Performance of the IS6110-TaqMan® assay in the diagnosis of extrapulmonary tuberculosis from different biological samples


[1]. Departamento de Imunologia, Centro de Pesquisas Aggeu Magalhães, Fundação Oswaldo Cruz, Recife, PE, Brasil.

Abstract

Introduction: This study evaluated the performance of the IS6110-TaqMan® assay in different types of biological samples and tissues for laboratory diagnosis of extrapulmonary tuberculosis. Methods: 143 biological samples and tissues from patients with suspected extrapulmonary tuberculosis from the health services of Recife/Pernambuco/Brazil were evaluated with the IS6110-TaqMan® assay. Results: The sensitivities of the IS6110-TaqMan® assay calculated for blood, urine, both blood and urine samples, tissue biopsies, extrapulmonary body fluid samples, and all samples from patients calculated together were 55.9%, 33.3%, 68.8%, 43.8%, 29.6%, and 73.7%, respectively, and the specificities were 80%, 100%, 78.6%, 100%, 100%, and 84.2%, respectively. Conclusions: The accuracy of qPCR was high in various clinical sample types. The analysis of more than one type of clinical sample collected from the same patient with extrapulmonary tuberculosis enhances the diagnostic power of the IS6110-TaqMan® assay when compared with the use of only one clinical sample.

Keywords: Mycobacterium tuberculosis complex. IS6110-TaqMan® assay. Molecular diagnosis. Extrapulmonary tuberculosis.

INTRODUCTION

Tuberculosis (TB) is a major public health problem of global importance, and it is the second leading cause of death worldwide, with 1.5 million people in 2014[1]. The World Health Organization (WHO) states that 80% of TB cases worldwide are concentrated in 22 countries, among which Brazil is in the 16th position[2]. In Brazil, 9,479 new cases of extrapulmonary TB occurred in 2014, with 562 cases in the State of Pernambuco alone[3].

Although pulmonary TB is the most common manifestation of the disease, approximately one million people (~15% of the total) develop extrapulmonary TB. In Brazil, the most frequent clinical forms are pleural, ganglionar (lymph node), osteoarticular, and urogenital tuberculosis[4].

Even though the detection of Mycobacterium tuberculosis at the initial phase of the disease is vital for effective treatment initiation, the effective diagnosis of extrapulmonary TB is challenging. This condition occurs mainly in cases where the disease is associated with other disorders. The extrapulmonary infections caused by the M. tuberculosis complex are frequently paucibacillary (with a lower number of bacteria in biological specimens) compared to the pulmonary form[5,6]. Besides, obtaining the patient’s extrapulmonary sample often requires invasive procedures and hospitalization, making it difficult to collect additional samples[6,7].

Although classical signs and symptoms of TB facilitate diagnosis, extrapulmonary TB often manifests with symptoms that are nonspecific or related to the site of infection, rendering a confirmation of the disease diagnosis difficult. Therefore, complementary tools are of critical importance in diagnosing this form of the disease[8]. Conventional laboratory exams include the direct microscopic detection of acid-fast bacilli (AFB) by Ziehl Nielsen staining and culture of clinical samples collected directly from the infection site, in the form of liquid and/or pleural biopsy, ganglion puncture, bone, urine, gastric wash, and others, in specific medium. The histopathological exam is also used for the detection of granulomatous lesions[9,9]. The examination of M. tuberculosis by culture tests remains the gold standard test for diagnostic confirmation[10]. However, the diagnostic sensitivity of conventional exams is compromised in paucibacillary patients, frequently resulting in a false negative outcome[11,12].
Nowadays, the most promising technique for the rapid diagnosis of TB is based on the polymerase chain reaction (PCR) method. Real-time PCR (qPCR) has an advantage over conventional PCR due to the ability to amplify and detect deoxyribonucleic acid (DNA) simultaneously through a fluorescence system. In addition, the quantification of nucleic acids is more precise and a highly reproducible using qPCR, as this method determines the values throughout the exponential phase of the reaction. Studies have demonstrated the efficacy of qPCR in detecting mycobacterial DNA in biological specimens, including pulmonary and extrapulmonary tissues.

