td>691 (90)</td>
</tr>
<tr>
<td>FluoroType positive, n</td>
<td>2</td>
<td>0</td>
<td>21</td>
<td>33</td>
<td>54 (7)</td>
</tr>
<tr>
<td>Total*, n (%)</td>
<td>34</td>
<td>1</td>
<td>709 (91)</td>
<td>36 (5)</td>
<td>804</td>
</tr>
<tr>
<td>Extra pulmonary</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Invalid, n</td>
<td>Data missing</td>
<td>Data missing</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>FluoroType negative, n</td>
<td>Data missing</td>
<td>Data missing</td>
<td>127</td>
<td>3</td>
<td>130 (92)</td>
</tr>
<tr>
<td>FluoroType positive, n</td>
<td>Data missing</td>
<td>Data missing</td>
<td>4</td>
<td>2</td>
<td>6 (4)</td>
</tr>
<tr>
<td>Total*, n (%)</td>
<td>Data missing</td>
<td>Data missing</td>
<td>136 (96)</td>
<td>5 (4)</td>
<td>141</td>
</tr>
<tr>
<td>Origin unknown</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FluoroType negative, n</td>
<td>1</td>
<td>Data missing</td>
<td>5</td>
<td>Data missing</td>
<td>5 (100)</td>
</tr>
<tr>
<td>Total*, n (%)</td>
<td>1</td>
<td>Data missing</td>
<td>5 (80)</td>
<td>Data missing</td>
<td>6</td>
</tr>
</tbody>
</table>

n= number of samples, % =how may percent in the specific category. *The total specimens in that category that was included in the final result and in the calculations.

Of all 951 specimens 820 samples were from Greek patients and 132 of the specimens were from patients that immigrant to Greece. In total 722 of the specimens from Greek patients were FluoroType negative and 42 FluoroType positive, 741 were culture negative and 23 culture positive. Of the specimens from immigrated patients 104 were both culture and FluoroType negative but 18 were FluoroType positive and 15 culture positive (Table 3)
Table 3. The data that was collected from both culturing and FluoroType analysis and also showing if the patient was from Greece or from an immigrant from another country than Greece.

<table>
<thead>
<tr>
<th></th>
<th>Contaminated, n</th>
<th>Not done, n</th>
<th>Culture negative, n</th>
<th>Culture positive, n</th>
<th>Total*, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Immigrants</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Invalid, n</strong></td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>FluoroType negative, n</td>
<td>6</td>
<td>0</td>
<td>100</td>
<td>4</td>
<td>104 (84)</td>
</tr>
<tr>
<td>FluoroType positive, n</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>14</td>
<td>18 (14)</td>
</tr>
<tr>
<td><em><em>Total</em> n (%)</em>*</td>
<td>6</td>
<td>0</td>
<td>104 (82)</td>
<td>18 (14)</td>
<td>131</td>
</tr>
<tr>
<td><strong>Greeks</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Invalid, n</strong></td>
<td>0</td>
<td>1</td>
<td>25</td>
<td>1</td>
<td>27</td>
</tr>
<tr>
<td>FluoroType negative, n</td>
<td>27</td>
<td>0</td>
<td>720</td>
<td>2</td>
<td>722 (91)</td>
</tr>
<tr>
<td>FluoroType positive, n</td>
<td>2</td>
<td>0</td>
<td>21</td>
<td>21</td>
<td>42 (5)</td>
</tr>
<tr>
<td><em><em>Total</em>, n (%)</em>*</td>
<td>29</td>
<td>1</td>
<td>741 (93)</td>
<td>23 (3)</td>
<td>820</td>
</tr>
</tbody>
</table>

n= number of samples, % = how may percent in the specific category. *The total specimens in that category that was included in the final result and in the calculations.

The calculation of the total FluoroType statistics result showed that the analytical sensitivity compared to culturing was 85,4%, the analytical specificity was 97,0 %, the PPV was 58,3% and the NPV was 99,3 % (Table 4).

Table 4. The calculate values of the sensitivity, specificity, positive and negative predictive values of for the FluoroType® MTB and microscopy for the diagnosis of tuberculosis

<table>
<thead>
<tr>
<th></th>
<th>FluoroType</th>
<th>Pulmonary</th>
<th>Extra Pulmonary</th>
<th>Greeks</th>
<th>Immigrants</th>
<th>Microscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Analytical sensitivity, %</strong></td>
<td>85,4</td>
<td>91,7</td>
<td>40,0%</td>
<td>91,3</td>
<td>77,8</td>
<td>81,8</td>
</tr>
<tr>
<td><strong>Analytical specificity, %</strong></td>
<td>97,0</td>
<td>98,0</td>
<td>97,7</td>
<td>97,2</td>
<td>96,2</td>
<td>97,1</td>
</tr>
<tr>
<td><em><em>Total PPV</em>, %</em>*</td>
<td>58,3</td>
<td>61,1</td>
<td>33,3</td>
<td>50,0</td>
<td>77,8</td>
<td>52,9</td>
</tr>
<tr>
<td><strong>Total NPV</strong>, %</td>
<td>99,3</td>
<td>99,5</td>
<td>97,7</td>
<td>99,7</td>
<td>96,2</td>
<td>99,3</td>
</tr>
</tbody>
</table>

