A novel lymphocyte transformation test (LTT-MELISA®) for Lyme borreliosis

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Abstract

Diagnosis of active Lyme borreliosis (LB) remains a challenge in clinically ambiguous, serologically indeterminant, and polymerase chain reaction-negative patients. Lymphocyte transformation tests (LTTs) have been applied to detect specific cellular immune reactivity, but their clinical application has been severely hampered by the poorly defined \textit{Borrelia} antigens and nonstandardized LTT formats used. In this study, we describe the development and clinical relevance of a novel LTT using a validated format (MELISA®) together with well-defined recombinant \textit{Borrelia}-specific antigens. From an initial screening of 244 patients with suspected \textit{Borrelia} infection or disease, 4 informative recombinant antigens were selected: OspC (\textit{Borrelia afzelii}), p41-1 (\textit{Borrelia garinii}), p41-2 (\textit{B. afzelii}), and p100 (\textit{B. afzelii}). Thereafter, 30 seronegative healthy controls were tested in LTT-MELISA® to determine specificity, 68 patients were tested in parallel to determine reproducibility, and 54 lymphocyte-reactive symptomatic patients were tested before and after antibiotic therapy to assess clinical relevance. Most (86.2%) of the 36.9% (90/244) LTT-MELISA®-positive patients were seropositive and showed symptoms of active LB. Specificity was 96.7% and reproducibility 92.6%. After therapy, most patients (90.7%) showed negative or markedly reduced lymphocyte reactivity correlating with clinical improvement. This novel LTT-MELISA® assay appears to correlate with active LB and may have diagnostic relevance in confirming LB in clinically and serologically ambiguous cases.

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1. Introduction

Lyme borreliosis (LB) is the most frequent vector-borne disease in Europe and the United States, and its prevalence appears to be rising (Centers for Disease Control and Prevention, 2004). The disease is transmitted by ticks harboring the infectious agent \textit{Borrelia burgdorferi} sensu lato. Although all 3 human pathogenic species \textit{B. burgdorferi} sensu stricto, \textit{B. afzelii}, and \textit{Borrelia garinii} cause LB in Europe, \textit{B. burgdorferi} sensu stricto appears to be the sole etiologic agent in the United States (reviewed in Stanek and Strle, 2003; Steere et al., 2004).

A multisystem disorder, LB produces a wide range of clinical manifestations. Only erythema migrans (EM), a transient reddening of the skin circumventing the tick bite site and developing within days to weeks after infection, may be pathognomonic, but at the same time, it occurs in only 60% of LB patients. All other extracutaneous symptoms are characteristic of a variety of diseases, rendering clinical diagnosis alone, except in the case of EM, as problematic (Stanek and Strle, 2003; Steere et al., 2004).

Routine laboratory testing is based on detecting the humoral immune response to \textit{Borrelia}-specific antigens in a 2-tiered format: screening with an enzyme-linked immunosorbent assay (ELISA) followed by confirmation of positive or borderline results in a more specific immunoblot or Western blot. However, although serologic analyses can confirm infection, they do not prove the existence of the disease. In
addition, serologic results are frequently negative in early Lyme disease, antibodies may persist for several years despite successful therapy, and interlaboratory serologic results may be discordant (Robertson et al., 2000; Wilske, 2003; Aguero-Rosenfeld et al., 2005). Microbial detection by culture or polymerase chain reaction (PCR) is highly specific but has a limited sensitivity (Dumler, 2001). Therefore, although infection with B. burgdorferi can often be determined, diagnosis of the disease itself remains challenging. At the same time, early therapeutic intervention appears to play a crucial role in preventing disease progression (Dattwyler et al., 1988).

During the 1990s, several groups attempted to improve the diagnosis of LB by examining the specific cellular immune reactivity to B. burgdorferi using various forms of the lymphocyte transformation test (LTT) (Table 1) (Dattwyler et al., 1988; Dressler et al., 1991; Krause et al., 1991; Yoshinari et al., 1991; Zoschke et al., 1991; Krause et al., 1992; Schempp et al., 1993; Roessner et al., 1994; Breier et al., 1995; Hupertz et al., 1996; Rutkowski et al., 1997). Clinical application of LTT for Lyme disease, however, proved limited because of generally poor sensitivity, specificity, and reproducibility, and its use has since been discredited limited because of generally poor sensitivity, specificity, and reproducibility, and its use has since been discredited (Wilske, 2003). Likely causes of these poor results include the use of 1) nonvalidated nonstandardized LTT formats with low lymphocyte concentrations (<250,000 cells per test), and 2) poorly defined antigenically heterogeneous “lysatcs” from whole B. burgdorferi or culture supernatants as antigens.