This study evaluated the performance of the IS6110-TaqMan assay, sensitive and specific, in detecting DNA of the Mycobacterium tuberculosis complex in different types of samples and biological tissues for laboratory diagnosis of extrapulmonary tuberculosis.

**METHODS**

**Study population and setting**

A prospective double-blind study was conducted with 57 patients of both sexes with suspected extrapulmonary TB in different clinical forms (pleural, cutaneous, bone, lymph node, miliary, meningoencephalitis, renal, intestinal, hepatic). These patients were sourced from public health services specialized in TB in the metropolitan region of Recife, State of Pernambuco, northeastern Brazil, between December 2010 and January 2012.

A combination of clinical, epidemiological, and laboratory criteria was used to define the diagnosis of extrapulmonary TB according to recommendations from the American Thoracic Society.

**Specimens analyzed**

Blood and urine samples were collected from each patient, as well as a third sample (a biopsy of tissues or another extrapulmonary body fluid), depending on the suspected clinical form of extrapulmonary TB. A total of 143 clinical samples were collected from 57 patients with initial suspected extrapulmonary TB: 49 blood samples, 46 urine samples, 27 biopsies of tissues, and 21 other extrapulmonary body fluids such as pleural, ascites, cerebrospinal and synovial fluid.

All clinical samples were collected before the initiation of specific anti-tuberculosis treatment. Culturing was performed on all samples, except for blood, followed by DNA extraction and IS6110-TaqMan® assay.

**Collection and laboratory processing of biological samples**

**Blood:** 4.5ml of blood was collected by venipuncture using tubes (Vacutainer®, Becton and Dickson, England) containing ethylenediaminetetraacetic acid (EDTA). After collection, blood was processed for the separation of its components [plasma and peripheral blood mononuclear cells (PBMCs)] with Ficoll – Paque™ PLUS (GE Healthcare Bio-Sciences, Upsala, Sweden) to minimize inhibitory effects on the IS6110-TaqMan® assay.

**Urine:** 10ml of morning samples were collected in a sterile tube on three consecutive days. Urine samples were initially decontaminated as per the Petroff protocol (NaOH 4%) and stored in a 0.9% saline solution.

**Biopsy (bone, lymph node, skin):** fragments were collected from the infection site and stored in a 0.9% saline solution.

**Extrapulmonary body fluids (cerebrospinal fluid, pleural, ascetic, and synovial fluids):** 2-10ml were collected in a dry and sterile tube.

**Culture:** cultures were established on Löwenstein-Jensen solid medium and incubated at 37°C for 6-8 weeks in a level 3 biosafety laboratory. This protocol was followed for all biological samples except for blood. Mycobacterium species were identified based on the growth rate and colony morphology. Tests of selective inhibition with para-nitrobenzoic acid (PNB) and thiophene-2-carboxylic acid hydrazide (TCH), niacin accumulation, and heat-stable catalase at 68°C were also performed.

**DNA extraction**

DNA from clinical samples was extracted using the QIAamp DNA Mini Kit (Qiagen, Duesseldorf, Germany), as per the manufacturer’s instructions. The reference strain of Mycobacterium tuberculosis (H37Rv) was grown on Löwenstein-Jensen (LJ) solid medium and the genomic DNA was extracted and purified using a Genomic Prep™ - Cells and Tissue DNA Isolation Kit (Amersham Biosciences, Piscataway, NJ, USA), as per the manufacturer’s instructions.

**qPCR conditions**

The PCR reactions were performed in real time using an ABI Prism 7500 Sequence Detection System (Applied Biosystems, California, USA) with TaqMan®-specific probes (and ROX as a passive reference), TAQM3 (5’-AGGGCAACCTGCGCCAG-3’) and TAQM4 (5’-GATCGCTGATCCGGCCA-3’) oligonucleotides, which amplify a target fragment of 122bp from IS6110. The cycling conditions followed the protocol described by Broccolo et al. Milli-Q water negative controls were included in all amplification reactions. All assays were performed in duplicate. The reactions included 1µl of DNA primers (300nm each), 1µl of probe (200 nM), 12.5µl of a TaqMan® Universal PCR Master Mix kit, 9µl of the DNA template, and dd-water, for a final volume of 25µl.

