Evaluation of a new microspot array for detection of IgG and IgM antibodies to Treponema pallidum in human serum samples

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INTRODUCTION

Treponema pallidum is the causative agent of Syphilis, a sexually transmitted globally spread disease in humans with increasing incidences in western industrial nations within the last 15 years. Laboratory diagnosis of Syphilis is performed by detection of T. pallidum specific antibodies in serum, plasma or liquor. A two-step algorithm comprising a first screening and a subsequent confirmatory test is recommended.

Actually line immunoassays (LIA) employing recombinant antigen lines on nitrocellulose strips are the established confirmatory test method, although result interpretation and automation is not as standardized as ELISA.

METHODS

An array-format based spot immunoassay (SIA) in 96well-microtitration plates (SeraSpot® Anti-Treponema-4 IgG/IgM) was established for the detection of IgG / or IgM antibodies directed against T. pallidum specific antigens Tpp15, Tpp17, Tpp47 and TmpA in human serum samples. Test specific controls are integrated in every array: A cut-off control, a negative control, and a sample control. Latter indicates the presence / absence of serum. Results are calculated in ratios via the staining intensities of the specific antigen spots relative to the cut-off spot.

TEST PROTOCOL

<table>
<thead>
<tr>
<th>Samples incubation</th>
<th>Conjugate incubation</th>
<th>Substrate reaction</th>
<th>Image analysis / data evaluation</th>
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</thead>
<tbody>
<tr>
<td>30'</td>
<td>30'</td>
<td>7'</td>
<td></td>
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<tr>
<td>(per 96well plate)</td>
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Bound antibodies from samples are detected by HRP-labeled anti-human IgG / IgM and chromogenic precipitating substrate solution. Immune complexes are visualized as pale blue to dark blue spots. Developed arrays are scanned with Seramun SpotSight® plate scanner and evaluated by software Seramun SpotSight® scan.

Samples: 1）Samples of patients with a positive IgG (n=232) and IgM (n=203) T. pallidum serology. 2）Healthy blood donors (n=382)

Kits: SeraSpot® Anti-Treponema-4 IgG/IgM (SP-010-4G, SP-010-4M, Seramun Diagnostica GmbH, Heidesee)

RESULTS

1. The sensitivity for the detection of IgG and IgM antibodies to T. pallidum antigens was determined with 98.6 % and 96.8 % resp. for the SIA in comparison to the referenced LIA. All SeraSpot® Anti-Treponema-4 IgG / IgM negative samples were retested by a 2nd commercially available LIA, leading to amended sensitivities of 98.6 % and 97.9 % resp..

2. The investigation of 382 healthy blood donor samples resulted in a specificity of 100 % for IgG and 97.9 % for IgM detection, resp.. The IgM positive samples were retested by a 2nd commercially available LIA, leading to an amended specificity of 98.7 %.

3. The coefficient of determination (r²) for correlation between manual assay processing and automated Dynex® DS2 assay processing with a selection of n = 96 (IgG) and n = 64 (IgM) samples was calculated with r² > 0.97 for IgG and r² > 0.96 for IgM detection.

4. The reproducibility of the spot immunoassay was verified via the calculation of intra-assay, inter-assay and inter-batch coefficients of variation (CV) with ≤ 10 %, ≤ 15 % and ≤ 20 % resp. for detection of IgG or IgM antibodies to the different antigens.

CONCLUSION

The SeraSpot® Anti-Treponema-4 IgG / IgM spot immunoassay is a reliable test system for the serological detection of T. pallidum infections and suitable for application under routine laboratory conditions. Both the assay procedure and data evaluation are easy to perform. In addition, the assay is very economical with regard to sample and reagent consumption. Due to the advantage of automation using standard ELISA technique the new method is superior to commonly used line immunoassays.