PERFORMANCE OF GeneXpert MTB/RIF IN THE DIAGNOSIS OF EXTRAPULMONARY TUBERCULOSIS IN MOROCCO

A. Aainouss*a,b, G. Momena,b, A. Belghiti,a, K. Bennani,c, A. Lamaammal,b, F. Chetioui,b, M. Messaoudi,b, M. Blaghena,d, J. Mouslima, M. Khyattib, M.D. El Messaoudib

*a Hassan II University, Casablanca, Morocco
*b Morocco Pasteur Institute, Casablanca, Morocco
*c Ministry of Health, Rabat, Morocco
*d University Couaib Doukkali, El Jadida, Morocco

Abstract. Tuberculosis (TB), a chronic bacterial disease caused by Mycobacterium tuberculosis, commonly affects the lung but can also affect other parts of the body (extrapulmonary tuberculosis, EPT). A rapid diagnosis is essential to initiate a specific and effective treatment. Although mycobacterial culture remains the gold standard for EPT diagnosis, molecular tools are attracting increasing interest. GeneXpert MTB/RIF, a rapid automated diagnostic test, allows the detection of Mycobacterium tuberculosis as well as mutations in the hot-spot region of the \( rpoB \) gene associated with Rifampicin resistance. The present study was performed to evaluate the performance of the GeneXpert MTB/RIF test for the diagnosis of EPT compared to the standard method. This prospective study was conducted on 304 clinical samples collected from 192 patients attending the Laboratory of Mycobacteria and Tuberculosis of Pasteur Institute of Morocco, between 2016 and 2017. Out of the 304 samples, 113 were pleural fluids decontaminated using the Petroff method and 191 were biopsies (78 lymph nodes and 113 pleural biopsies) decontaminated using the Löwenstein method. Mycobacterium tuberculosis detection and identification were performed on all samples using smear microscopy, Löwenstein–Jensen medium culture and the GeneXpert MTB/RIF test. Our results showed that 54.5% (103/189) were men and 45.5% (86/189) were women. The age of patients ranged from 2–78 years and the majority of patients was in the age group 25–45 years. Extrapulmonary samples were derived from lymph nodes, pleural fluids and pleural tissues, with a percentage of 25.66, 37.17 and 37.17%, respectively. Interestingly, the sensitivity of the GeneXpert was 51.4% for all samples and 83.3% for lymph nodes. In conclusion, the present study revealed that the performance of the GeneXpert test depends highly on the type of sample, with a high sensitivity observed for lymph nodes. Additionally, we clearly showed that the GeneXpert MTB/RIF test presents limitations in the diagnosis of pleural TB. Thus, we recommend the coupled use of the GeneXpert MTB/RIF and the conventional techniques for EPT diagnosis.

Key words: extrapulmonary tuberculosis, paucibacillary tuberculosis, GeneXpert, diagnosis, sensitivity.
Резюме. Туберкулез (ТБ), хроническое бактериальное заболевание, вызываемое Mycobacterium tuberculosis, обычно поражает легкие, но также может затрагивать и другие анатомические области (внелегочный туберкулез, ВЛТ). Для начала специфического эффективного лечения необходимо проведение быстрой диагностики. Хотя культивирование микобактерий остается золотым стандартом для диагностики ВЛТ, все больший интерес вызывают молекулярные методы. GeneXpert MTB/RIF — быстрый автоматизированный диагностический тест, который позволяет обнаруживать M. tuberculosis, а также мутации в «горячем участке» гена rpoB, связанные с устойчивостью к рифампицину. Настоящее исследование было выполнено для оценки эффективности теста GeneXpert MTB/RIF для диагностики внелегочного туберкулеза в сравнении со стандартным методом. Данное проспективное исследование было проведено на 304 клинических образцах, собранных у 192 пациентов, посещавших лабораторию микобактерий и туберкулеза Института Пастера в Марокко в период с 2016 по 2017 гг. Из 304 образцов 113 составляла плевральная жидкость, очищенная с использованием метода Петрова, а 191 образец биопсии (78 лимфатических узлов и 113 биопсий плевры), деконгламетированные с использованием метода Левейнштейна. Обнаружение и идентификацию Mycobacterium tuberculosis проводили для всех образцов с использованием микроскопии мазка, культивирования на среде Левенштейн–Йенсена и теста GeneXpert MTB/RIF. Наши результаты показали, что 54,5% (103/189) составляли мужчины и 45,5% (86/189) — женщины. Возраст пациентов составлял от 2 до 78 лет, большинство пациентов были в возрастной группе от 25 до 45 лет. Внелегочные образцы были взяты из лимфатических узлов, плевральной жидкости и плевральных тканей с процентным соотношением 25,66, 37,17 и 37,17% соответственно. Интересно, что чувствительность GeneXpert составила 51,4% для всех образцов и 83,3% для лимфатических узлов. Таким образом, настоящее исследование показало, что эффективность теста GeneXpert значительно зависит от типа образца, при этом высокая чувствительность наблюдается для лимфатических узлов. Кроме того, мы показали, что тест GeneXpert MTB/RIF имеет ограничения в диагностике плеврального туберкулеза. Мы рекомендуем совместное использование GeneXpert MTB/RIF и традиционных методов для диагностики внелегочного туберкулеза.