In the present study, we describe a novel LTT that incorporates 2 improvements to obviate the abovementioned problems. First, a standardized LTT format using a higher cell concentration (1 x 10^6 cells per test), memory lymphocyte immunostimulation assay (MELISA®), was applied (Stejskal et al., 1994). Its technical validity and clinical relevance for detecting cellular immune reactivity to other antigens has been extensively documented (Stejskal et al., 1986, 1990, 1996, 1999; Tibbling et al., 1995; Stejskal, 1997; Sterzl et al., 1999; Regland et al., 2001; Valentine-Thon and Schiwara, 2003; Prochazkova et al., 2004; Kakuschke et al., 2005). Second, only well-characterized recombinant Borrelia-specific antigens were used (Jauris-Heipke et al., 1993a, 1993b; Luft et al., 1993; Robinson et al., 1993; Wilske et al., 1993a, 1993b; Wilske, 2003). The specificity, reproducibility, and clinical potential of this novel LTT-MELISA® for LB are presented.

2. Materials and methods

2.1. Recombinant Borrelia antigens

Eight recombinant Borrelia-specific antigens, developed in collaboration with the Max von Pettenkofer Institute, National Reference Center for Borreliae, University of Munich, Munich, Germany (Jauris-Heipke et al., 1993a, 1993b; Luft et al., 1993; Robinson et al., 1993; Wilske et al., 1993a, 1993b; Wilske, 2003), were obtained from MIKROGEN (Neuried, Germany). These antigens are used in MIKROGEN’s commercial ELISA and Western blot tests, are derived from all 3 genospecies, include the immunodominant epitopes of both early (IgM) and late (IgG) response to the disease, and were tested here in serial dilutions of the following initial concentrations: OspA (B. burgdorferi, 50 μg/mL), OspC (B. garinii, 45 μg/mL), OspC (B. afzelii, 100 μg/mL), p41 (B. afzelii, 50 μg/mL), p41-internal fragment-1 (B. garinii, 50 μg/mL), p41-internal fragment-2 (B. afzelii, 100 μg/mL), p18 (B. afzelii, 100 μg/mL), and p100 (B. afzelii, 100 μg/mL). These concentrations were established in pilot studies to be neither cytotoxic nor mitogenic for human lymphocytes (data not shown).

2.2. Patient samples

Blood was submitted in citrate phosphate dextrose adenine (CPDA) monovettes (Sarstedt, Nümbrecht, Germany) from cooperating practices and sent to arrive in our laboratory within 24 h of drawing. Peripheral blood mononuclear cells (PBMCs) were isolated on Ficoll–Histopaque (Sigma-Aldrich Chemie, Taukirkhe, Germany) immediately upon arrival in the laboratory and either used in LTT-MELISA® directly or stored in 20% medium (RPMI-1640 containing HEPES [Life Technologies, Karlsruhe, Germany], 8 mg/L gentamicin [Sigma-Aldrich Chemie], 2 mmol/L L-glutamine [Biochrom seromed, Berlin, Germany], and 20% pooled, heat-inactivated human AB serum [Cambrex Bio Science Verviers S.p.r.l., Verviers, Belgium]) overnight at 4 °C.

For initial screening, PBMCs from 244 consecutive patients (102 male, 142 female; average age, 49 years; range, 2–80 years) with suspicion of Lyme infection and/or Lyme disease, with or without prior antibiotic therapy, were tested in LTT-MELISA® against 4 to 8 different recombinant Borrelia antigens. Nearly all samples were

