

# An IgM capture enzyme linked immunosorbent assay to detect IgM antibodies to treponemes in patients with syphilis

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**SUMMARY** A new IgM capture enzyme linked immunosorbent assay (ELISA) was compared with the 19S(IgM) fluorescent treponemal antibody absorption (19S(IgM)FTA-ABS) test for detecting IgM antibodies to treponemes. Serum samples from 180 people, 109 with various stages of untreated syphilis, 45 with treated syphilis, and 26 non-infected, were investigated.

In all diagnostic groups of syphilis the reactivity of the IgM capture ELISA was similar to that of the 19S(IgM)FTA-ABS test except in untreated neurosyphilis, for which the IgM capture ELISA was significantly less sensitive. The IgM capture ELISA was very sensitive in congenital (100%, 5/5) and primary (82%, 18/22) syphilis, but less sensitive in secondary (60%, 12/20), latent (53%, 16/30), neurosyphilis (34%, 11/32), and treated (11%, 5/45) syphilis.

False positive IgM capture ELISA results were not found in five people who gave false positive Venereal Disease Research Laboratory (VDRL) reactions or in 21 neonates born to mothers adequately treated for syphilis before or during pregnancy. This indicated that the IgM capture ELISA was very specific.

The course of antitreponemal IgM reactivity after treatment of early infectious syphilis was followed up in six patients. The quantity of IgM antibody declined in nearly all patients after treatment, but still remained detectable in five patients up to six months after treatment. In contrast, non-treponemal antibodies measured by the VDRL test disappeared in four out of six patients within five months from starting treatment.

In conclusion, the IgM capture ELISA may be useful for easy and sensitive detection of IgM antibodies to treponemes in patients with congenital and primary syphilis. A positive test result in these cases indicates that patients should receive treatment if they have not been treated recently. The test is not, however, recommended to replace the VDRL test to monitor patients treated for syphilis.

IgM class antibodies to treponemes are usually the first to be produced during treponemal infection. IgG class antibodies appear rapidly thereafter, and soon become predominant.<sup>1</sup> Testing for antitreponemal IgM has been thought to be useful for detecting active disease early,<sup>2</sup> evaluating treatment,<sup>1,3</sup> and screening for congenital syphilis in neonates.<sup>4</sup>

Some investigators<sup>2,5</sup> have indicated a need to separate the IgM fraction from serum before testing, to avoid false negative results caused by IgG masking IgM and to avoid false positive results caused by rheumatoid factor. Several methods to accomplish this have been described. They include ultracentrifugation,<sup>1</sup> ion exchange,<sup>6</sup> size exclusion,<sup>5</sup> and affinity chromatography.<sup>6</sup> These techniques are used to separate antitreponemal IgM before testing by immunofluorescence or haemagglutination.

In The Netherlands the 19S(IgM) fluorescent treponemal antibody absorption (FTA-ABS) test, using size exclusion chromatography to isolate IgM

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antibodies, is generally accepted as the standard procedure for detecting antitreponemal IgM. Application of this test is, however, limited by the need for special equipment and by technical complexity. Technically simpler methods suitable for detecting antitreponemal IgM routinely have been proposed. They include the IgM solid phase haemadsorption assay (SPHA),<sup>7</sup> the enzyme linked immunosorbent assay (ELISA) to detect antiaxial filament IgM (IgM-ELISA),<sup>2</sup> and the *Treponema pallidum* specific IgM haemagglutination assay (TP-IgM-HA).<sup>8</sup> Because of doubts raised about the sensitivity of the SPHA<sup>9</sup> and the lack of commercially available reagents for the IgM-ELISA and the TP-IgM-HA, these tests have never been generally accepted as suitable for detecting IgM antibodies to treponemes.

In the present study we compared a recently introduced IgM capture ELISA (Captia Syphilis-M; Mercia Diagnostics, Guildford, England) with the 19S(IgM)FTA-ABS test to detect IgM antibodies to treponemes in patients with treated or untreated syphilis.