Ключевые слова: внелегочный туберкулез, олигобациллярный туберкулез, GeneXpert, диагностика, чувствительность.

Introduction

Tuberculosis (TB) remains a serious global health problem and is one of the leading causes of death worldwide [21]. In Morocco, 30,897 TB cases were declared in 2017, with an estimated prevalence of 88 cases per 100,000 inhabitants [10]. The most common site of infection with Mycobacterium tuberculosis (MTB) worldwide is the lung, although dissemination can occur to any part of the body, resulting in extrapulmonary tuberculosis (EPT) [18]. Worldwide, EPT accounted for 15% of the 7 million TB incident cases notified in 2018, with proportions ranging from 8% in the World Organization Health (WHO) Western Pacific Region to 24% in the Eastern Mediterranean Region (EMR) [25]. Among the EMR countries, Tunisia has the highest proportion of EPT cases (60%) [1]. In Morocco, EPT accounts for 46% of all TB cases, with lymph node EPT being the most common localization followed by pleural EPT [1, 7, 10]. Due to the paucibacillary nature of the non-respiratory specimens, diagnosis of EPT remains challenging. Therefore, molecular tools using nucleic acid amplification methods has been recommended by the WHO for rapid diagnosis of TB infection [9, 22].

Among these molecular technologies, GeneXpert MTB/RIF, a rapid technology developed by Ceiphed (Sunnyvale, CA, USA) is the only fully automated cartridge based real time PCR, implementing molecular beacon technology which can detect MTB complex DNA and Rifampicin (RIF) resistance in less than two hours [17]. Although the use of GeneXpert MTB/RIF is not recommended for the diagnosis of TB with all non-respiratory specimens, this test have been used by WHO for the diagnosis of EPT since 2013 [24]. GeneXpert MTB/RIF has been validated in several studies using an excessively small sample size of EPT, resulting in a large different range of sensitivities and specificities. This difference may also be due to the studied patient population, processing methods as well as the composite reference standard (CRS) [17].

In this field, the present study was designed to evaluate the performance of the Xpert MTB/RIF assay for the detection of M. tuberculosis com-
plex (MTBC) and RIF resistance in lymph node, pleural fluids and pleural biopsy using a large simple size. We also compared the diagnostic performance of GeneXpert MTB/RIF with that of conventional culture, CRS and patient’s clinical findings.

Materials and methods

This study was conducted in the laboratory of mycobacteria and tuberculosis of Pasteur Institute of Morocco. The clinical samples were collected from 192 patients with suspicion of EPT based on clinical criteria and EPT positive patients in post-therapeutic follow-up. The collected specimens consisted of 191 biopsies (pleural biopsy \( n = 113 \), lymph nodes \( n = 78 \)) and 113 pleural fluids. For suspected pleural TB patients, two specimens were collected (biopsy and pleural fluid).

Laboratory processing of specimens. Decontamination and concentration of clinical specimens is an important and critical step in the isolation of mycobacteria. The modified Petroff method was used for the decontamination of pleural fluids. Briefly, the sample was homogenized for 15 min in a shaker using an equal volume of NaOH (4%). After centrifugation at 3000g for 20 min, the deposit was neutralized with 20 mL of sterile distilled water. After another centrifugation, the sediment was inoculated in solid media (Löwenstein–Jensen) and the smear and Xpert MTB/RIF were performed [23].

