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A Solid-Phase Indirect Haemadsorption Assay (SPIHA) for Detection of Immunoglobulin M Antibodies to Toxoplasma gondii: Application to Diagnosis of Acute Acquired Toxoplasmosis

Ein Indirekter Festphasen-Hämadsorptionstest (SPIHA) zum Nachweis von IgM-Antikörpern gegen Toxoplasma gondii: Anwendung in der Diagnostik akuter Toxoplasma-Infektionen

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Abstract

A solid-phase indirect haemadsorption assay (SPIHA) for the detection of immunoglobulin M (IgM) antibodies to *Toxoplasma gondii* is described. Polystyrol microtiter plates are coated with anti-human IgM (μ-chain-specific) antibodies and then sequentially allowed to react with patient's serum and sheep erythrocytes sensitized with soluble antigen of *Toxoplasma gondii*. A total of 111 sera were tested in fluorescent antibody test (FAT and IgM-FAT), complement fixation test (CFT), indirect haemagglutination assay (IHA), and in SPIHA. 47 sera were from individuals with a suspected or verified acute *Toxoplasma* infection. In most of these cases the SPIHA allowed a clear interpretation with respect to the status of the infection, even when the IgM-FAT was not conclusive. In contrast to IgM-FAT, rheumatoid factor or exceedingly high specific IgG antibodies did not interfere with results in SPIHA. A laboratory-acquired infection enabled us to demonstrate the course of immune response measured by SPIHA as well as by IgM-FAT, FAT, CFT, and IHA. The method proposed here is well appropriated to IgM detection, simple to perform, inexpensive, and thus representing an alternative to IgM-FAT, convenient for the routine laboratory.

Zusammenfassung

Ein Indirekter Festphasen-Hämadsorptionstest (SPIHA) zum Nachweis von IgM-Antikörpern gegen *Toxoplasma gondii* wird beschrieben. Der Test umfaßt 1. die selektive Adsorption der IgM-Antikörper aus dem Patientenserum an Mikrotiterplatten, die mit antihumanen IgM-Antikörpern beschichtet sind, und 2. die Reaktion der spezifischen IgM-

Antikörper mit Erythrozyten, welche mit Antigen von *Toxoplasma gondii* sensibilisiert sind. Insgesamt wurden 111 Seren im Indirekten Immunfluoreszenztest (FAT und IgM-FAT), in der Komplementbindungsreaktion (CFT), im Indirekten Hämagglutinationstest (IHA) und im SPIHA getestet. 47 Seren davon stammten von Personen mit einer vermuteten oder verifizierten akuten *Toxoplasma*-Infektion. In den meisten dieser Fälle erlaubte der SPIHA eine klare Aussage über den Status der Infektion, und zwar auch in jenen Fällen, in denen mittels IgM-FAT keine schlüssige Aussage möglich war. Im Gegensatz zum IgM-FAT konnte im SPIHA keine Beeinflussung der Ergebnisse durch Rheumafaktor oder durch hohe Titer spezifischer IgG-Antikörper festgestellt werden. Eine Laborinfektion ermöglichte uns die Beobachtung des Verlaufs der Immunantwort nach der Infektion, gemessen im SPIHA, IgM-FAT, FAT, CFT und IHA. Die hier dargestellte Methode ist für den IgM-Nachweis gut geeignet, sie ist einfach durchzuführen und außerdem auch billig. Alle für die Durchführung des Tests notwendigen Materialien sind im Handel erhältlich. Somit stellt diese Methode eine für das Routinelabor geeignete Alternative zum IgM-FAT dar.

Introduction

The detection of specific immunoglobulin M (IgM) antibodies is a well known tool for the diagnosis of a variety of acute infectious diseases. With respect to toxoplasmosis the detection of specific IgM and, hence, the identification of a recently acquired *Toxoplasma* infection may be important in three clinical situations (*Welch* et al., 1980): 1) in patients with debilitating or life-threating illness, 2) in patients with syndromes such as lymphadenopathy that must be distinguished from lymphoproliferative disorders, and 3) in pregnant women.