Additionally, a qPCR assay targeting the housekeeping gene β-actin was performed. PCR reactions included 12.5µl TaqMan®, 2.5µl of TaqMan® Endogenous Controls (Applied Biosystems, California, USA) (500nM probe and 300nM each primer) and 9µl of the DNA template plus Milli-Q water, for a final volume of 25µl. PCR conditions were: initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15s, and annealing and amplification at 60°C for 1 min.

**Dilution curve with a reference strain of Mycobacterium tuberculosis (H37Rv) in urine and blood samples**

Urine and blood samples were collected from one individual who was considered clinically healthy, in other words, without clinical manifestations, without a history of contact with a bacilliphilous person, and negative on laboratory exams (urine culture and tuberculin test). To one aliquot of these samples, the reference strain of M. tuberculosis (H37Rv) was added to a final
concentration of 1 on the McFarland standard scale, then serial 10-fold dilutions were performed to achieve concentrations ranging from $3 \times 10^7$ to $3 \times 10^{10}$ bacilli/ml. The minimum detection sensitivity of the assay was 15 fg of DNA for both samples, corresponding to 3 bacilli/ml. This limit was defined by the amplification of the lowest dilution of the target DNA over the formation of primer dimers (the negative control of the reaction). To evaluate the positivity of biological samples, the threshold value of amplification ($C_T$) was established for positive and negative cases. The resulting $C_T$ was 25 (25-33) and 34 (34-38) for the positive and negative tests, respectively (data not shown).

Statistical analysis

Culture and/or clinical responses to specific treatments were primarily considered the gold standard for performance calculations. The IS6110-TaqMan® assay was also evaluated considering the results of the cultured samples as a gold standard (except for blood, for which no culturing was done). For the IS6110-TaqMan® assay, the sensitivity, specificity, and positive and negative predictive values were calculated, with a 95% confidence interval (CI) identified for each parameter. The database was constructed using Statistical Packages for the Social Sciences (SPSS) statistical software for Windows (Version 18, IBM Corporation, Armonk, NY, USA) which was also used to conduct statistical analyses, including cross tables and frequency calculations. For accuracy tests, the authors used the free software OpenEpi (Open Source Epidemiologic Statistics for Public Health, Version 3.01. Update 2013, available online at: www.OpenEpi.com).

For the blood samples, the IS6110-TaqMan® assay was positive when at least one blood phase, PBMC and/or plasma, was positive; negative results were obtained when both were negative. The performance of the IS6110-TaqMan® assay was calculated in parallel for both blood phases.

In blood and urine samples, the performance of the IS6110-TaqMan® assay was calculated alone and in parallel (blood and/or urine positive or both negative).

The performance of the IS6110-TaqMan® assay was calculated for the combination of all biological sample types (blood and/or urine and/or biopsy or extrapulmonary liquid) from the same patient. In this case, a positive IS6110-TaqMan® assay was observed when at least one clinical sample had a positive result. Results were negative when all the patient’s biological samples were negative.

Ethical considerations

This research was approved by the Ethics Committee of the Aggeu Magalhães Institute/FIOCRUZ (IAM/FIOCRUZ).

RESULTS

Clinical, epidemiological and laboratory findings

Out of the 57 participating patients with suspected extrapulmonary TB, 38 (66.7%) had their final diagnosis defined as extrapulmonary TB (G1), and 19 (33.3%) patients were classified as non-TB (G2) with another kind of disease (different from TB) and were considered a control group. Clinical forms of TB registered in the study are described in Table 1. Additionally, in the control group, the following diagnoses were observed: lymphoma, bone cancer, sarcoidosis, and bacterial meningitis.

From the total number of patients included in the study, 32 (56.1%) were male. The age ranged from 1 to 89 years old, with an average of 34 years old. The majority (75%) of tested samples were negative for *M. tuberculosis* in culture.