Lymph nodes and pleural biopsies were decontaminated using the Löwenstein method. The samples were cut into small pieces with a sterile scalpel or scissors and homogenized in a sterile porcelain mortar using 5 mL of sterile distilled water. Four mL of sulfuric acid H2SO4 4% were then added and incubated at room temperature for 10 minutes. The sample was neutralized with 3.5 mL of NaOH (6%) and the solution was then put in a centrifuge tube with a screw cap after adding 20 mL of sterile distilled water. After centrifugation at 3000g for 20 min, the sediment was inoculated in LJ, the smear and GeneXpert MTB/RIF were made.

Smears microscopy. Smears microscopy was used to detect Acid-Fast Bacilli (AFB) by the conventional Ziehl–Neelsen method. A semi quantitative grading system was used to report the number of AFB observed in the stained smear of each sample. When no AFB was seen after examining 300 fields, the smear was annotated “no AFB seen”.

Solid media (Löwenstein–Jensen). To isolate and semi quantify growth of Mycobacteria, for each specimen two Löwenstein–Jensen (LJ) medium were inoculated with 0.2 mL of the decontaminated sediment and incubated at 37°C for 60 days. The isolates were then identified using colony morphology, pigmentation, niacin test and nitrate reductase tests.

GeneXpert MTB/RIF assay. The GeneXpert test was performed as previously described [23]. The technique covers an area of 81 base pairs of the \( rpoB \) gene, between the codon 507 and 533, which encodes for the B subunit of RNA polymerase. Five molecular beacon type genetic probes were used with a different fluorophore. Briefly, the GeneXpert reagent was added in a 2:1 ratio to the decontaminated and concentrated specimen. The sample container was mixed twice during 15 min incubation period at room temperature. Finally, 2 mL of the treated sample was transferred into the GeneXpert cartridge, loaded into the GeneXpert device and the automatically generated results were read after 2 hours.

Statistical analysis. The results were analyzed using the Excel software 2016 and VassarStats (http://vassarstats.net/clin1.html). Sensitivity and specificity of GeneXpert MTB/RIF regarding type of sample (pleural fluid, pleural tissue for suspect pleural TB, and lymph nodes for suspect lymphadenitis TB) were calculated. GeneXpert MTB/RIF diagnostic accuracy was calculated in comparison to LJ culture, which is the reference standard for EPTB. Therefore, in this study we evaluate the use of GeneXpert MTB/RIF using a CRS composed of smear microscopy, culture (LJ) and clinical findings. Any patient that was positive for any one component of the CRS was considered TB cases. Contaminated cultures and indeterminate results (Error, Invalid, No result) were excluded and not retested.

The gold standard for the diagnosis of TB pleuritic remains the detection of MTB in pleural fluid and/or pleural biopsy specimens, either by microscopy and/or culture. We used two types of sample for the diagnosis of pleural TB, if culture from pleural tissue or fluid was positive for MTB, the patient was considered positive for TB. The GeneXpert MTB/RIF result should be considered as bacteriological confirmation of TB if the sample was collected from a patient who was not recently receiving treatment with anti-TB drugs [6].

Results

Between 2016 and 2017, 192 patients were included and a total of 304 extrapulmonary samples were processed for GeneXpert. All samples were also processed for smears microscopy and culture in solid media. Among the 192 studied patients, 78 (40.62%) were patients with lymph node TB and the remaining 114 (59.37%) were patients with pleural TB. The isolates were then identified using colony morphology, pigmentation, niacin test and nitrate reductase tests, which confirmed that all isolates are \( M. \) \( tuberculosis \), \( M. \) \( tuberculosis \) gene, between the codon 507 and 533, which encodes for the \( \beta \) subunit of RNA polymerase. Five molecular beacon type genetic probes were used with a different fluorophore. Briefly, the GeneXpert reagent was added in a 2:1 ratio to the decontaminated and concentrated specimen. The sample container was mixed twice during 15 min incubation period at room temperature. Finally, 2 mL of the treated sample was transferred into the GeneXpert cartridge, loaded into the GeneXpert device and the automatically generated results were read after 2 hours.