The most common serological tests for the detection of human IgM antibodies to *Toxoplasma gondii* use anti-human IgM conjugates in "indirect assays" such as fluorescent antibody test (FAT) (*Remington* et al., 1968), enzyme-linked immunosorbent assay (ELISA) (*Voller* et al., 1976), radioimmunoassay (RIA) (*Gehle* et al., 1976). However, false-positive reactions may be obtained in these tests due to the presence of rheumatoid factor (RF) (*Camargo* et al., 1972; *Hyde* et al., 1975) or antinuclear antibodies (ANA) (*Araujo* et al., 1971). Conversely, false-negative results or weakened positive reactions are likely to occur in sera presenting high concentrations of specific, competitive anti-parasite IgG antibodies which rapidly saturate the binding sites of the antigen and thereby inhibit the binding of specific IgM (*Cohen* et al., 1967; *Filice* et al., 1980; *Franco* et al., 1981; *Pyndiah* et al., 1979).

Recently several assays have been performed in toxoplasmosis serology making use of a solid-phase immunological entrapment of IgM: double sandwich IgM-ELISA (*Duermeyer* et al., 1980; *Naot* and *Remington*, 1980; *Remington*, 1982), IgM-immunosorbent agglutination assay (*Desmonts* et al., 1981; *Desmonts*, 1982), reverse enzyme immunoassay (*Franco* et al., 1981; *Walls* and *Franco*, 1982). These tests are described to be more sensitive and more specific than the IgM-FAT, although easy to perform and inexpensive.

Our aims were, firstly, to take advantage of the initial step of these assays in which at first the patient's IgM and subsequently the patient's anti-parasite IgM are selected; thus, interference of RF or ANA as well as antibody competition due to excess of specific IgG is hindered.

Secondly, we attempted to find a test system which is not so much time consuming and intensive in work as e.g. the double sandwich IgM-ELISA is.

For this purpose we intended to apply a haemadsorption technique (which has

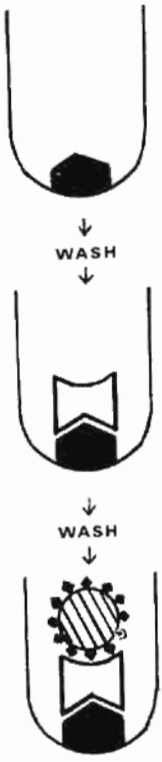
been described for detection of Rubella IgM antibodies by *Krech* and *Wilhelm*, 1979; *Van der Logt* et al., 1981, and for detection of *Treponema pallidum*-specific IgM by *Schmidt*, 1980) in toxoplasmosis serology.

Materials and Methods

Microtiter plates: disposable U-shaped polystyrol plates (Nunc, Kamstrup, Denmark).

Specific antiserum to human IgM: IgG fraction (μ-chain-specific) of rabbit anti-human IgM.

Specimen 1: commercially available antiserum to human IgM (Behringwerke, Marburg, FR Germany).



1) Anti-human IgM antibodies are fixed to the wells

2) Addition of human serum. (If IgM antibodies are present, adherence to anti-IgM molecules takes place. Non-IgM-serum components will be washed off)

3) Erythrocytes, sensitized with soluble antigen of *Toxoplasma gondii*, are added. (In case of anti-*Toxoplasma* IgM positive sera the sensitized erythrocytes are fixed to the specific antibodies and so uniformly distributed over the entire surface of the well. If no specific IgM antibodies are present, the sensitized erythrocytes drop down to the center of the well)

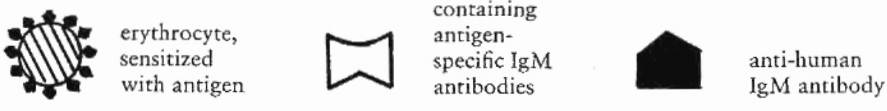


Fig. 1. Outline of the SPIHA procedure for detection of IgM antibodies to *Toxoplasma gondii*.

Specimen 2: highly purified antiserum, containing 90% immunoreactive antibodies to human IgM, kindly provided by Behringwerke.

Human sera: 92 sera of pregnant women, submitted to our laboratory for diagnosis in toxoplasmosis surveillance, 15 sera containing RF, and 4 sera containing ANA were tested in FAT, IgM-FAT, CFT, IHA, and in SPIHA.

Sera with rheumatoid factor were kindly supplied by Dr. *J. Kovarik,* II. Med. Univ. Klinik, Vienna; sera containing ANA were obtained from the II. Univ. Hautklinik, Vienna. Sera with IgM antibodies to Rubella virus were kindly provided by Prof. Dr. *Ch. Kunz,* Inst. f. Virologie, Univ. Vienna.

