Development and evaluation of a monoclonal antibody inhibition enzyme linked immunosorbent assay to diagnose syphilis

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SUMMARY A highly specific inhibition enzyme linked immunosorbent assay (ELISA) using murine monoclonal antibodies to treponemes has been developed to diagnose syphilis. The monoclonal antibodies used in this study were reactive to antigens of both Treponema pallidum and Treponema pertenue and not to antigens of non-pathogenic treponemes. Inhibition of the binding of monoclonal antibody to the treponemal antigens was successful with serum antibodies of patients with syphilis in an inhibition ELISA using monoclonal antibodies raised against T. pallidum antigens with molecular weights of 42 and 47 kilodaltons. In contrast, the binding of monoclonal antibodies obtained by immunising mice with treponemal membrane protein TmpB, derived from recombinant DNA was not inhibited by serum antibodies from patients with syphilis. The sensitivity of the inhibition ELISA using monoclonal antibody against the 47 kilodalton T. pallidum antigen was 93% in 58 serum samples from patients with untreated syphilis. The sensitivity was 79% if the monoclonal antibody against the 42 kilodalton T. pallidum antigen was used. By a combination of the test results obtained in these two inhibition assays a sensitivity of 97% in the 58 serum samples from untreated patients and 64% in 64 from treated patients was obtained. The specificity of the inhibition ELISA performed with either monoclonal antibody was 100% in 500 serum samples from non-infected people. The specificity in 432 non-infected patients attending a sexually transmitted disease clinic was 98.8% for the monoclonal antibody against the 42 kilodalton antigen, 99.5% for the monoclonal antibody against the 47 kilodalton antigen, and 98.4% for the combined antibodies. The sensitivity and specificity of the inhibition ELISA using the combination of test results obtained by the application of the monoclonal antibodies against the 42 kilodalton treponemal membrane protein, TmpA, and against the 47 kilodalton T. pallidum antigen were comparable with those of the Treponema pallidum haemagglutination assay (TPHA) and the fluorescent treponemal antibody sorbent (FTA-ABS) test for diagnosing early untreated disease. The inhibition ELISA offers the potential for additional confirmation of early untreated syphilis. Its use for confirming late untreated syphilis is still under investigation. The test is highly specific for pathogenic treponemes and does not need sorbens.

Most infectious diseases lead to an immune response that elicits antibodies against different antigenic determinants of the causative organism. The range of determinants to which antibodies are raised is large and includes pathogen specific epitopes as well as determinants that are common to (un)related bacteria. The immune response to common antigens in patients with syphilis leads to non-specific reactions in diagnostic tests using Treponema pallidum subsp pallidum (T. pallidum) as antigen. In these serological tests a sorbent is therefore often used to remove serum antibodies that are not specific for T. pallidum.1-3 The effectiveness of such a sorbent is not well defined, which stresses the need for serological tests using well defined specific antigens. Immunoassays using purified antigens4 or recombinant proteins5-6 and immunoblotting techniques5 have been proposed as serological tests for syphilis without the need for a sorbent.

The application of monoclonal antibodies (MoAbs) to detect treponemal antibody may provide a highly specific tool for diagnosing treponematoses. Such a
Development and evaluation of a MoAb inhibition ELISA to diagnose syphilis

Patients, materials, and methods

**Antigens**

*T. pallidum* subsp. *pallidum*, *T. pallidum* subsp. *pertenue* (*T. pertenue*), and *T. phagedenis*, biotype Reiter, were used to evaluate the specificity of the different monoclonal antibodies used in this study. *T. pallidum* (Nichols strain) was cultured by serial passage in rabbit testes. Treponemes were extracted from the rabbit testicular tissue and subsequently purified by being urogafin density centrifuged, as described previously. Preparations of 10⁷ treponemes/ml in phosphate buffered saline (PBS) were stored at -70°C. *T. pertenue*, strain Gauthier, kindly provided by P. Hindersson, Copenhagen, was essentially prepared in the same way as *T. pallidum*. *T. phagedenis* was cultured in Brewer's thioglycollate medium with 10% (v/v) heat inactivated rabbit serum as described previously.

