Kinetics and time dependence of the differential agglutination of acetone [AC]- and formalin [HS]-fixed *Toxoplasma* tachyzoites by serum of mice with experimental toxoplasmosis

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Abstract Researches to specify a serologic diagnostic test capable of determining the stage of toxoplasmosis, whether recent or latent, have been hampered by lack of knowing the real time of infection. Studying the precise kinetics of the differential agglutination of acetone [AC]-fixed versus that of formalin [HS]-fixed tachyzoites (differential agglutination test or AC/HS test) by sera of mice during the course of toxoplasmosis and assessment of its value in the differentiation between recent and latent infections in mice were the aims of the present work. Experimental toxoplasmosis was induced in mice, sera were collected sequentially and AC/HS test, FAST-ELISA to determine levels of IgM and IgG and microscopic examination of brain for *Toxoplasma* cysts were done. Both AC and HS specific patterns in the AC/HS test were noted to be dependent on the time from the onset of infection. Acute patterns of the AC/HS test were observed early in infection till before the appearance of brain cysts. Non-acute patterns were obtained late on 28th day post infection coinciding with the disappearance of IgM, persistence of IgG and presence of cysts in brains. The AC antibody was high in the recent phase of infection, and then it declined to be replaced by high sustained level of HS antibody late in infection. In conclusion, in the presence of both IgM and IgG, the appearance of either equivocal pattern or the non-acute pattern in the AC/HS test is significant in ruling out acute infection in mice.

Keywords *Toxoplasma gondii* · Differential agglutination test · AC/HS test · Experimental toxoplasmosis

Introduction

Toxoplasma gondii is an intracellular protozoan parasite that is able to infect all warm-blooded animals and causes toxoplasmosis. The disease is generally benign and has little clinical relevance in immunocompetent individuals. However, it can be serious or fatal in children infected during their intra-uterine development and in immunocompromised patients in the absence of early diagnosis and adequate treatment. Infection generally occurs through ingestion of either oocysts shed in cat faeces, or viable tissue-cysts present in undercooked meat. During acute infection, tachyzoites, the rapidly multiplying stage of the parasite, can invade and proliferate in all nucleated cells by active penetration and formation of a parasitophorous vacuole. Replication leads to host cell death and rapid invasion of neighboring cells. After repeated replication, host cells are disrupted and tachyzoites disseminate via the bloodstream and can invade many tissues such as the central nervous system, eye, skeletal, heart muscle and placenta (Garcia 2003). However, the cell-mediated immune response induces stage conversion of the rapidly multiplying tachyzoites into bradyzoites with formation of tissue cyst containing up to thousands of bradyzoites. Such cysts are the hallmark of chronic infection and are present for the life of the host (Montoya and Liesenfeld 2004).

In the early 1980s, it was observed that the agglutination of acetone treated *T. gondii* (AC) tachyzoites was remarkably and surprisingly different from that of Formalin (HS) fixed parasites (Thulliez et al. 1986). This

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phenomenon has been attributed to the variation in IgG profiles in response to shifting *Toxoplasma* surface antigens as the infection progresses from an acute to a more chronic stage. The AC preparation contains stage-specific antigens which are recognized by immunoglobulin G (IgG) antibodies formed against *Toxoplasma* tachyzoites early in infection (Suzuki et al. 1988). These antibodies have specificities different from those formed later in infection. The differential agglutination of acetone [AC]-fixed versus that of formalin [HS]-fixed tachyzoites is used in the differential agglutination (AC/HS test) a serological test that compares the titers obtained with Formalin-fixed tachyzoites (HS antigen) with those obtained with acetone- or methanol-fixed tachyzoites (AC antigen) (Montoya et al. 2007). The test was used to diagnose toxoplasmic encephalitis in AIDS patients (Suzuki et al. 1988) and toxoplasmic lymphadenitis (Montoya et al. 2007) and is one of a group of tests forming *Toxoplasma* serum profile (Kaul et al. 2004) including a double sandwich IgM ELISA and the Sabin-Feldman dye test in combination with IgG avidity results to distinguish between recently acquired and distant infections in pregnant females (Liesenfeld et al. 2001; Remington et al. 2006). Results of the differential agglutination test are recorded as acute or non-acute patterns (Montoya et al. 2007). The absence of knowledge about the accurate time of infection in humans did not allow for an estimate of the accurate time of conversion of the pattern in the AC/HS test from acute to non-acute (Montoya et al. 2007).

The aim of the present study was to record the precise kinetics of the differential agglutination (of acetone [AC]-fixed versus that of formalin [HS]-fixed tachyzoites) test (AC/HS test) and its value in the differentiation between recent and latent toxoplasmosis in mice.

Materials and methods

Mice

Six to eight weeks old, Swiss *Albino* mice, weighing 25–30 g, supplied by Schistosoma Biological Supply Center, Theodor Bilharz Research Institute Giza, Egypt, were maintained and bred under conventional conditions using pathogen-free diets and bedding materials. A total of 300 mice were used in the study. All were serologically negative for toxoplasmosis.