Rheumatoid factor was determined and adsorbed by latex agglutination test (Latex-RF Reagent, Behringwerke), consisting of an aqueous suspension of polystyrol particles coated with human IgG. ANA were detected by an indirect fluorescent antibody test, using glass slides coated with baby hamster kidney cells (Behringwerke).

Antigen: IHA-reagent, consisting of stabilized sheep erythrocytes sensitized with soluble *Toxoplasma* antigen (Cellognost Toxoplasmosis, Behringwerke).

Coating buffer: 0.08 M carbonate buffer, pH 9.6.

Washing buffer (PBS-T-BSA): phosphate-buffered saline (PBS), pH 7.2 + 0.05% Tween 20 (T) + 2% bovine serum albumine (BSA).

SPIHA procedure (Fig. 1): Wells of microtiter plates were coated with 50 μl of rabbit antiserum to human IgM diluted in 0,08 M carbonate buffer (coating buffer). After overnight incubation at + 4 °C, the plates were washed three times for 5 min each in PBS-T-BSA and shaken dry. 50 μl of the serum dilution (geometric dilution row in PBS-T-BSA) were added to washed wells. Plates were incubated for 2 h at + 37 °C and then washed again three times and shaken dry. Thereafter sequentially 50 μl of IHA-buffer solution, pH 8.1 (Behringwerke), and 10 μl of *Toxoplasma* IHA-reagent were added. The optimal dilution of the *Toxoplasma* IHA-reagent as well as the optimal dilution of the rabbit antiserum to human IgM was determined in preliminary block titrations.

Plates treated in the above described manner were covered and allowed to stand overnight at room temperature in a vibration-free place. For reading the results the sedimentation pattern of the erythrocytes was determined from below by means of a mirror.

In case of IgM positive sera the sensitized erythrocytes are fixed to the specific antibodies and uniformly distributed over the entire surface of the U-shaped well. In case of IgM negative sera the sensitized erythrocytes drop down to the center of the well and a clear ring formation can be seen.

Other serological tests: FAT and CFT: performed according to the recommendations of the Bundesgesundheitsamt of FR Germany (Bundesgesundheitsblatt, 1976).

IgM-FAT: performed according to *Remington* et al. (1968)

IHA: Cellognost Toxoplasmosis (Behringwerke).

Results

Optimal conditions for SPIHA: In preliminary experiments we found the concentration of erythrocytes and the quality of the anti-human IgM antibody to be critical for obtaining good results. A too small amount of erythrocytes used in the test led to false-positive or indeterminable results, whereas too many erythrocytes caused false-negative results. Therefore, the adequate concentration of erythrocytes should be defined by block titration.

Two specimens of anti-human IgM antibodies were examined for the test: 1) a commercially available, 2) a highly purified antibody. Only the latter showed satisfying results in SPIHA.

Specificity: Several controls were carried out to assure the specificity of the test system:

a) Anti-human IgM antibodies fixed to the plates and sensitized erythrocytes did not react nonspecifically in the absence of patient's serum.

b) When we used uncoated plates, no positive reactions occured with *Toxoplasma* IgM positive sera in SPIHA. This assured that patient's antibodies do not fix to microtiter plates nonspecifically during incubation time.

c) In order to exclude the possibility that antibodies of the IgG class are bound nonspecifically in the test system, a serum sample obtained from a patient with acute toxoplasmosis containing both specific IgM and IgG was diluted 10^{-3} in PBS and incubated for 1 h at 37 °C with different dilutions of anti-human IgM antibody (10^{-1}, 10^{-2}, 10^{-3}). After centrifugation for 10 min at 1000 g the serum was allowed to react in SPIHA.

Treatment of serum with 10^{-1} and 10^{-2} anti-μ antibodies resulted in negative reactions (all IgM antibodies had been precipitated), while serum pretreated with 10^{-3} anti-μ was still positive (not all IgM antibodies had been inhibited).

d) Four sera with IgM antibodies to Rubella virus and two sera with IgM antibodies to Echinococcus were tested in SPIHA to examine cross-reactivity to other acute infectious diseases. All sera gave negative results.

e) A control test with nonsensitized erythrocytes ran concomitantly on each plate at one serum dilution of each serum sample to check for nonspecific agglutinins.