Before use in ELISA or immunoblotting experiments, the treponemes were suspended in TRIS buffer (0.01 mol/l TRIS hydrochloric acid, pH 7.4, and 0.15 mol/l sodium chloride) and submitted to ultrasonic treatment three times for 15 seconds each using a Branson sonifier at 20 kHz, 50 W. The sonicates were stored at -20°C until used.

**Serum samples**

Serum samples from 58 patients with untreated syphilis had been stored at -20°C. Fresh samples submitted for syphilis serology tests were obtained from 496 patients (with or without syphilis) attending the sexually transmitted disease (STD) clinic at the University Hospital in Rotterdam. Sixty four of the fresh samples submitted for syphilis serology tests, which were obtained from patients who had been treated for syphilis, were reactive in at least one treponemal test. Five hundred samples from non-infected people were obtained from blood donors attending the Rotterdam blood bank. The patients with syphilis were classified clinically and serologically as described previously. All samples were tested in the TPHA (Japan Lyophilization Company) and the FTA-ABS and VDRL tests.

To investigate the possible cross reactivity of samples from patients with other spirochetoses in the inhibition ELISA, six samples reactive in an immunofluorescence test to *Borrelia burgdorferi* and seven reactive in an ELISA to *Leptospira* strain Wijnberg, serotype *icterohaemorrhagiae*, were also tested in the inhibition ELISA (courtesy of Dr W J Terpstra, WHO/FHO collaborating centre for serology and research on leptospirosis, Amsterdam).

**Preparing and characterising monoclonal antibodies (MoAbs)**

Details of producing and selecting MoAbs have been described elsewhere. Hybridoma cultures of antibody to *T. pallidum* were selected by *T. pallidum* ELISA, as reported by Van Eijk et al. MoAbs C3E5, D6F7C6, and 1-14M1 were prepared from mice that had been immunised with *T. pallidum* and MoAbs 15, 21, 29, 30, and 34 were prepared from mice that had been injected with the 34 kilodalton recombinant DNA derived treponemal membrane protein TmpB.

The MoAbs were purified by adding saturated ammonium sulphate solution to the ascites fluids to make a final saturation of 50%, which was mixed for one hour at 4°C by slow rotation. The insoluble proteins were pelleted by being centrifuged for 10 minutes at 10 000 × g. The pellet was rinsed with 50% saturated ammonium sulphate and centrifuged again. The final pellet was dissolved in 10 mmol/l sodium phosphate buffer (disodium hydrogen and sodium dihydrogen phosphates and 0.01 mmol/l calcium chloride, pH 6.8) and dialysed overnight against the same buffer. Final purification was accomplished by
high performance liquid chromatography using a hydroxylapatite column (100 × 7.8 mm, LKB, Sweden). Up to 2-5 mg monoclonal antibody dissolved in 2 ml of a 10 mmol/l sodium phosphate buffer was loaded into the column. A 10 to 350 mmol/l sodium phosphate buffer gradient was run in 30 minutes at a flow rate of 1 ml/minute. The fractions that contained protein, as estimated by absorption at 280 nm, were collected and used for further study.

Monoclonal antibody was labelled with horseradish peroxidase according to the method of Nakane and Kawaoi.29 MoAbs were characterised with the FTA test, TPHA, and ELISA using T pallidum and T phagedenis, biotype Reiter, antigens. The titre of each undiluted MoAb in the T pallidum ELISA was defined as the MoAb dilution that showed half the extinction of the undiluted MoAb solution.

The suitability of the MoAbs for possible use in the inhibition ELISA was studied by inhibiting the binding of the conjugated MoAb by its homologous unlabelled counterpart that had been bound to the immobilised antigen. Binding of conjugated MoAb was inhibited by the unlabelled homologous MoAb in all cases except for D6F7C6, which was therefore excluded from further experiments.