### Table 1

Examples of applications of LTT to LB in the 1990s

<table>
<thead>
<tr>
<th>Publication</th>
<th>No. of samples</th>
<th>No. of antigens</th>
<th>Type of antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dattwyler et al. (1988)</td>
<td>35</td>
<td>1</td>
<td>Whole B. burgdorferi</td>
</tr>
<tr>
<td>Dressler et al. (1991)</td>
<td>178</td>
<td>3</td>
<td>Whole/sonicated B. burgdorferi</td>
</tr>
<tr>
<td>Krause et al. (1991)</td>
<td>74</td>
<td>1</td>
<td>Whole B. burgdorferi</td>
</tr>
<tr>
<td>Yoshinari et al. (1991)</td>
<td>24</td>
<td>6</td>
<td>Polypeptide fractions</td>
</tr>
<tr>
<td>Zoschke et al. (1991)</td>
<td>26</td>
<td>1</td>
<td>Whole B. burgdorferi</td>
</tr>
<tr>
<td>Krause et al. (1992)</td>
<td>61</td>
<td>3</td>
<td>Whole B. burgdorferi + 2 recombinant antigens</td>
</tr>
<tr>
<td>Schempp et al. (1993)</td>
<td>12</td>
<td>2</td>
<td>Whole/sonicated B. burgdorferi</td>
</tr>
<tr>
<td>Roessner et al. (1994)</td>
<td>12</td>
<td>4</td>
<td>Whole/sonicated B. burgdorferi + 3 recombinant antigens</td>
</tr>
<tr>
<td>Breier et al. (1995)</td>
<td>44</td>
<td>1</td>
<td>Whole B. burgdorferi</td>
</tr>
<tr>
<td>Hupertz et al. (1996)</td>
<td>103</td>
<td>2</td>
<td>Whole B. burgdorferi</td>
</tr>
<tr>
<td>Rutkowski et al. (1997)</td>
<td>16</td>
<td>1</td>
<td>Whole B. burgdorferi</td>
</tr>
</tbody>
</table>

* Includes patients and controls.
from patients residing in Germany; 5 were from patients in England. For these preliminary tests, the conventional cutoff (stimulation index [SI] ≥ 3 in at least 1 reaction, see herein below) was applied. Based on the results obtained, 4 antigens were selected for all subsequent analyses: OspC (B. afzelii), p41-1, p41-2, and p100.

Serologic data were available from 157 of these 244 patients and were either provided by the physician or determined in our Department of Serology with the ELISA screening assay Enzygnost® Borrelia for IgG and/or IgM (Dade Behring Marburg, Marburg, Germany) and the confirmatory recomBlot Borrelia IgG/IgM (MIKROGEN).

Healthy controls were 30 laboratory coworkers with no recent history of tick bites, no clinical suspicion of Lyme disease, and seronegative in both ELISA and Western blot. These probands (5 male, 25 female; average age, 43 years; range, 22–60 years) were tested once or more than once to the 4 selected recombinant Borrelia antigens, each in 3 dilutions.

Replicate tests from 68 randomly selected patients were performed on the same day by the same or by 2 different technicians to assess reproducibility.

Finally, to analyze the kinetics of LTT-MELISA® reactivity after antibiotic therapy, 1 or more follow-up blood samples were obtained from 54 initially reactive symptomatic patients (22 male, 32 female; average age 50 years, range 11–74) at various time points after completion of the therapy.

2.3. LTT-MELISA® for LB

The LTT-MELISA® was performed essentially as described in detail for detecting metal sensitivity (Valentine-Thon and Schiwara, 2003) with 1 major modification: instead of metal solutions, the 24-well cell culture plates were precoated with recombinant Borrelia antigens in 50 µL/well medium without human serum. The coated plates were wrapped in parafilm and stored at −20 °C until use.

Briefly, 1 x 10^6 PBMCs (monocyte-reduced by plastic adherence) in 1 mL of 10% medium were pipetted into the wells of a 24-well cell culture plate precoated with Borrelia antigens in 3 dilutions each and incubated for 5 days at 37 °C with 5% CO₂. Three negative controls (lymphocytes in 10% medium without antigen) and 1 positive control (lymphocytes in 10% medium plus 2 µg/mL pokeweed mitogen; Sigma-Aldrich Chemie) were included on each plate. On day 5, the cells were pulsed for 5 h with 3 µC methyl-[^3]H-thymidine (Amersham Buchler, Brunswick, Germany; specific activity, 185 GBq/mmol) and the radioactivity measured in a liquid scintillation counter (1450 Microbeta TriLux; Wallac Distribution, Freiburg, Germany). Counts per minute (cpm) were converted to an SI representing the cpm in the test well divided by the average cpm in negative control wells. Conventionally, a cutoff of SI ≥ 3 in at least 1 well defined a positive patient; SI ≥ 10 was considered “strong positive”.