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The demographic and clinical characteristics of 192 patients enrolled in this study are reported in table 1 showing that 103 were men and 86 were women (sex ratio: 1.20). The mean age of patients was 28 years with extreme ages of 2 and 78 years old. Interestingly, our results showed that 67 (35.4%) patients were young adults (15–25 years old) and...
39.15% (74/189) were between 25 and 45 years old. Extrapulmonary samples were collected from lymph nodes, pleural fluids, and pleural tissues, with a percentage of 25.66%, 37.17% and 37.17%, respectively. For 3/304 (0.9%) samples the results of GeneXpert were inconclusive, and these samples were excluded from further analysis. Overall, 50 out of the 301 studied specimens (16.61%) were positive for MTB by culture and 35 (11.63%) by the GeneXpert assay. Using the GeneXpert assay, the highest positivity rate was observed with lymph node specimens (33.33%), while the highest positivity rate was observed with pleural tissues (25.66%) by culture (Table 1). Our data further show that out of the 75 lymph nodes, 5 (6.6%) were AFB positive by conventional smear microscopy and 11 (14.6%) were positive for MTB by culture. Of 75 lymph nodes tested by GeneXpert, 25 (33.3%) were positive for TB (Fig.).

Among 113 pleural tissues studied, 3 (2.6%) were smear microscopy positive, 10 (8.8%) were positive for mycobacteria by culture and 4 (3.5%) were GeneXpert test positive (Fig.).

Of 113 pleural fluids tested, conventional culture showed the highest positivity rate (25.6%), followed by the GeneXpert assay, which was found positive in 6 pleural fluid specimens (5.3%), while only 3 pleural fluid specimens (2.6%) were positive by conventional smear (Fig.).

The GeneXpert test also provides a semi-quantitative report of the number of DNA copies detected in the sample. As expected it was “very low” or “low”, in the large majority (97.14%) of the samples that scored positive.

Based on the two reference standard (CRS and culture), TB diagnosis was confirmed in 68 patients (36%), 30 patients (40%) had a lymphadenitis TB and 38 (33.3%) had a pleural TB.

The sensitivity, specificity, positive and negative predictive values (PPV and NPV respectively) of the GeneXpert MTB/RIF test and smear microscopy are detailed in Table 3.

**Comparison with the culture method.** The GeneXpert assay showed the sensitivity and the specificity of 18% [95% CI, 9–32%] and 89.6% [95% CI, 85–93%], respectively. The sensitivity of smear examination compared to culture was 16% [95% CI, 7–29%] and the specificity was 98.8% [95% CI, 96–99%]. As presented in Table 2, our data show that the performance of the GeneXpert test varies according to the nature of the sample. In fact, the sensitivity of the GeneXpert test was in decreasing order of 60% [95% CI, 27–86%] for lymph nodes, 10.3% [95% CI, 2–28%] for tissue pleural and 0% [95% CI, 0–34%] for pleural fluids.

**Comparison with the Composite Reference Standard (CRS).** The comparison of the sensitivity of the GeneXpert and the smear microscopy shows an overall sensitivity of 51.4% [95% CI, 39–63%] and 16.18% [95% CI, 8–27%], respectively, when compared to the CRS. The performance of the GeneXpert test depended on the type of sample, the test sensitivity rate was highest for lymph nodes (83%) [95% CI, 64–93%], followed by pleural biopsy (15.8%) [95% CI, 6–32%] and pleural fluid (10.5%) [95% CI, 3–25%].

**Discussion**

Because of the paucibacillary nature of the non-respiratory specimens, diagnosis of EPT remains challenging and associated with low sensitivity to conventional methods; smears microscopy and culture.

**Table 1. Demographic and clinical characteristics of patients included in the study and results of the technics**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, years</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2–15</td>
<td>27</td>
<td>14.3</td>
</tr>
<tr>
<td>15–25</td>
<td>67</td>
<td>35.4</td>
</tr>
<tr>
<td>25–45</td>
<td>74</td>
<td>39.2</td>
</tr>
<tr>
<td>45–60</td>
<td>12</td>
<td>6.3</td>
</tr>
<tr>
<td>&gt; 60</td>
<td>9</td>
<td>4.8</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>103</td>
<td>54.5</td>
</tr>
<tr>
<td>Female</td>
<td>86</td>
<td>45.5</td>
</tr>
<tr>
<td><strong>Specimen type</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph node</td>
<td>75</td>
<td>24.9</td>
</tr>
<tr>
<td>Pleural tissue</td>
<td>113</td>
<td>37.5</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>113</td>
<td>37.5</td>
</tr>
<tr>
<td><strong>Smear result</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>11</td>
<td>3.7</td>
</tr>
<tr>
<td>Negative</td>
<td>290</td>
<td>96.3</td>
</tr>
<tr>
<td><strong>Culture result</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>50</td>
<td>16.6</td>
</tr>
<tr>
<td>Negative</td>
<td>251</td>
<td>83.4</td>
</tr>
<tr>
<td><strong>GeneXpert result</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>35</td>
<td>11.6</td>
</tr>
<tr>
<td>Negative</td>
<td>266</td>
<td>88.4</td>
</tr>
</tbody>
</table>