SDS-PAGE AND WESTERN BLOTTING
Sonicated T pallidum, T pertenue, and T phagedenis were dissolved in 3% sodium dodecyl sulphate (SDS) by being heated in a boiling water bath for three minutes under reducing conditions using 2% 2-mercaptoethanol. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using 13% slab gels (17 × 12 cm) as described by Laemmli.30 Immunoblotting was performed to identify the components of the pathogenic and non-pathogenic treponemes to which the monoclonal antibodies were directed. Electrophoretic transfer of proteins to nitrocellulose was performed with a transblot apparatus (Biorad, USA) using the method of Burnette.31 After the proteins had been transferred, the nitrocellulose was cut into separate strips. Each strip was soaked in PBS containing 0-05% Tween 20 (polysorbate 20) (PBS-T) for 15 minutes and incubated for one hour with MoAb diluted 1:100 in PBS-T. After being rinsed in PBS-T for 10 minutes, the nitrocellulose strips were incubated for one hour with 1:1000 dilution of antibodies to mouse (RIVM, The Netherlands) or human (Nordic, The Netherlands) immunoglobulin labelled with horseradish peroxidase. The nitrocellulose strips were rinsed again, the substrate solution, which contained 0-6 mg/ml tetramethyl-benzidine, 2 mg/ml diocylsodiumsulphosuccinate and 0-015% (v/v) hydrogen peroxide in citrate/phosphate buffer (5 mmol/ml citric acid, 10 mmol/l disodium hydrogen phosphate, pH 5-0, and 25% (v/v) ethanol), was added, and they were incubated for 10 minutes.

TITRATION OF MONOCLONAL ANTIBODIES IN T pallidum ELISA
The optimum dilution of each monoclonal antibody labelled with horseradish peroxidase was assessed by titration in the T pallidum ELISA. For this purpose a microtitration plate precoated with T pallidum ultrasonicate was incubated for one hour with labelled MoAb in increasing dilutions. Ten minutes after adding 0-1 ml substrate solution (0-1 mg/ml tetramethylbenzidine, 0-009% hydrogen peroxide, and 0-11 mol/l sodium acetate, adjusted with citric acid to pH 5-5), the reaction was stopped by adding 3 mol/l sulphuric acid. The extinctions were measured and plotted against the appropriate dilution of the labelled monoclonal antibody.

INHIBITION ELISA
Preliminary experiments had shown that maximum inhibition of monoclonal antibody binding to the antigens by serum antibodies was achieved by incubating a low dilution of the patient’s serum before incubating the MoAb. The patient’s serum diluted to the same extent had also to be added to the MoAb solution during its incubation. To incorporate these conditions, the inhibition ELISA was performed as follows: 0-1 ml of T pallidum sonicate (2-7 × 10⁶ treponemes/ml PBS) was coated on to the wells of a microtitration plate (96 wells, Dynatech, Germany) by incubating the plates for one hour at 37°C. The plate was rinsed for 30 seconds in a continuous low pressure flow of rinsing buffer (TS-buffer containing 0-05% Tween 20). The patient’s serum diluted 1:5, 1:50, and 1:100 in PBS-T was then added to the wells and incubated for one hour at 37°C. The plates were rinsed with PBS-T and appropriately diluted test serum mixed with labelled MoAbs at the indicated working dilution was added to the wells and incubated for one hour at 37°C. The plates were rinsed with PBS-T, and 0-1 ml substrate solution was added to the wells and incubated. The reaction was stopped by adding 50 µl of 3 mol/l sulphuric acid. The extinction was measured with a multiscan (Titertek) at 450 nm.

Appropriate control samples were included in each assay. The reactivity of each MoAb was checked by incubating without serum the appropriate MoAb labelled with horseradish peroxidase. The specificity of the reaction was checked using alpha-fetoprotein labelled with horseradish peroxidase as a control on each plate.