**Performance of the IS6110-TaqMan® assay in various clinical samples in patients with and without extrapulmonary TB**

The sensitivity, specificity, and positive/negative predictive values of the IS6110 TaqMan® assay on blood, urine, biopsy samples, and other body fluids, analyzed alone or in parallel, are described in Table 2.

Considering the results of the cultured samples as a gold standard, the performance of TaqMan® ranged between 18-34% (sensitivity) and 75-100% (specificity). For urine samples, the sensitivity was 18.2% (CI = 4.6-41.0), and the specificity was 75% (CI = 60.9-81.9). For biopsy tissues, these values were 34% (CI = 9.5-57.2) and 88.2% (CI = 65.7-96.7), respectively, and for extrapulmonary body fluids, 33.3% (CI = 9.7-70) and 100% (CI = 78.5-100), respectively.

Considering all clinical samples from the same patients calculated in parallel, the sensitivity of the TaqMan® assay compared with culturing was 64.3% (CI = 39.2-89.4), and the specificity was 51% (CI = 36.2-66.1). These results are detailed in Table 3.

**Table 1: Clinical forms of extrapulmonary tuberculosis.**

<table>
<thead>
<tr>
<th>Clinical Form</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pleural</td>
<td>14</td>
<td>36.8</td>
</tr>
<tr>
<td>Peripheral lymph node</td>
<td>5</td>
<td>13.2</td>
</tr>
<tr>
<td>Meningoencephalitis</td>
<td>2</td>
<td>5.3</td>
</tr>
<tr>
<td>Bone</td>
<td>8</td>
<td>21.1</td>
</tr>
<tr>
<td>Cutaneous</td>
<td>3</td>
<td>7.9</td>
</tr>
<tr>
<td>Renal</td>
<td>1</td>
<td>2.6</td>
</tr>
<tr>
<td>Intestinal</td>
<td>2</td>
<td>5.3</td>
</tr>
<tr>
<td>Hepatic</td>
<td>1</td>
<td>2.6</td>
</tr>
<tr>
<td>Miliary</td>
<td>2</td>
<td>5.3</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>100.0</td>
</tr>
</tbody>
</table>

DISCUSSION

The development of sensitive and specific tools for the diagnosis of tuberculosis, mainly for paucibacillary forms such as extrapulmonary TB, remains a great challenge. The reasons include the difficulty in accessing most lesions and the
TABLE 2: Performance of the IS6110-TaqMan® assay in clinical sample between patients with extrapulmonary TB (G1) and No TB group (G2), using culture and/or clinical diagnosis with the therapeutic response as gold-standard.

<table>
<thead>
<tr>
<th>Clinical samples</th>
<th>Patients (n)</th>
<th>IS6110-TaqMan® assay</th>
<th>Predictive value</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>G1</td>
<td>G2</td>
<td>Sensitivity</td>
<td>Specificity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>34</td>
<td>55.9% (19/34)</td>
<td>80% (12/15)</td>
<td>86.4% (19/22)</td>
<td>44.4% (12/27)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CI = 39.4–71.1</td>
<td>CI = 54.8–93.0</td>
<td>CI = 66.7–95.3</td>
<td>CI = 27.6–62.7</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>33</td>
<td>33.3% (11/33)</td>
<td>100% (13/13)</td>
<td>100% (11/11)</td>
<td>37.1% (13/35)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CI = 19.8–50.4</td>
<td>CI = 77.2–100.0</td>
<td>CI = 74.1–100.0</td>
<td>CI = 23.2–53.7</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood + urine</td>
<td>32</td>
<td>68.8% (22/32)</td>
<td>78.6% (11/14)</td>
<td>88% (22/25)</td>
<td>52.4% (11/21)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CI = 51.4–52.1</td>
<td>CI = 52.4–52.4</td>
<td>CI = 70–95.8</td>
<td>CI = 59.6–85.8</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biopsy</td>
<td>16</td>
<td>43.8% (7/16)</td>
<td>100% (11/11)</td>
<td>100% (7/7)</td>
<td>55% (11/20)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CI = 23.1–66.8</td>
<td>CI = 74.1–100.0</td>
<td>CI = 64.6–100.0</td>
<td>CI = 34.2–74.2</td>
</tr>
<tr>
<td>Other extrapulmonary body fluids</td>
<td>15 7</td>
<td>29.6% (6/15)</td>
<td>100% (7/7)</td>
<td>100% (6/6)</td>
<td>31.8% (7/16)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CI = 13.8–50.0</td>
<td>CI = 64.6–100.0</td>
<td>CI = 61–100.0</td>
<td>CI = 16.4–52.7</td>
</tr>
</tbody>
</table>