#### Patients, materials, and methods

##### SERUM SAMPLES

Serum samples from 154 patients with syphilis were obtained from the sexually transmitted disease clinic at the University Hospital, Rotterdam, and the Department of Neurology, the Free University, Amsterdam. One hundred and nine of these patients were untreated. The samples from the 45 patients treated for syphilis were collected at various intervals after the start of treatment. The diagnosis and classification of syphilis was made according to criteria described previously.<sup>10</sup> All samples were reactive in at least the FTA-ABS test.

Consecutive samples from five patients with primary syphilis and one patient with early latent syphilis, which were collected at the start of treatment and at various intervals after treatment, were used to evaluate IgM antibody to treponemes after treatment. The samples were tested in the same run by the IgM capture ELISA and the Venereal Disease Research Laboratory (VDRL) test, and on the same day by the 19S(IgM)FTA-ABS test. The serum samples of all patients were reactive in the FTA-ABS and VDRL tests at the start of treatment. In addition samples from five patients with solitary false positive VDRL test reactions were used. Samples from five neonates with clinical signs indicative of congenital syphilis were obtained from the Baragwanath Hospital in Johannesburg. Samples giving positive results in the *T pallidum* haemagglutination assay (TPHA) and the FTA-ABS test were obtained from 21 neonates without clinical signs indicative of syphilis who attended

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All serum samples were tested using the TPHA (Japan Lyophilisation Company, Japan), the FTA-ABS test,<sup>11</sup> the 19S(IgM)FTA-ABS test, the VDRL test,<sup>11</sup> and the antitreponemal IgM capture ELISA. Rheumatoid factor was detected by a latex agglutination test (Rapitex, Behring Werke, West Germany). All samples were stored at  $-20^{\circ}\text{C}$ .

##### 19S(IgM)FTA-ABS test

IgM was separated by gel filtration chromatography using high performance liquid chromatography (HPLC; LKB, Sweden). A 50  $\mu\text{l}$  volume of serum, prefiltered through a 0.2  $\mu\text{m}$  filter (Millex G-V, Millipore), was passed through a 7.5  $\times$  75 mm column (TSK GSWP; Toya Soda, Japan) that had been equilibrated with 0.25 mmol/l phosphate buffered saline (PBS), pH 7.2, at a flow rate of 1 ml/minute. The eluate was monitored at 280 nm, and fractions of 50  $\mu\text{l}$  were collected. The IgM fraction was partially excluded by the gel. The mean IgG contamination in the fraction containing IgM was less than 25 mg/l and the molar IgM/IgG ratio was 200-fold greater in favour of IgM than before fractionation. The IgM was diluted seven times. Concentrations of IgM and IgG were measured by a fluoroimmunoassay.<sup>12</sup>

The IgM fractions were investigated with an indirect immunofluorescent assay according to the protocol of the FTA-ABS test.<sup>11</sup> To minimise the risk of false positive and negative test results because of contaminating IgG, IgG was investigated separately using the FTA test with the appropriate anti-IgG conjugate. In all cases except one, as indicated, no fluorescence was observed.

##### Antitreponemal IgM capture ELISA

IgM antibodies to treponemes were detected by a capture ELISA procedure using test kits that included microtitration plates coated with antihuman IgM, positive and negative control serum samples, dilution buffer, wash buffer, tracer complex, and substrate. The test was performed according to the manufacturer's instructions. Briefly, microtitre plate wells coated with rabbit antihuman IgM were incubated for one hour at 37°C with 100  $\mu\text{l}$  serum diluted 1/50 in dilution buffer. Control serum samples (positive at high and low titres and negative) were included in every experiment. After the wells had been emptied and washed five times with 300  $\mu\text{l}$  wash buffer, the plates were incubated for one hour with 100  $\mu\text{l}$  tracer complex diluted 1/20. The tracer complex consisted of *T pallidum* antigens, a biotinylated antiaxial filament monoclonal IgM antibody, and streptavidin conjugated with horseradish peroxidase (HRP). The monoclonal antibody appeared to be of the IgM class by radial immunodiffusion. The wells were washed again as mentioned

above, and 100 µl substrate was added. The reaction was stopped after 30 minutes by adding 25 µl of 2 mol/l sulphuric acid. The plates were read on a multiscan reader (Titertek) at 450 nm. The cut off point between positive and negative was set at the absorbance of the low titre positive control according to the manual supplied by the manufacturer. Serum samples showing an absorbance within about 10% of the low titre positive control were tested again.