Results
SPECIFICITY OF MONOCLONAL ANTIBODIES
To define the reactivity and specificity of MoAbs
reactive to *T. pallidum* antigens, eight MoAbs directed
to treponemal antigens of molecular weights 34, 42,
and 47 kilodaltons were investigated in the ELISA,
TPHA, FTA test, and western blot using *T. pallidum*,
*T. pertenu*, and *T. phagedenis* antigens.

Table 1 shows the results of evaluating the eight
monoclonal antibodies in the ELISA using *T. pallidum*
or *T. phagedenis* antigens and in the FTA test and the
TPHA using *T. pallidum* antigens. None of the MoAbs
reacted with *T. phagedenis* antigens in the ELISA. All
but one (1–14M1) reacted in the *T. pallidum* ELISA,
FTA test, and TPHA. MoAb 1–14M1 reacted in the *T.
pallidum* ELISA and TPHA, but not in the FTA test.

The reactivity of the MoAbs to *T. pallidum*,
*T. pertenu*, and *T. phagedenis* was analysed by immuno-
 blotting. Figure 1 shows the results of the immuno-
 blotting with *T. pallidum* antigens. MoAbs C3E5 and
D6F7C6 reacted with both *T. pallidum* and *T. pertenu*
antisgens of 47 kilodaltons, and MoAb 1–14M1 with 42
kilodalton antigens. MoAbs 15, 21, 29, 30, and 34
reacted with 34 kilodalton antigens of *T. pallidum*
and *T. pertenu*. None of the MoAbs was reactive to
*T. phagedenis* antigens in immunoblotting (results not shown).

**TITRATION OF MONOCLONAL ANTIBODIES**
A titration curve for each MoAb was constructed
using the *T. pallidum* ELISA. For MoAbs 15, 21, 29,
30, and 34 a maximum extinction plateau was main-
tained at dilutions of up to 1:800, for MoAbs C3E5,
D6F7C6, and 1–14M1 a plateau was maintained at
dilutions of up to 1:3200, 1:1600, and 1:800 respectively.
At higher dilutions a sharp decline in extinction
was noticed. The steepest part of these slopes
represented the optimum dilutions for inhibition
experiments. The working dilution for each MoAb was therefore
chosen where its titration curve started to slope. These
titration curves were highly reproducible. Several serum samples from patients
with syphilis in the inhibition ELISA using MoAbs at different dilutions
to confirm the correct working dilution were tested.
No improved inhibition was obtained at other
dilutions.

**DISCRIMINATION BETWEEN POSITIVE AND
NEGATIVE RESULTS IN THE INHIBITION ELISA**
The inhibition of MoAb binding by serum samples
from patients with syphilis compared with samples
from non-infected people was measured by evaluating
the test results in 20 serum samples from patients
with untreated syphilis, and 100 control samples. All
samples were tested at three different dilutions (1:5,
1:50, and 1:100).

Wide variation in the extinctions of samples with
little or no inhibitory capacity made it impossible to
correlate the test results to the extinction that was
obtained with the labelled MoAb alone. We initially

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**Table 1** Comparison of reactivity of monoclonal antibodies (MoAbs) in various tests using Treponema pallidum or *T. phagedenis*, biotype Reiter, antigens

<table>
<thead>
<tr>
<th>MoAb</th>
<th>Molecular weight (kilodaltons) of reactive antigen</th>
<th>ELISA titre</th>
<th>FTA</th>
<th>TPHA titre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>T. pallidum</em></td>
<td><em>T. phagedenis</em></td>
<td><em>T. pallidum</em></td>
</tr>
<tr>
<td>15</td>
<td>34</td>
<td>1/25600</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>21</td>
<td>34</td>
<td>1/800</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>29</td>
<td>34</td>
<td>1/800</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>30</td>
<td>34</td>
<td>1/800</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>34</td>
<td>34</td>
<td>1/800</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>C3E5</td>
<td>47</td>
<td>1/1600</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>D6F7C6</td>
<td>47</td>
<td>1/1600</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>1–14M1</td>
<td>42</td>
<td>1/51200</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

ELISA = enzyme linked immunosorbert assay. FTA = fluorescent treponemal antibody test. TPHA = *T. pallidum* haemagglutination assay.