Furthermore, a positive and negative control were performed on each microtiter plate. A pool of sera which were highly positive for *Toxoplasma* IgG but negative for IgM served as negative control. A serum sample from a patient with clinically proved acute toxoplasmosis served as positive control.

Reproducibility: 15 sera were tested several times on different days. The variation in titers observed for positive test sera was not greater than fourfold. Negative sera remained negative at any time.

Results of SPIHA in sera: We selected 92 RF-negative sera of pregnant women, submitted to our laboratory for diagnosis in toxoplasmosis surveillance. All sera were tested in FAT, IgM-FAT, CFT, IHA, and SPIHA and arranged in three groups: 1) sera of uninfected individuals, 2) sera of individuals with a chronic *Toxoplasma* infection, 3) sera of individuals with a suspected or verified acute *Toxoplasma* infection. Assignment to these groups was carried out according to serology as de-

Table 1. Results of FAT, IgM-FAT, CFT, IHA, and SPIHA in sera from uninfected individuals and individuals with chronic *Toxoplasma* infection

Source of sera	No. tested	No. of sera (titer or range of titers[1])				
		FAT	IgM-FAT	CFT	IHA	SPIHA
Uninfected individuals	12	12 (neg)	12 (neg)	12 (neg.)	12 (neg.)	12 (neg.)
individuals with chronic Toxoplasma infection	33	1 (16) 7 (64) 25 (256)	33 (neg.)	17 (neg.) 9 (5) 6 (10) 1 (20)	8 (64) 10 (256) 9 (1000) 6 (4000)	24 (neg.) 3 (16) 2 (32) 1 (64) 3 (256)

[1] reciprocal

Table 2. Results of IgM-FAT, SPIHA, and FAT in sera from individuals with a suspected or verified acute *Toxoplasma* infection

Serum No.	Titer[1]		
	IgM-FAT	SPIHA	FAT
1	256	2000	4000
2	256	8000	1000
3	256	8000	1000
4	256	4000	256
5	256	2000	256
6	64	1000	4000
7	64	16000	1000
8	64	16000	1000
9	64	8000	1000
10	64	4000	1000
11	64	4000	1000
12	64	8000	256
13	64	8000	256
14	64	4000	256
15	64	2000	256
16	64	2000	256
17	64	2000	256
18	64	2000	256
19	64	2000	256
20	64	2000	256
21	64	1000	256
22	64	16	64
23	16	2000	1000
24	16	4000	256
25	16	4000	256
26	16	2000	256
27	16	1000	256
28	16	128	1000
29	16	256	256
30	16	neg.	64
31	16	neg.	64
32	16	256	64
33	16	neg.	64
34	neg.	1000	4000
35	neg.	1000	4000
36	neg.	16	64000
37	neg.	neg.	4000
38	neg.	neg.	4000
39	neg.	256	4000
40	neg.	neg.	4000
41	neg.	16	1000
42	neg.	neg.	1000
43	neg.	256	1000
44	neg.	neg.	1000
45	neg.	neg.	1000
46	neg.	256	1000
47	neg.	neg.	1000

[1] reciprocal

scribed elsewhere (*Aspöck,* 1982; *Flamm* and *Aspöck,* 1981) or according to known clinical course of illness.

As can be seen in table 1 all sera obtained from uninfected individuals gave negative results in FAT, IgM-FAT, CFT, IHA, and SPIHA (FAT, IgM-FAT, IHA, and SPIHA titers below 1:16 were considered negative).

33 sera were from individuals with a chronic *Toxoplasma* infection (Table 1). 24 of these sera were negative in IgM-FAT and SPIHA as well. 9 sera yielded weakly positive titers in SPIHA, while IgM-FAT was negative (FAT, CFT, IHA titers: see Table 1).

47 sera were from individuals with a suspected or verified acute *Toxoplasma* infection (Table 2): 21 sera (No. 1–21) yielded coincidently high titers in IgM-FAT and SPIHA. One serum (No. 22) was 1:64 positive in IgM-FAT but only 1:16 positive in SPIHA. Five out of eleven sera with IgM-FAT titers of 1:16 (No. 23–33) were highly positive in SPIHA, while six sera yielded only low titers (up to 1:256) or were even negative. Two sera were found to be negative in IgM-FAT (No. 34, 35) but highly positive in SPIHA. 12 sera (No. 36–47) negative in IgM-FAT gave also negative or slightly positive results in SPIHA (CFT, IHA titers: not shown).