**Figure.** Showing distribution of samples according to the positivity of smears microscopy, culture and Xpert test
Negative results in smear microscopy and/or culture cannot therefore exclude the presence of TB [16], while positive smear examination cannot distinguish between non-tuberculosis mycobacteria and MTB. In addition, culture which is the gold standard for TB diagnosis, takes 2–8 weeks to get the results, impacting negatively the time to treatment decision [11]. Therefore, molecular techniques play an important role in rapid diagnosis of some EPT forms, although the sensitivity is poor for some of them [8]. GeneXpert assay in the diagnosis of lymphadenitis TB from lymph nodes (83%). These results are consistent with those of previous studies conducted in Tunisia by Ghariani et al. [5] who reported a value of 87.5%.

In our study, 14.6% (11/76) lymph node biopsies were found positive for TB by culture. According to previous studies, TB positivity rates of lymph node biopsies by culture varied from 10.3% to 45%. A positivity rate of 10.3% was reported in a study conducted in Morocco [1], 10.8% in Tunisia [13] and 45% in South Africa [26]. Furthermore, our results on TB detection by culture from pleural biopsies and pleural fluids show a positivity rate of 25.6% and 8.8%, respectively. This is quite similar to what was found in a study conducted by Du et al. [3] who reported a value of 31.7% for pleural biopsies and 17.4% for pleural fluids.

Our findings on the performance of the GeneXpert test compared to CRS (51.4%) correlate with study conducted by Moure et al., who reported a value of 58% [15] and study conducted by Rakotoarivelô et al. in Antananarivo, the capital city of Madagascar, who reported a value of 65% [20]. When GeneXpert MTB/RIF was compared to culture for the detection of resistance to RIF, we found a much lower sensitivity (18%) than other studies that report a high sensitivity ranging from 79% to 82% [20, 22, 27].

Our data also showed a higher sensitivity of the GeneXpert MTB/RIF assay in the diagnosis of lymphadenitis TB from lymph nodes (83%). These results are consistent with those of previous studies conducted in Tunisia by Ghariani et al. [5] who reported a value of 87.5%.

In the present study, the lowest GeneXpert sensitivity was observed for the diagnosis of pleural TB, which was quite similar to the study conducted in Spain by Porcel et al. [19], reporting a value of 15% and the study conducted in South Africa by Friedrich et al. [4], who reported a value of 25%. This low sensitivity of GeneXpert for the diagnosis of pleural TB, could be due to a very low number of bacteria or the presence of an inhibitory substance in the sample, which may have inhibited the amplification of MTB without any effect on the internal control of PCR [2].

Previous studies conducted on the performance of GeneXpert MTB/RIF showed a variable sensitivity of the test [12]. This difference may be explained by various factors including the heterogeneity of the studied populations, the type of EPT, the quality and nature of samples and the gold standard used in the study [12].

Among negative culture specimens, 10.35% (26/251) were found to be positive for GeneXpert MTB/RIF.

Table 2. Performance of the GeneXpert MTB/RIF test with respect to different sample types compared to culture and CRS

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>Culture as a reference standard</th>
<th>CRS as a reference standard</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity % (95% CI)</td>
<td>Specificity % (95% CI)</td>
</tr>
<tr>
<td>Lymph node</td>
<td>60 (27–86)</td>
<td>70.3 (57–80)</td>
</tr>
<tr>
<td>Pleural tissue</td>
<td>10.3 (2–28)</td>
<td>96.4 (89–99)</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>0 (0–34)</td>
<td>96.1 (89–98)</td>
</tr>
</tbody>
</table>

Table 3. Performance of GeneXpert MTB/RIF and smear microscopy compared to culture and CRS