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**Fig 1** Monoclonal antibody (MoAb) reactivity to Treponema pallidum on western blots (2.5 × 10⁹ treponemes per lane). Lanes 1 and 2 show reactivity of MoAbs C3E5 and D6F7C6, lane 3 shows reactivity of MoAb 1–14M1, lanes 4 to 8 show reactivity of MoAbs 15, 21, 29, 30, and 34 raised to the 34 kilodalton treponemal membrane protein B (TmpB) derived from recombinant DNA.
observed, however, that an increase of extinction of at least 0.5 occurred between the lowest and highest dilution of each serum sample from a patient with syphilis. Such an increase was not observed in any control serum tested. Samples showing an increase of extinction of at least 0.5, when diluted as stated, were therefore considered to be positive. In further experiments some of the samples from patients with syphilis showed an increase of extinction of less than 0.5. These samples also showed an extinction of less than 0.3 when diluted at the three different dilutions (fig 2). They were tested again at higher dilutions, when an increase of 0.5 was always observed. Such samples were considered to show strongly positive results in the inhibition ELISA.

Table 2  Results of the monoclonal inhibition ELISA with serum samples from patients with syphilis and non-infected people

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No of samples</th>
<th>MoAb 1–14M1</th>
<th>MoAb C3E5</th>
<th>Both MoAbs</th>
<th>TPHA</th>
<th>FTA-ABS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary syphilis</td>
<td>15</td>
<td>13 (87)</td>
<td>12 (80)</td>
<td>14 (93)*</td>
<td>14</td>
<td>93 (75)</td>
</tr>
<tr>
<td>Secondary syphilis</td>
<td>18</td>
<td>15 (83)*</td>
<td>18 (100)</td>
<td>18 (100)*</td>
<td>18</td>
<td>100 (100)</td>
</tr>
<tr>
<td>Early latent syphilis</td>
<td>25</td>
<td>18 (72)</td>
<td>24 (96)*</td>
<td>24 (96)</td>
<td>25</td>
<td>100 (100)</td>
</tr>
<tr>
<td>Non-infected blood donors</td>
<td>500</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0 (2)</td>
</tr>
</tbody>
</table>

*Three samples in these groups did not show 0.5 extinction but had ≤0.3 extinction.
†One sample did not show 0.5 extinction increase but had ≤0.3 extinction.
For meanings of abbreviated test names see table 1.

Fig 2  Reactivity (extinction at 450 nm) of monoclonal antibodies 1–14M1 (left) and C3E5 (right) in the inhibition ELISA with serum samples that gave (■—■) moderately positive results, ( △—△ ) strongly positive results, and (○—○) negative results in serological tests for syphilis.

INHIBITION OF MoAb BINDING BY SERUM SAMPLES FROM PATIENTS WITH SYphilis

All MoAbs were evaluated in the inhibition ELISA. None of the MoAbs directed against the 34 kilodalton TmpB were inhibited by serum from patients (results not shown). All MoAbs raised against the 34 kilodalton TmpB were therefore excluded from further investigations. Pronounced inhibition was observed with MoAbs C3E5 and 1–14M1.

Figure 2 shows the results of typical experiments with a serum sample strongly reactive in the inhibition ELISA (from a patient with secondary syphilis, TPHA 2+, FTA-ABS 3+, VDRL 1:4), a moderately reactive sample (from a patient with treated syphilis, TPHA 2+, FTA-ABS 3+), and a non-reactive sample using MoAb 1–14M1 or C3E5 labelled with horseradish peroxidase. The non-reactive serum sample showed high extinctions at all dilutions. When diluted fivefold the moderately reactive sample appreciably inhibited the binding of the labelled conjugate and resulted in low extinctions, but when diluted 100-fold this sample did not substantially inhibit the binding of labelled MoAb. The strongly reactive serum inhibited the binding of the labelled MoAb to a high extent, and low extinctions were observed for all three dilutions.