For quality control, cells from the 5-day cultures were additionally analyzed morphologically after staining cyto-spin preparations with rapid differential hematology staining solutions (Dade Behring Marburg) to confirm the presence of lymphoblasts in positive results and of viable small lymphocytes (noncytotoxicity and nonmitogenicity) in negative results. Valid results were those showing an average background proliferation of <3000 cpm, a strong positive control response of SI >30, and a morphologic confirmation.

3. Results

3.1. LTT-MELISA® reactivity in 244 patients

Ninety (36.9%) of the 244 patients initially tested were positive to 1 (n = 39) or more (n = 51) recombinant Borrelia antigens. Frequency (%) and strength of the responses (average SI ± SD) to the individual antigens varied: OspA (6.5, 3.5 ± 0.3), OspC B. garinii (2.2, 10.6), OspC B. afzelii (13.5, 14.6 ± 22.6), p41 (10.2, 8.7 ± 7.1), p41-1 (29.2, 7.0 ± 4.5), p41-2 (5.0, 7.7 ± 4.5), p18 (11.0, 4.9 ± 2.3), and p100 (13.5, 4.9 ± 2.1). Based on these results, OspC (B. afzelii) was selected as a marker of early infection (Jauris-Heipke et al., 1993b; Wilske et al., 1993a; Robinson et al., 1993) for all further testing.

Of the 90 LTT-MELISA®–positive patients, 56 of the 65 (86.2%) for whom serology was available were positive for antibodies to Borrelia in ELISA and/or Western blot, confirming at least a prior infection with B. burgdorferi. All
of those for whom clinical data were available \( n = 35 \) showed current symptoms suggestive of Lyme disease, primarily EM, joint pain, and chronic arthritis. Nine LTT-MELISA\(^*\)-positive patients were seronegative: 7 showed symptoms of Lyme disease; 1 was asymptomatic but had had a tick bite 12 days before testing, was treated with antibiotics, and became LTT-MELISA\(^*\) negative upon follow-up testing; and 1 reported no clinical data.

Of the 154 (63.1\%) LTT-MELISA\(^*\)-negative patients, fewer (70.7\%) were seropositive (65/92 for whom serology was known). Of those for whom clinical symptoms were reported (87), 58.6\% were currently asymptomatic (i.e., had spontaneous- or treatment-resolved LB) and 41.4\% presented with uncharacteristic symptoms (headache, fatigue, joint and muscle pain, eczema, facialis paresis, burnout syndrome).

### 3.2. LTT-MELISA\(^*\) reactivity in healthy controls

Most seronegative healthy control subjects were repeatedly negative in LTT-MELISA\(^*\) to the 4 recombinant *Borrelia* antigens tested (Table 2). Three individuals responded once weakly positive (SI = 3.73, 4.43, 6.32) to a single dilution of 1 antigen, and 1 individual responded weakly (SI = 3.91, 3.92) to 2 antigens in 1 dilution each. Based on these results, a new more rigorous working cutoff was defined as follows: all results of SI \(< 3\); patient is “negative”; a single reaction of SI \(\geq 3\); patient considered “equivocal”; multiple reactions of SI \(\geq 3\) (i.e., for more than 1 dilution of a single antigen or for more than 1 antigen): patient is “positive”; multiple reactions of SI \(\geq 10\); patient is “strong positive”. Applying this new cutoff, only 1 control subject was positive, and the specificity was therefore 96.7\%.

### 3.3. Reproducibility

Ten patients were concordant positive (new cutoff) and 53 patients concordant negative for a concordance rate of 92.6\% (63/68). In the 5 discordant patients, 9 of 11 positive discordant values were in a low-positive range (SI = 3.32–4.94); no discordant result showed a “high positive” value of SI \(\geq 10\).

All 5 discordant patients had possible current or resolving LB based on recent tick bites followed by clinical symptoms (\( n = 4 \)), borderline serology (\( n = 2 \)), or strong concordant positive results in previous LTT-MELISA\(^*\) tests before therapy (\( n = 1 \)).