The WHO serum tested for reference was highly positive in SPIHA (1:2000) but only 1:16 positive in IgM-FAT. These findings are in accordance with those made by *Desmonts,* 1982 by means of the IgM-immunosorbent agglutination assay. The low IgM-FAT titer has been attributed to antibody competition.

Comparison of SPIHA and IHA in cases of acute toxoplasmosis: When we compared test results of SPIHA and IHA in sera from individuals with a recent history of toxoplasmosis (confirmed by IgM-FAT titers of 1:64 and 1:256; sera No. 1–22, Table 2) we could not find any correlation (Table 3). In SPIHA all sera but one yielded highly positive results, whereas most of the sera tested in IHA had titers of 1:64. However, we found six sera to be negative and some sera that were highly positive in IHA.

Results of SPIHA in sera containing RF or ANA: 19 serum samples that were positive for RF or for ANA, obtained from individuals with rheumatoid arthritis or systemic lupus erythematosus, were tested for *Toxoplasma* antibodies in FAT, IgM-FAT, CFT, IHA, and SPIHA (Table 4).

9 of the sera were found positive in IgM-FAT, while only one was positive in SPIHA. The positive reaction in SPIHA remained positive also after preadsorption

Table 3. Results of IHA and SPIHA in sera from individuals with an acute *Toxoplasma* infection

Source of sera	No. tested	Titer[1]	
		IHA	SPIHA
Individuals with recently acquired Toxoplasma infection	22	6 (neg.) 12 (64) 1 (256) 2 (1000) 1 (4000)	1 (16) 2 (1000) 8 (2000) 4 (4000) 5 (8000) 2 (16000)

[1] reciprocal

with RF latex particles. After treatment of the 9 IgM-FAT positive sera with IgG coated latex particles 8 became negative, the one serum positive in SPIHA remained positive in IgM-FAT as well.

Sera with ANA did not cause false-positive results, neither in IgM-FAT nor in SPIHA.

Immune response after a laboratory-acquired Toxoplasma infection, demonstrated in SPIHA, IHA, FAT, IgM-FAT, and CFT: During our studies for this report a laboratory-acquired *Toxoplasma* infection occured in our department. So we had the opportunity to examine the course of immune response measured by SPIHA in comparison with development of antibody levels in other tests (Fig. 2): Antibodies to *Toxoplasma gondii* could be detected one week after infection by FAT and IgM-FAT, and at a low level by SPIHA, while titers in CFT did not appear before the second week. IHA was the latest test that became positive.

Table 4. Results of serological tests in sera containing RF or ANA

Patient No.	RF[2]	ANA[3]	Serological test result – titer[1] FAT	IgM-FAT	CFT	IHA	SPIHA
1	pos.	neg.	64	16	neg.	64	neg.
2	pos.	neg.	256	16	neg.	256	neg.
3	pos.	neg.	neg.	neg.	neg.	neg.	neg.
4	pos.	neg.	64	16	neg.	64	256
5	pos.	neg.	64	16	5	64	neg.
6	pos.	neg.	64	neg.	neg.	64	neg.
7	pos.	neg.	256	64	neg.	64	neg.
8	pos.	neg.	neg.	neg.	neg.	neg.	neg.
9	pos.	neg.	64	neg.	neg.	64	neg.
10	pos.	neg.	neg.	neg.	neg.	neg.	neg.
11	pos.	neg.	256	16	neg.	64	neg.
12	pos.	neg.	64	16	neg.	64	neg.
13	pos.	neg.	256	64	neg.	1000	neg.
14	pos.	neg.	256	16	neg.	256	neg.
15	pos.	neg.	neg.	neg.	neg.	neg.	neg.
16	neg.	pos.	64	neg.	neg.	64	neg.
17	neg.	pos.	256	neg.	5	1000	neg.
18	neg.	pos.	neg.	neg.	neg.	neg.	neg.
19	neg.	pos.	64	neg.	neg.	64	neg.