SENSITIVITY AND SPECIFICITY OF THE INHIBITION ELISA

Table 2 shows the results of testing 58 serum samples from patients with early syphilis and 500 from non-infected people in the inhibition ELISA, TPHA, and FTA-ABS test to assess the sensitivity and specificity of the inhibition ELISA. All 58 samples from patients with untreated early syphilis reacted in at least the FTA-ABS test. More samples from patients with secondary and latent syphilis reacted in the inhibition ELISA using labelled MoAb C3E5 than when MoAb 1–14M1 was used which resulted in sensitivities of 98% (mean 100% for secondary syphilis and 96% for latent syphilis) with C3E5 and 77% (mean 83% for secondary syphilis and 72% for latent syphilis) with 1–14M1. For primary syphilis, the inhibition ELISA
using MoAb C3E5 was less sensitive (80%) than with MoAb 1-14M1 (87%). In the entire group of untreated patients with early syphilis the sensitivity of the inhibition ELISA using labelled MoAb C3E5 only (93%) was comparable with that of the TPHA (98%) and the FTA-ABS test (100%). The combined results of both C3E5 and MoAb 1-14M1 inhibition ELISAs (97%) were also comparable with those of the TPHA and the FTA-ABS test.

The specificity of the inhibition ELISA using either MoAb (C3E5 or 1-14M1) for testing 500 samples from healthy people was 100%, which was comparable with the specificities of the TPHA (99.8%) and the FTA-ABS test (99.6%).

To detect possible cross reactivity of serum samples from patients with other spirochaetoses in the inhibition ELISA, we tested the six samples reactive in the *Borrelia burgdorferi* immunofluorescence test and the seven samples reactive in the *Leptospira icterohaemorrhagiae* ELISA. These 13 samples gave negative results in syphilis serology tests (results not shown). The six samples reactive in the *Borrelia burgdorferi* immunofluorescence test did not react in the inhibition ELISA using labelled MoAbs C3E5 or 1-14M1, but one out of seven samples reactive in the *Leptospira* ELISA also reacted in the inhibition ELISA using either labelled MoAb.

**Table 3  Reaction pattern in results of serological tests on serum samples submitted for syphilis serology**

<table>
<thead>
<tr>
<th>TPHA or FTA-ABS</th>
<th>Inhibition ELISA</th>
<th>No of samples</th>
<th>Patients with known treated syphilis</th>
<th>People without evidence of syphilis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-14M1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>22</td>
<td>22</td>
<td></td>
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<tr>
<td>Positive</td>
<td>Negative</td>
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<tr>
<td>Negative</td>
<td>Negative</td>
<td>425</td>
<td>425</td>
<td></td>
</tr>
</tbody>
</table>

See tables 1 and 2 for meanings of abbreviated test names.

**Discussion**

In the present study an inhibition ELISA for the serodiagnosis of syphilis using antitreponemal MoAbs is reported. Eight MoAbs were investigated for their suitability in the inhibition ELISA. Three MoAbs had been raised against *T. pallidum* organisms and five were raised against the 34 kilodalton recombinant protein, TmpB. All eight MoAbs were reactive in the *T. pallidum* ELISA, which indicated that the *T. pallidum* antigens used in the inhibition ELISA contained the epitopes to which the MoAbs were reactive. Furthermore, the MoAbs reacted in the TPHA and the FTA test with the exception of MoAb 1-14M1 in the FTA test. Although MoAb 1-14M1 was raised against the 42 kilodalton protein TmpA, which is associated with treponemal membrane, it did not react with presumed exposed treponemal surface antigens in the FTA test. As MoAbs directed to the 34 kilodalton TmpB were not inhibited by serum from patients with syphilis, they were not suitable for use in the inhibition ELISA. Homologous inhibition of labelled MoAb by its unlabelled counterpart was shown for all MoAbs except MoAb D6F7C6, which indicated that the labelling of D6F7C6 may have altered the structure of its antigen binding sites.