*positive predictive value, **negative predictive value

Among the pulmonary samples the FluoroType statistics showed that the analytical sensitivity was 91,7%, the analytical specificity was 98,0 %, the PPV was 61,1 % and the NPV was 99,5 %. The extrapolummary samples showed an analytical sensitivity at 40,0%, the analytical specificity was 97,7 %, the PPV was 33,3 % and the NPV was 97,7 %. Among the Greek samples the FluoroType statistics showed that the analytical sensitivity was 91,3%, the analytical specificity was 97,2 %, the PPV was 50,0 % and the NPV was 99,2 %. Among
immigrant the analytical sensitivity was 77,8% the analytical specificity was 96,2 % the PPV was 77,8% and the NPV was 96,2 % (Table 4).

The calculation of statistic values of the Microscopy was made the same way as the statistical values from the FluoroType. the total Microscopy statistics result showed that the analytical sensitivity compared to culturing was 81,8%, the analytical specificity was 97,1 %, the PPV was 52,9% and the NPV was 99,3 % (Table 4).
Discussion

In this study the diagnostic accuracy of the FluoroType® MTB was evaluated compared to the reference method, cultivation on LJ medium and/or liquid culture system MGIT Bactec 960.

Of all the evaluated specimens FluoroType detected more positive result than the culture. This indicates that the FluoroType® MTB is more sensitive to detect the TB bacilli than the “gold standard method” if you compare the result. Anyhow, this sensitivity can be both problematic and positive for the clinician that are responsible for the patients. Positive in which way that the FluoroType may detect bacilli that the references methods do not and problematic in that way that the FluoroType might detect death bacilli that not cause a current TB infection in the patient.

The analytical specificity for this study was high which indicates that patients that are TB negative are not given a TB diagnosis which is a good thing. Though there was a few, three samples to be correct, that were false negative with the FluoroType but had a positive culture result. Though there was less than five colonies that could be detected on the culture. Which indicates that there only was a few bacilli in the patient sample to begin with, and/or none or very few bacilli in the part of the sample that was analyzed by the FluoroType which can be an explanation that the TB could not be detected with the assay.

The previous evaluation studies that have been made by Hoffman et el., and Eigner et al., both showed an analytical specificity at 98.9 % respective 96,4 % each (15-16), which is similar to the result in this evaluation study and this similarity indicated that analytical specificity of the FluoroType® MTB is between 96,4 – 98,9 % but more evaluations studies is needed for confirmation.

The analytical sensitivity was high and this result is similar to previous evaluation studies made by Eigner et al., and Hoffman et el., with an analytical sensitivity on 84,6% respective 88,1 % (15-16). This similarity indicated that analytical sensitivity of the FluoroType® MTB is between 84,6 – 88,1 % but more evaluations studies is needed for confirmation.
However, a high analytical sensitivity that give many true positive result often also give false positive results (17) and since TB is a serious illness (1-9, 15-16) there is important to find all true positive and false negative. Therefor it is positive with a sensitive method but it also means that not just this assay can be the only ground to give a patient a positive TB diagnosis. Anyhow, the FluoroType is showed to be good method to imply a TB diagnosis in just a few hours.

Among the discrepancies there was a few false positive among the FluoroType positive result, which who was culture negative. The false FluoroType positive result that came from patients with previous TB that were under treatment indicates that the FluoroType detected DNA from dead bacteria. Since the culturing was negative and that fact that the patients were under treatment. The false FluoroType positive that later was confirmed negative with either FluoroType or another molecular assay can have different reasons and explanation for being false positive. For example contamination from other positive samples.

The result between different types of samples was showed to be different from each other when look through the analytical sensitivity. The analytical sensitivity for Extrapulmonary specimens was as expected low (6) compared to pulmonary specimens. However, the analytical specificity and NPV was almost the same between pulmonary and extrapulmonary samples.

Though there was showed to be a difference between FluoroType positive Greeks and immigrants. Which indicated that the samples from immigrants that were analyzed came from immigrants that might have heritage in countries were the prevalence of TB is higher than in Greece which is a low indecent country (3).

Something else that is worth noticing is that the FluoroType® MTB has similar calculated statistical values as the microscopy which is one of the classical ways of diagnosing tuberculosis (9).
Conclusion

The result in this study shows that the FluoroType® MTB has proven to be an accurate assay to detect *Mycobacterium tuberculosis*. The result indicate that the FluoroType® MTB is an accurate assay to detect the bacilli but should not be alone to determine if a patient have TB or not.

Scientific output

The importance of a fast and accurate assay to detect *Mycobacterium tuberculosis* is much needed in the society today since the number of infected patients is not just increasing in developing countries but in industrialized countries, such as Greece, as well.
Acknowledgments

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