[1] reciprocal
[2] positive titer = $\geqslant 20$
[3] positive titer = > 160

Discussion

In an effort to find an easy-to-perform IgM detecting assay for the routine laboratory which is both highly sensitive and specific, we developed a *Toxoplasma* IgM-SPIHA that requires less time to be performed than the double sandwich IgM-ELISA or the reverse enzyme immunoassay (although the important initial step,

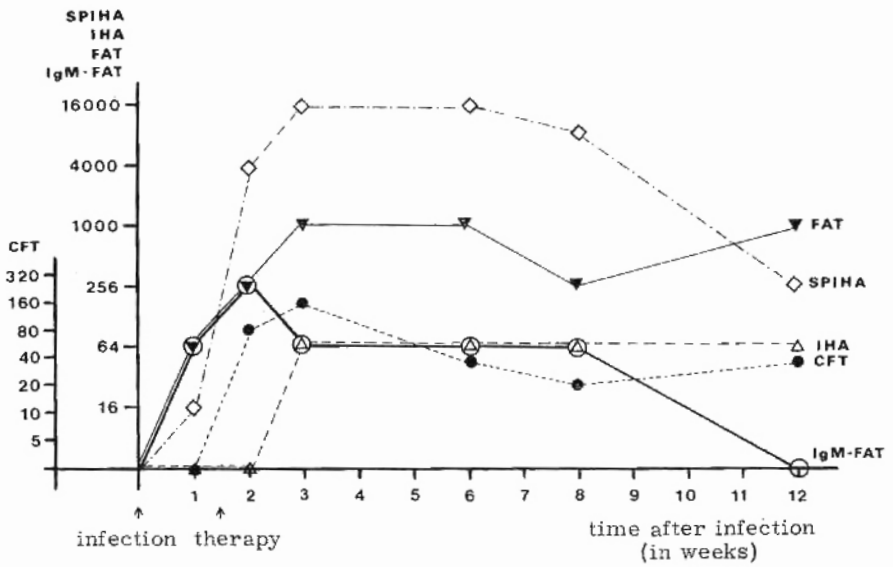


Fig. 2. Course of immune response after a laboratory-acquired *Toxoplasma* infection; serum samples tested in SPIHA, IHA, FAT, IgM-FAT and CFT (titers reciprocal).

the selective adsorption of human IgM antibodies onto microtiter plates precoated with anti-human IgM, is the same as in those tests). All reagents being necessary for the SPIHA procedure are commercially available. This represents a further advantage of the SPIHA compared with the double sandwich IgM-ELISA and the reverse enzyme immunoassay, as these tests require a parasite culture and antigen preparation. An additional peroxidase-labeling of the antigen is necessary for the reverse enzyme immunoassay. Yet the labeling of antigen is somewhat problematic: Contamination of the antigen by either mouse protein from animal-grown organisms or tissue cells from cultured organisms result in nonspecific reactions and in an increased background (*Walls* and *Franco*, 1982). As a second problem the use of such an impure and heterogeneous antigen involves the need of a high amount of expensive enzyme (*Walls* and *Franco*, 1982). Compared with the IgM-immunosorbent agglutination assay, the SPIHA allows a better evaluation of the test results due to a greater reading accuracy by the use of sensitized erythrocytes.

When we compared the SPIHA with the IgM-FAT we found the SPIHA to be specific, highly sensitive and well appropriated for IgM demonstration (Table 2): In 21 sera (No. 1–21) of individuals with a suspected or verified acute toxoplasmosis the test results obtained by IgM-FAT were confirmed by highly positive titers in SPIHA.

In one serum (No. 22) a rare combination of antibody titers was observed (1:64 in FAT and also in IgM-FAT, only 1:16 in SPIHA). This serum was obtained one week p.i. from a technician of our laboratory who had stuck herself into the finger with a needle contaminated with trophozoites of Toxoplasma gondii (clinical symptoms: fever, lymphadenopathy, headache). The SPIHA became highly positive not before the second week (Fig. 2).

This example demonstrates that we cannot rule out the possibility that a low titer in SPIHA may reflect an acute toxoplasmosis. Nevertheless, there is only a small chance that a low titer in SPIHA reflects an acute infection, as IgM antibodies rise quickly within two weeks after infection to a very high level (Fig. 2). On the other hand, IgM antibodies to *Toxoplasma gondii* are present at a low level for several months after the onset of the infection (*Braveny,* 1979; *Naot* et al., 1982).