Regarding its specificity in people assumed to be healthy, the inhibition ELISA using MoAbs C3E5 and
1–14M1 was comparable with the specificities of the TPHA and the FTA-ABS test. In the 432 serum samples from patients attending the STD clinic, the specificity of the inhibition ELISA was lower. This may have been because incomplete disease histories were obtained from these patients, who were at high risk for acquiring STDs.

In patients with untreated syphilis the sensitivities of the inhibition ELISA were 79% using MoAb 1–14M1, 93% using C3E5, and 97% using both. The sensitivity of the inhibition ELISA using both MoAbs was therefore comparable with those of the TPHA and the FTA-ABS test. The use of MoAb C3E5 alone provided a test with 93% sensitivity. This, in combination with the test results obtained with MoAb 1–14M1, slightly enhanced the sensitivity for patients with primary syphilis, but should be confirmed by testing more serum samples.

The ideal inhibition assay takes advantage of a MoAb that is able to react at all stages of syphilis. Such a MoAb was not found in this study. Several possible explanations exist for the differences in reactivity between the MoAbs in the inhibition ELISA. The avidity of the MoAb may be higher than that of the polyclonal serum directed to the common determinant, which results in less inhibition. Serum antibodies reactive to the common determinant may be present in small amounts. Serum antibodies directed to the appropriate determinant may not be present, depending on the stage of syphilis.

The epitope to which the MoAb was reactive may possibly not be presented to the host immune system during natural infection. This possibility may apply to the MoAbs raised against the recombinant protein TmpB. The use of these MoAbs in the inhibition ELISA indicated that they could not be inhibited by serum from patients with syphilis. This could have been due to an antibody response of mice to epitopes of the recombinant treponemal protein, TmpB, that differed from the epitopes of the naturally occurring TmpB from treponemes. In a direct ELISA using TmpB as antigen, reactions were seen in only a limited number of serum samples from patients with untreated syphilis that gave positive results in other tests for syphilis, which indicated that certain patients do not produce antibodies to the 34 kilodalton protein TmpB (Schouls et al, submitted for publication). This confirms the poor antibody response to TmpB in man.

The use of more than one MoAb in the inhibition ELISA may overcome the disadvantages of using a single MoAb. As can be expected from this theoretical viewpoint, however, the specificity of the test would decline for each additional MoAb that is used. This was shown by combining the results of the inhibition ELISA using MoAbs C3E5 and 1–14M1 to screen a high risk population. In 64 serum samples from patients with treated syphilis that were not reactive in the VDRL but were reactive in at least one treponemal test, the sensitivity of the inhibition ELISA using MoAb 1–14M1 was appreciably less than the sensitivity using MoAb C3E5.

The fact that MoAb 1–14M1 was less effective in diagnosing treated syphilis than MoAb C3E5 agrees with the observation that the serum antibodies reactive to the 42 kilodalton TmpA derived from recombinant DNA showed a rapid decline in reactivity soon after the patients were treated.28

Technically, non-reactive serum samples showed high variation of extinction in the inhibition ELISA. This was circumvented by testing each serum sample at three different dilutions. The dilutions that were chosen were based on the limited number of samples tested. An improvement in future experiments may be the inclusion of dilutions higher than 1:100 of each serum specimen.

The application of MoAbs in an inhibition assay offers the opportunity to show antibody response to distinct epitopes. Although there is a considerable similarity between the antigenic structures of pathogenic treponemes, epitopes of different pathogenic treponemal species may differ. In future, monoclonal antibodies directed to such epitopes may provide an inhibition ELISA to differentiate between various pathogenic treponemes.

In conclusion, the inhibition ELISA using the combination of test results of two MoAbs directed to 42 and 47 kilodalton treponemal proteins may be a highly specific and sensitive test for diagnosing early untreated syphilis. Because of its high specificity, the inhibition ELISA may have potential for confirming early untreated syphilis. Furthermore, the inhibition ELISA forms a model for the study of a wider panel of monoclonal antibodies whose inhibition pattern may possibly give guidance to the stage of syphilis and treatment status.

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References

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