**STATISTICAL ANALYSIS**

The sign test was used to compare the results of the different tests.

**Results**

**IGM ANTIBODIES TO TREPONEMES IN PATIENTS WITH UNTREATED SYPHILIS**

Serum samples from 109 patients with untreated syphilis were investigated with the IgM capture ELISA and the results compared with those of the 19S(IgM)FTA-ABS test (table 1). Both tests showed comparable sensitivity at all stages of untreated syphilis except for neurosyphilis. The sensitivity of the IgM capture ELISA was high in congenital (100%) and primary (82%) syphilis, but less in secondary (60%) and latent syphilis (53%). In patients with neurosyphilis the reactivity of the IgM capture ELISA was significantly lower (34%) than that of the 19S (IgM)FTA-ABS test (72%). Of the 12 samples from patients with neurosyphilis that were reactive in the 19S(IgM)FTA-ABS test but not the IgM capture ELISA, two had absorbance values within 10% below those of the low titre positive control. Retesting those samples did not alter the results. Fluorescence in the 19S(IgM)FTA-ABS test was strong in seven out of these 12 samples and weak in the remaining five. All 32 samples from patients with neurosyphilis gave negative results when tested for the presence of rheumatoid factor.

Table 1 *IgM antibodies to Treponema pallidum in serum samples from 109 patients with untreated syphilis comparing results in IgM capture ELISA with those in 19S(IgM)FTA-ABS test*

Diagnostic category of syphilis	No	No positive in:		Percentage agreement
		ELISA	FTA-ABS	
Primary	22	18	18	100
Secondary	20	12	14	80
Latent	30	16	18	87
Congenital	5	5	5	100
Neurosyphilis	32	11	23	63

ELISA = enzyme linked immunosorbent assay.  
FTA-ABS = fluorescent treponemal antibody absorption (test).

**IGM ANTIBODIES TO TREPONEMES IN PATIENTS WITH TREATED SYPHILIS AND NON-INFECTED PEOPLE**

Table 2 shows the incidence of IgM antibodies to treponemes in 71 serum samples from patients with adequately treated syphilis, people showing false positive VDRL reactions, and non-infected neonates who had positive treponemal reactions. The patients with treated syphilis were divided into two categories; those with positive treponemal test results and a positive VDRL reaction, and those with only positive treponemal test results. Of the 23 samples from patients with treated syphilis who showed positive VDRL reactions, three were positive in both the IgM capture ELISA and the 19S(IgM)FTA-ABS test and two were each reactive in one test only. Of the 22 samples from treated patients with a negative VDRL reaction, only one gave a positive result in the IgM capture ELISA. No evidence of rheumatoid factor was found in any of these serum samples. IgM antibodies to treponemes were not found in the five people who had false positive VDRL reactions. The 21 serum samples from neonates of mothers adequately treated for syphilis before or during pregnancy also gave negative results in both IgM tests. All neonates, however, gave positive results in the TPHA and the FTA-ABS test.

**COURSE OF REACTIVITY TO IGM ANTIBODIES TO TREPONEMES AFTER TREATMENT**

To assess the influence of treatment on reactivity in the IgM capture ELISA, consecutive serum samples from six patients with early infectious syphilis were investigated for up to 10 months after the start of treatment. The figure compares the results of the IgM capture ELISA with those of the 19S(IgM)FTA-ABS and

Table 2 *IgM antibodies to Treponema pallidum in serum samples from 45 patients with treated syphilis and 26 non-infected people comparing results in IgM capture ELISA with those in 19S(IgM)FTA-ABS test*

Diagnostic category and results of other tests	No	No positive in:	
		ELISA	FTA-ABS
Treated syphilis: TPHA, FTA-ABS, and VDRL positive	23	4	4
Treated syphilis: TPHA and FTA-ABS positive, VDRL negative	22	1	0
False positive VDRL	5	0	0
Non-infected neonates: TPHA and FTA-ABS positive	21	0	0

ELISA = enzyme linked immunosorbent assay.  
FTA-ABS = fluorescent treponemal antibody absorption (test).  
TPHA = T pallidum haemagglutination assay.  
VDRL = Venereal Disease Research Laboratory (test).