TB: tuberculosis; G1 (Group 1): patients with extrapulmonary TB; G2 (Group 2): patients without TB (with a diagnosis other than TB); CI: confidence interval.

TABLE 3: Accuracy of the IS6110 TaqMan® assay in all biological sample types, analyzed together, per patient.

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Accuray</th>
<th>Sensitivity = 73.7% (CI = 59–85)</th>
<th>Specificity = 84.2% (CI = 62.4–94.5)</th>
<th>Positive predictive value = 90.3% (CI = 75.1–96.7)</th>
<th>Negative predictive value = 61.5% (CI = 42.5–77.6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS6110 TaqMan® assay</td>
<td>Positive</td>
<td>28</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>10</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>38</td>
<td>19</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TB: tuberculosis; CI: confidence interval.

The fact that these are commonly patients with a low circulating bacterial load. In these cases, conventional exams of AFB and culture – the latter being considered a gold standard, usually yield negative results. In extrapulmonary TB, only one quarter of the cases can be diagnosed by bacteriological confirmation. From the 34 blood samples of patients with extrapulmonary TB, 15 were negative in the IS6110-TaqMan® assay. These false negative results might be related to the small quantities of targeted DNA present on the clinical samples or even the presence of PCR inhibitors. On the other hand, three blood samples were positive in the IS6110-TaqMan® assay, but these patients were finally diagnosed as non-TB, so the molecular results were false positives. Therefore, it is possible that qPCR may detect the presence of native genetic material from dead or latent organisms, generating discordant results with the gold standard used in the study. A study performed by Lima et al. analyzed blood samples from patients under 15 years of age with extrapulmonary TB with nested PCR and showed similar results to the present study. Among the 33 urine samples from patients with extrapulmonary TB, 22 were negative in the IS6110-TaqMan® assay. These false negative results were probably due to the presence of components such as salts, enzymes, and other bacteria, which can compromise the purification process of DNA and therefore inhibit the amplification reaction in urine. In this study, there were no false positive urine results from the IS6110-TaqMan® assay.
When the IS6110-TaqMan® assay for urine samples was compared with two different gold-standard criteria (1: culture and/or response to antitubercular therapy; 2: culture alone), the sensitivity increased by 15.1% when compared to the gold standard 1. These findings reinforce the limitation of culturing in cases of patients with paucibacillary forms. Studies show variability in positive results of the culture test (12% to 80%) in a range of clinical samples and biological tissues.

Most biological samples analyzed in this study were blood and urine (66.4%), which are considered samples from ambulatory collection and represent a less invasive procedure for the patient. When blood and urine samples were evaluated in combination, the sensitivity of the molecular test (68.8%) increased by approximately 20% when compared with the results of the tests performed alone (55.9% and 33.3%, respectively).

Studies have shown that, despite the low sensitivity of molecular methods on urine samples, when these samples were analyzed in combination with blood samples, the sensitivity was increased by around 8-10%. Lima et al. evaluated a molecular diagnostic test for extrapulmonary TB in blood and urine samples. The sensitivity of the assay using combined samples (calculated in parallel) was 71.9%, but when using only urine samples, the sensitivity was 40.6%.

Rebollo et al. evaluated blood and urine samples from patients diagnosed with TB using a simple PCR system, with the positive culture for each sample as the gold standard, and found a lower (42%) sensitivity. Despite their use of a conventional PCR system less sensitive than the IS6110-TaqMan® assay, the authors highlight the importance of using more than one type of sample from each patient with suspected TB, as this approach can increase the sensitivity.