The fact that the SPIHA gave a clearly positive reaction a few days later than the IgM-FAT might be due to the source of antigen: In IgM-FAT and FAT surface antigen is used, while in SPIHA, IHA, CFT, and ELISA soluble antigens are reactive to the antibodies. When *Ambroise-Thomas* et al. (1978) used a "whole mixed antigen" in IHA, they were able to detect antibodies in sera of acute infected individuals as early as in FAT. Therefore, we assume that the use of another type of antigen (e.g. whole mixed antigen) in SPIHA may also expedite the antibody demonstration.

In 11 sera (No. 23–33) the IgM-FAT was only 1:16 positive. IgM-FAT titers below 1:64 are not accepted as an indication for acute toxoplasmosis by many authors (*Aspöck,* 1982; *Braveny,* 1979; *Naot* and *Remington,* 1980) so that these sera could not be clearly identified by IgM-FAT. A convincing interpretation with respect to the onset of the infection was, however, possible by SPIHA; a SPIHA titer of $> 1:256$ was considered highly suggestive of recent infection.

Among the sera No. 34–47 the eventuality of a recent infection had to be taken into consideration on the basis of results obtained by FAT, although these sera were negative in IgM-FAT. In 12 of these sera the SPIHA gave negative or slightly positive reactions, thus confirming test results obtained by IgM-FAT. Therefore, the suspicion on recent infections could be dropped. In two sera (No. 34, 35), however, the IgM-FAT failed to detect IgM antibodies, whereas the SPIHA was highly positive. This test result – in correlation with highly positive titers in FAT and CFT – led us to the assumption that these sera gave false-negative results in IgM-FAT due to antibody competition. (Unfortunately, no more serum was available for further confirmation by chromatographic separation.)

In some sera from chronically infected individuals we were able to find low antibody titers in SPIHA but not in IgM-FAT (Table 1). This can be attributed to the higher sensitivity of the SPIHA and to the prolonged persistance of IgM antibodies in toxoplasmosis mentioned before.

In SPIHA IgM and IgG antibodies are separated in the initial step of the assay, and thus cannot compete for antigenic binding sites. No interference of rheumatoid factor occured in SPIHA, whereas in IgM-FAT most (but not all) of RF positive sera gave false-positive results. One RF positive serum which yielded a positive result also in SPIHA (Table 4) remained anti-*Toxoplasma* IgM positive after preadsorption with IgG coated latex particles. Therefore, it was considered as a truly positive reaction for anti-*Toxoplasma* IgM.

RF may be a source of false-positive reactions even in tests such as the double sandwich IgM-ELISA: *Schmitz* et al. (1980) found a strong nonspecific binding of the RF-IgM with peroxidase-labeled human or animal IgG. *Naot* et al. (1981) observed that in sera containing both rheumatoid factor and antinuclear antibodies false-positive results may occur due to interactions of RF-ANA-complex with the Fc-portion of the antigenspecific IgG antibody used for the enzyme conjugate. In

SPIHA, however, these nonspecific reactions cannot occur, since no labeled antibodies are used as a conjugate.

In a comparative study of SPIHA and IHA we could not find any correlation (Table 3). This can be simply explained by the fact that in SPIHA and IHA different classes of antibodies are reacting. A laboratory-acquired infection, the course of which we observed in detail (Fig. 2) illustrates the difference between antibody levels in IHA and SPIHA. Antibody titers measured in IHA showed only a slow rise some weeks after infection. Therefore, in agreement with results of previous studies (*Balfour* et al., 1980; *Picher* and *Aspöck*, 1981; *Welch* et al., 1980), we consider the IHA as inadequate for detection of a recently acquired *Toxoplasma* infection. In contrast, antibodies in SPIHA reached their maximum already three weeks after infection and decreased after six weeks (but were still present when IgM-FAT already became negative).

The Solid-Phase Indirect Haemadsorption Assay provides a simple, sensitive and accurate detection of specific IgM antibodies. The test is well appropriated for the routine laboratory (even for the short-equipped), and thus representing an alternative method to IgM-FAT. The advantages of this assay suggest that the SPIHA should be applied also for the diagnosis of other acute parasitic diseases.

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