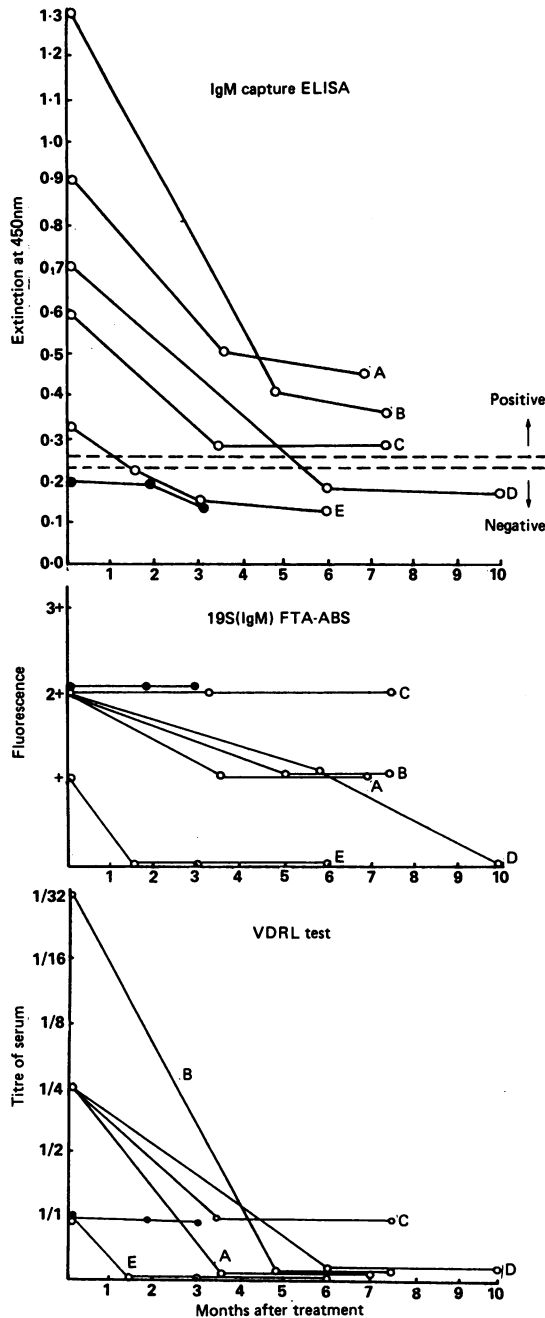


Figure Course of IgM reactivity in the IgM capture enzyme linked immunosorbent assay (ELISA), the 19S(IgM) fluorescent treponemal antibody absorption (FTA-ABS) test, and the Venereal Disease Research Laboratory (VDRL) test after treating five patients (A-E) with primary and one patient (●—●) with early latent syphilis.

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VDRL tests at various intervals after the start of treatment.

In all patients with primary syphilis the titre of IgM in both tests for IgM antibodies to treponemes declined as a result of treatment. After six months, however, three out of the six patients still gave positive IgM capture ELISA results and four gave positive 19S(IgM)FTA-ABS test results. All five patients with primary syphilis showed a positive VDRL reaction at the start of treatment, but five months after treatment only one still showed a positive result. A patient suffering from early latent syphilis was followed up for three months after treatment and was found to give negative results in the IgM capture ELISA and positive results in both the 19S(IgM)FTA-ABS and VDRL tests.

### Discussion

Treponeme specific IgM can most reliably be detected in the 19S(IgM) fraction after its separation from serum.<sup>2,5,7-9</sup> The 19S(IgM)FTA-ABS test is widely used to show treponeme specific IgM, but the time consuming operation of this method makes a more simple procedure desirable.