### 3.4. Longitudinal case studies

One or more blood samples could be obtained from 32 LTT-MELISA\(^*\)-positive (new cutoff) patients with clinical suspicion of active LB at various time points after antibiotic therapy. All showed a significant reduction of lymphocyte reactivity, almost always correlating with clinical improvement (Table 3). In 25 patients, reactivity to all antigens became negative (typical cases shown in Fig. 1A–C). The time to achieving complete negativity varied from “during therapy” to up to 1 year, usually 4 to 8 weeks, after completion of treatment. In the remaining 7 patients, reactivity was still positive to some antigens but markedly lower than before therapy (typical cases shown in Fig. 1D, E).

Similarly, 17 of 22 LTT-MELISA\(^*\) “equivocal” symptomatic patients with suspicion of active LB became negative after therapy (typical case shown in Fig. 1F); 5 remained unchanged.

### 4. Discussion

The present study describes the development, specificity, reproducibility, and clinical relevance for LB of a novel LTT based on 2 improvements. First, a standardized validated LTT was applied. Developed in the 1980s, the MELISA LTT modification has since been shown to improve the sensitivity and specificity of conventional LITTS for detecting metal sensitivity by utilizing a higher number of lymphocytes per well (\( 1 \times 10^6 \)), preselected nonmitogenic pooled human serum, nonmitogenic and noncytotoxic concentrations of antigens, and reduced numbers of monocytic cells (Stejskal et al., 1994, 1999; Valentine-Thon and Schiwara, 2003). Furthermore, quality control is enhanced by supplementing the radiologic with a morphologic analysis of proliferating cells to reduce possible false-positive or false-negative results (Stejskal et al., 1999). Its technical validity and clinical relevance for detecting and monitoring immunologic sensitization to drugs, medications, and heavy metals have been well documented (Stejskal et al., 1986, 1990, 1994, 1996, 1999; Tibbling et al., 1995; Stejskal, 1997; Sterzl et al., 1999; Regland et al., 2001; Valentine-Thon and Schiwara, 2003; Prochazkova et al., 2004; Kakuschke et al., 2005). The present study describes the 1st application of LTT-MELISA\(^*\) to detect cellular immune reactivity to infectious organisms.

In the 2nd improvement, only well-characterized recombinant *Borrelia* antigens used in commercial serologic tests were used. In most previous studies, the antigenic source was bacterial lysates or culture supernatants (Table 1). Besides containing known mitogenic substances (e.g., bacterial lipopolysaccharides) and multiple antigenic epitopes, such sources exclude the in vivo expressed antigens recently demonstrated to improve the serologic diagnosis of *Borrelia* infections (Wilske et al., 1993a; Schulte-Spechtel et al., 2003; Wilske, 2003; Aguero-Rosenfeld et al., 2005). The use of recombinant antigens in LITTS, therefore, can reduce nonspecific background proliferation and at the same time improve the specificity and clinical relevance of the results. On the other hand, in 2 more recent studies using exclusively recombinant *Borrelia* antigens, specificity was improved but was still problematic (Bauer et al., 2001; Kalish et al., 2003). Apparently, the use of recombinant antigens together with an optimized LTT format is critical.

Because the 4 antigens selected in this study are derived from *B. afzelii* and *B. garinii* but do not include *B. burgdorferi sensu stricto*, their relevance for detecting LB...
in countries with low or nonexistent prevalences of *B. afzelii* and/or *B. garinii* (i.e., United States) is unclear. In 124 tests of patients outside Germany, 40 were reactive and included patients from England (*n* = 28), Luxembourg (*n* = 4), Switzerland (*n* = 2), France (*n* = 2), Austria (*n* = 1), Holland (*n* = 1), as well as the United States (*n* = 2) (data not shown). Results from the latter 2 patients support a potential diagnostic relevance even in the United States. Alternatively, these 2 seropositive patients (frequent travelers) may have become infected outside the United States. A study with LB patients from within the United States should clarify this important question.