In this study, the sensitivity and specificity of the IS6110-TaqMan® assay in extrapulmonary body fluid samples were 28.6% and 100%, respectively. Since the number of bacilli is lower in various extrapulmonary body fluids, it is known that false negative results arise mainly from the paucibacillary nature or even from the irregular distribution of bacilli in body fluids, likely explaining the low sensitivity found in this study. Rosso et al. evaluated pleural fluid samples with the IS6110-TaqMan® assay and obtained a better sensitivity (40%), probably because of the greater number of patients included in their study. Other research has also demonstrated a better performance of molecular tests in body fluids as compared with this study.

We also evaluated the performance of the IS6110-TaqMan® assay in tissue samples. The sensitivity and specificity in this context were 43.8% and 100%, respectively. Out of the 16 tissue samples in group G1, the IS6110-TaqMan® assay was not able to detect Mycobacterium tuberculosis in 9 samples; these results were considered false negatives. Possible causes were the quality and/or quantity of collected material, the presence of few bacilli on biopsied tissue, and collection outside the site of infection, as well as the inhibitors present in the sample. No false positive results were found, showing that this test may aid in the confirmation of the disease.

Sun et al. evaluated the performance of qPCR on 90 kidney biopsies and found a higher sensitivity than this study (83.3%), probably due to the inclusion of 30 patients with renal TB. Another work, which evaluated pleural fragments by qPCR using the same molecular target and extraction method, yielded similar results to this research (a sensitivity of 52.8% and specificity of 93.3%). In agreement with our study, Meghdadi et al., using PCR targeting IS6110, demonstrated a sensitivity and specificity of 48.6% and 100%, respectively.

In the different sample types evaluated, culture results demonstrated inferior performance in defining the final diagnosis of patients when compared with the gold standard 1. These results show the limitation of culture tests in the diagnosis of paucibacillary forms of TB, such as the extrapulmonary TB.

The main aim of this study was to evaluate the performance of detection of the IS6110 DNA sequence of Mycobacterium tuberculosis by qPCR in the laboratory diagnosis of extrapulmonary TB. Determination of sensitivity values for qPCR on each type of clinical sample yielded values ranging from 26.7% (in “other extrapulmonary body fluids”) to 55.9% (in blood samples), when analyzed alone. However, when the sensitivity of the IS6110-TaqMan® assay was evaluated considering the combination of all clinical samples from a patient, it increased to 73.7%. A decrease in specificity (84.2%) was observed, however, compared to the analysis of clinical samples individually, particularly blood samples.

In regions where TB is endemic, negative results from the IS6110-TaqMan® assay must not be interpreted as excluding the possibility of the disease. We observed that the positive predictive values obtained were equal to or higher than 86% in all analyses, reinforcing the confirmatory power of the assay in the detection of Mycobacterium tuberculosis in patients with suspected extrapulmonary TB, despite the small sample sizes. However, the predictive values for the various tests analyzed (Table 2 and Table 3) cannot be inferred for the general population. These values are not comparable because the prevalence of TB is not the same in all situations.

Our results demonstrate the importance of investigating more than one type of clinical sample collected from the same patient with suspected extrapulmonary tuberculosis, which might improve the predictive power of the IS6110-TaqMan® assay.

The IS6110-TaqMan® assay can be an important tool to facilitate the laboratory diagnosis of extrapulmonary TB, mainly in the most severe cases of the disease, to initiate specific therapy. However, more research is needed to clarify the value of this new technique as a diagnostic test in the clinical setting.

Acknowledgements

We thank the staff of the Immunoepidemiology Laboratory and the professionals involved.
Conflict of interest

The authors declare that there is no conflict of interest.

Financial support

This study was funded by Programa de Excelência em Pesquisa/ Clinical Research (Grant number: 402058/2012-7), Research Program for the Single Health Service: Shared Health Management 2012 – Pernambuco and Instituto Aggeu Magalhães/Fundação Oswaldo Cruz.

REFERENCES