The IgM capture ELISA shares with other recently developed tests the specific procedure of IgM immune adsorption (the binding of IgM antibodies to a solid phase that has been coated previously with antibodies to IgM raised in animals.<sup>13</sup> However, detecting immune adsorbed IgM antibodies specific to treponemes using red blood cells sensitised to *T pallidum* antigens<sup>7</sup> or by ELISA<sup>2</sup> may be insensitive.<sup>13</sup> Although detecting adsorbed IgM by sensitised red blood cells is very specific, this technique is insensitive because the blood cells adhere insufficiently in the haemadsorption step.<sup>9,13</sup> In contrast to the SPHA, high sensitivity can be expected in the IgM capture ELISA because of the separation of IgM from IgG and the use of a biotin-avidin complex, which amplifies the reaction of antigen with antibody.

Regarding the specificity of a capture ELISA used to detect IgM, rheumatoid factor can cause false positive results because, bound to the anti-IgM antibodies coated to the solid phase, rheumatoid factor can directly bind the crystallisable fragment of the IgG class conjugate.<sup>2,14</sup> In the IgM capture ELISA false positive results are not likely to occur in this way because a tracer complex is used instead of an IgG class conjugate.

In early stages of syphilis the sensitivity of the IgM capture ELISA was 93% compared with the 19S(IgM)FTA-ABS test. In the case of neurosyphilis, however, the sensitivity of the IgM capture ELISA only reached 47% compared with that of the 19S(IgM)FTA-ABS test. In addition, the consecutive

serum samples from one patient with latent syphilis, which were collected at the onset of treatment and thereafter, did not react in the IgM capture ELISA although they showed strong fluorescence in the 19S(IgM)FTA-ABS test. Several explanations may be relevant for the differences in the sensitivity of the two tests in the patient with latent syphilis and those with neurosyphilis. Firstly, the immune response in the patients with latent syphilis could be directed against a limited range of *T pallidum* antigens. If this range did not include the *T pallidum* axial filament (32–39 kilodalton) components, to which the monoclonal antibody used in the IgM capture ELISA was directed, negative results could be expected. However, western blot analysis of IgM fractions from this patient did not show a different reaction pattern in the 32–39 kilodalton antigen range from that of a patient suffering from primary syphilis (results not shown). Performing the IgM capture ELISA on these serum samples using a monoclonal antibody directed to the 47 kilodalton antigen, which had been labelled by the manufacturer under the same conditions as the monoclonal antibody to axial filament, did not give positive reactions either. Secondly, the difference in sensitivity of the IgM capture ELISA and the 19S(IgM)FTA-ABS test could be due to the fact that some epitopes of *T pallidum* antigens first become immunogenic in late syphilis and that IgM antibodies raised to these epitopes may be detected in a immunofluorescence test and not in an ELISA, as has been pointed out by Pedersen *et al.*<sup>2</sup>

In our hands the IgM capture ELISA was less successful than the currently used VDRL test in monitoring the effect of treatment. We do not know the reason for this, but the serum of treated patients with syphilis may contain 8S(IgM) treponemal antibodies long after 19S(IgM) antibodies have disappeared.<sup>13</sup> Detecting 8S(IgM) antibodies by an IgM detection test may therefore mask the success of treatment as indicated by the absence of 19S(IgM) antibodies. The IgM capture ELISA probably cannot distinguish between 19S(IgM) and 8S(IgM) antibodies,<sup>13</sup> and therefore serum samples from which 19S(IgM) antibodies are absent but that still contain 8S(IgM) antibodies may react in the IgM capture ELISA.

In conclusion, the IgM capture ELISA in its present form can be a useful test in early stages of syphilis and for screening for congenital syphilis. It might, however, be possible to improve the test for late syphilis, especially neurosyphilis, by setting the cut off point between positive and negative results to a lower value. Treatment monitoring with the IgM capture ELISA is not very useful because IgM antibodies to

treponemes can be detected for longer after treatment than non-treponemal antibodies reactive in the VDRL test. The VDRL test is therefore able to detect the response to treatment more rapidly than a test detecting treponemal IgM. Finally, the absence of IgM antibodies to treponemes does not imply that syphilis is absent.<sup>2</sup>

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