Although most of the LTT-MELISA®–reactive patients among the 244 initially tested were seropositive and symptomatic, supporting a good correlation between cellular reactivity and both *Borrelia* infection and, apparently, active LB, 9 lymphocyte reactive patients were seronegative. Seven of these showed clinical symptoms characteristic of LB and may exemplify the controversial “seronegative LB patients” reported in previous LTT studies, in particular, in Dattwyler et al. (1988). Seronegativity may result from immunosuppression, delayed humoral response, sequestration of antibodies, or inadequate assay systems (Stanek and Strle, 2003; Wilske, 2003; Aguero-Rosenfeld et al., 2005). The latter is supported by our own observations that many ELISA-negative patients are, in fact, positive in Western blot (unpublished data, see also patients 1 and 5). The current 2-tiered assay system will fail to identify a *Borrelia* infection in such patients, because Western blot is generally performed (i.e., reimbursed!) only when ELISA is positive or equivocal. ELISA-negative persons with clinical suspicion of LB should, therefore, except in unequivocal EM, be tested in Western blot or, alternatively, in an optimized LTT.

Based on the results obtained with control persons, a new working cutoff was defined (i.e., patient positive only with multiple reactions of SI ≥ 3) in analogy to that applied for identifying beryllium sensitization with the beryllium lymphocyte proliferation test (Be-LPT) (United States Department of Energy, 2001; Stange et al., 2004). The high specificity obtained (96.7%) is in contrast to the poor specificity of most previous studies and underlines the relevance of a standardized LTT format with an appropriate cutoff in addition to recombinant antigens. “Equivocal” patients (only 1 reaction of SI ≥3) may be showing “false positive” or true very low-level specific cellular reactivity, and should be repeat tested. Repetitive “equivocal” results appear to support active LB. Patient 6 in this study, for example, had classic early stage LB (EM) with seroconversion, tested only “equivocal” (not positive) in 3 successive blood samples, and became LTT-MELISA® negative, seronegative, and asymptomatic after antibiotic therapy.

The good reproducibility rate of 92.6% based on replicate plate testing of 68 patients supports the reliability of the assay system. This rigorous approach (as opposed to testing replicate wells of the same plate) was deliberately designed to account for the many variations arising from the multiple manual steps in LTT-MELISA®. The 5 “discordant” patients showed low-level responses characterizing the “gray zone” typical of most laboratory tests. A similarly high reproducibility rate of 94% was reported for the LTT-MELISA® as applied to detecting metal sensitivity (Valentine-Thon and Schiwa, 2003), whereas comparable reproducibility data for other LTTs have been generally lacking. Recently, in an extensive analysis of Be-LPT results obtained from split samples, an interlaboratory agreement of up to 64.7% and intralaboratory agreement of up to 91.9% was reported (Stange et al., 2004).

Although the sensitivity of LTT-MELISA® could not be statistically evaluated in this study (appropriate samples for PCR or culture authentication were not available from most patients), the clinical relevance of this assay system for identifying patients who can benefit from antibiotic therapy was clearly demonstrated. The large majority of lymphocyte-reactive symptomatic patients showed a significant reduction in lymphocyte reactivity correlating with clinical improvement after 1 or 2 courses of treatment. The 7 patients still (albeit less) reactive (e.g., patients 4 and 5) may require more time for depletion of specific memory cells. Similarly, the 5 “equivocal” patients who remained unchanged may still respond with time, be therapy resistant, have reinfections, or have an alternative etiology for their clinical state. Although the kinetics of both humoral and cellular immunologic responses is clearly variable, T-cell reactivity appears to diminish more rapidly than antibody titers in most patients. Consequently, the assay system described here may provide an early marker of therapeutic success. At the same time, persistent lymphocyte reactivity after a 1st course of therapy (as seen in patients 2, 3, 4, and 5) may indicate the need for more intensive and/or alternative antimicrobial treatment. Reduced (but not negative) lymphocyte reactivity after therapy was reported in 3 cases by Breier et al. (1995) and in 6 cases by Krause et al. (1991), but not in most of the 39 cases described more recently by Vaz et al. (2001). Major methodological, protocol, and cutoff differences in the latter study probably account for this discrepancy.

Because of the complexity of lymphocyte proliferation assays and the controversy surrounding their use for diagnosing LB, we strongly recommend that the LTT-MELISA® described here, or comparable tests, be applied only in accredited laboratories with proven cell culture expertise. Such laboratories should establish their own working cutoff for defining a positive patient, perform rigorous reproducibility testing at regular intervals for quality control, and interpret the results in conjunction with available anamnestic, clinical, and serologic data. Under these conditions, LTT-MELISA® or equivalent LTTs should have diagnostic relevance for clarifying serologically and clinically ambiguous cases of LB and, where appropriate, in confirming therapeutic success.
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