<table>
<thead>
<tr>
<th>Techniques</th>
<th>Culture as a reference standard</th>
<th>CRS as a reference standard</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity % (95% CI)</td>
<td>Specificity % (95% CI)</td>
</tr>
<tr>
<td>Smear microscopy</td>
<td>16 (7–29)</td>
<td>98.8 (96–99)</td>
</tr>
<tr>
<td>GeneXpert MTB/RIF</td>
<td>18 (9–32)</td>
<td>89.6 (85–92)</td>
</tr>
</tbody>
</table>

Table 4. Performance of GeneXpert MTB/RIF and smear microscopy compared to culture and CRS

<table>
<thead>
<tr>
<th>Techniques</th>
<th>Culture as a reference standard</th>
<th>CRS as a reference standard</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity % (95% CI)</td>
<td>Specificity % (95% CI)</td>
</tr>
<tr>
<td>Smear microscopy</td>
<td>16 (7–29)</td>
<td>98.8 (96–99)</td>
</tr>
</tbody>
</table>
A similar finding was observed by Mechal et al. who reported that 10.6% of positive cases in GeneXpert MTB/RIF were negative by culture [14]. This could be due to the paucibacillary nature of extrapulmonary clinical specimens and/or the severe regime of decontamination of clinical samples decreasing the viability of mycobacterial strains.

In the present study, culture in solid media showed a moderate sensitivity of 47% for ETB. This could be explained by the fact that the decontamination step may have resulted in lowering the bacillary load and consequently the reduction of test sensitivity [22].

In conclusion, our study clearly revealed that the high sensitivity and specificity of GeneXpert, coupled with its simplicity and speed, make this technique the most useful tool for a rapid diagnosis of lymph node TB. Additionally, we found that use of the GeneXpert assay presents limitations in the diagnosis of pleural TB. Therefore, we recommend the coupled use of GeneXpert MTB/RIF and conventional technique for EPT diagnosis.

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References

Авторы: 
Айнус А., аспирант, центр медицинской биологии лаборатории микобактерий и туберкулеза, Институт Пастера Марокко, г. Касабланка, Марокко; аспирант кафедры биологии, лаборатория экологии и окружающей среды, отделение микробиологии научного факультета Бен М’Сик, Университет Хасана II, г. Касабланка, Марокко; 
Белгити А., к.н., лаборатория экологии и окружающей среды, отделение микробиологии научного факультета Бен М’Сик, Университет Хасана II, г. Касабланка, Марокко; 
Бенани К., магистр здравоохранения, департамент эпидемиологии и контроля заболеваний, Министерство здравоохранения, г. Рабат, Марокко; 
Ламамал А., инженер-экспериментатор лаборатории микобактерий и туберкулеза, Институт Пастера Марокко, г. Касабланка, Марокко; 
Щетенч Ф., инженер-экспериментатор лаборатории микобактерий и туберкулеза, Институт Пастера Марокко, г. Касабланка, Марокко; 
Мессауди М., инженер-экспериментатор лаборатории микобактерий и туберкулеза, Институт Пастера Марокко, г. Касабланка, Марокко; 
Благи А., PhD Student, Medical Biology Center, Laboratory of Mycobacteria and Tuberculosis, Morocco Pasteur Institute, Casablanca, Morocco; 
Мессазвуди М., PhD, Laboratory of Mycobacteria and Tuberculosis, Morocco Pasteur Institute, Casablanca, Morocco; 
Ауинус А., PhD Student, Medical Biology Center, Laboratory of Mycobacteria and Tuberculosis, Morocco Pasteur Institute, Casablanca, Morocco; 
Авторы: 
Айнус А., аспирант, центр медицинской биологии лаборатории микобактерий и туберкулеза, Институт Пастера Марокко, г. Касабланка, Марокко; 
Белгити А., к.н., лаборатория экологии и окружающей среды, отделение микробиологии научного факультета Бен М’Сик, Университет Хасана II, г. Касабланка, Марокко; 
Мессауди М., инженер-экспериментатор лаборатории микобактерий и туберкулеза, Институт Пастера Марокко, г. Касабланка, Марокко; 
Мессазвуди М., PhD, Laboratory of Mycobacteria and Tuberculosis, Morocco Pasteur Institute, Casablanca, Morocco; 
Кьятти М., к.н., ведущий научный сотрудник лаборатории вирусной онкологии, Институт Пастера Марокко, г. Касабланка, Марокко; 
Эль Мессазвуди М.Д., магистр здравоохранения, зав. лабораторией микобактерий и туберкулеза, Институт Пастера Марокко, г. Касабланка, Марокко.

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