Infection

The cysts used for the experimental infection were obtained from the brains of *Albino* mice infected with the KSU strain of *T. gondii*. The strain was, kindly supplied

from Parasitology Department, Faculty of Veterinary Medicine, Zakazik University, Egypt and maintained by inoculated mice passage. Infected mice were sacrificed after 8–12 weeks of infection by cervical dislocation; the brains were removed and homogenized in phosphate buffered saline (PBS) pH 7.2. (Sigma Chemical Co., St. Louis, USA), Mice were infected orally with 30 cysts using an orogastric gavage.

Samples

In the first week post-infection, serum samples were collected daily from 10 mice then every other day till the appearance of *Toxoplasma* brain cysts then weekly. Sera were stored at 20°C until used.

Examination for cysts in the brain of the mice

Brains of the mice were removed and homogenised individually in PBS (pH 7.2) and the suspension was placed on a slide, mounted under a cover slip, and viewed under a light microscope.

IgM and IgG detection by Falcon Assay Screening test (FAST-ELISA)

Antigen for FAST-ELISA was prepared according to Erlich et al. (1983), obtained tachyzoites (RH strain—Diagnostic and Research Unit, Parasitology Department, Faculty of Medicine—Ain shams University-Cairo, Egypt) were washed by centrifugation in PBS at $2,000\times g$ for 10 min. Pellet was suspended in distilled water then sonicated in a Soniprep 150 sonicator (England) at 20 watts for 30 s at 4°C followed by centrifugation at $2,000\times g$ for 5 min. The protein concentration in the supernatant was measured according to Bradford (1976). FAST-ELISA was carried out according to the procedure described by Hancock and Tsang (1986) with some modifications. Beads were sensitized with tachyzoite antigen at 4 µg/ml in sensitization buffer (0.05 M Tris-HCl, 0.3 M KCl, 2 mM EDTA, pH 8.0) 2 h at room temperature, then washed in PBS with 0.05% Tween 20 (PBS-TW). Antigen coated beads were incubated in sera diluted (1:30) in PBS-TW for 15 min at room temperature. The antibody binding was identified with either goat anti-mouse IgG or IgM peroxidase conjugate (1:1,000, 1:3,000, respectively, incubated for 15 min) and visualized by incubation for 5 min in TMB (tetramethylbenzidine) Microwell Peroxidase Substrate System. The absorbance at 650 nm was measured using Uvmax, Kinetic microplate reader, USA. And the values of optical density (O.D.) greater than the mean +double the standard deviation of negative control sera were considered positive.

The differential agglutination test (or AC/HS test)

The HS and AC tests were run with Formalin-fixed (HS test) and acetone-fixed (AC test) tachyzoites (HS and AC antigens).

Preparation of antigens

Two antigens were prepared: the HS and AC antigens using the RH strain of *T. gondii* (Diagnostic and Research Unit, Parasitology Department, Faculty of Medicine—Ain Shams University-Cairo, Egypt) were prepared as described previously (Suzuki et al. 1988; Dannemann et al. 1990). In brief, harvested tachyzoites, from peritoneal cavity of previously infected mice with RH strain, were liberated from host cells by passing the peritoneal exudates through 21-gauge needle. For HS antigen preparation, tachyzoites were washed twice by centrifugation in PBS containing 1% bovine serum albumin (PBS/BSA) (Sigma), Formalin was added to a final concentration of 20% of the mixture while the suspension was under continuous agitation for 10 min, thereafter, the solution was kept for overnight (at least for 16 h) at 4°C. For AC antigen, the same procedure of preparing the HS-antigen was carried but Formalin was substituted with acetone; after washing in PBS, acetone was added to the parasite suspension in PBS/BSA to provide a final concentration of 30% acetone in the mixture. Agitation was maintained for 10 min to allow complete mixing. Then, the solution was kept at 4°C for 36 h. The following day, Formalin- and acetone-fixed parasites were washed separately twice in PBS/BSA. The pellet of tachyzoites was adjusted to contain 3×10^7 tachyzoites/ml suspended in PBS containing 1% BSA and was stored at 4°C till used. Both antigens retained its antigenic properties for over 1 year.

Conventional direct agglutination test

Before carrying out the AC/HS test all sera were tested in the conventional direct agglutination test as screening test; sera that were negative in the conventional agglutination test were not evaluable in the AC/HS test. Direct agglutination test using Formalin-fixed tachyzoites was performed as described previously (Desmonts and Remington 1980). Direct agglutination test is used for the detection of toxoplasma IgG antibodies, IgM-mediated agglutination is suppressed by using a diluting buffer containing 2-mercaptoethanol. The test is performed in microtiter polystyrene plates with round (U-shaped) wells (Dynatech Laboratories, Inc., Chantilly, Va.). For screening the sera, conventional direct agglutination test using Formalin fixed tachyzoites was performed on each serum sample starting from concentration 1 in 20 (100 µl serum + 1.9 ml PBS

containing 0.2 M 2-mercaptoethanol) for 7 dilutions (up to 1/1,280). The test was performed in microtitre plate. 50 µl of each dilution of the sera were transferred into the microtitre plate. 50 µl of the tachyzoites suspension were added to each well, the plate was covered with a self-adhesive sheet, mixed thoroughly and left to stand at room temperature overnight. *Toxoplasma* negative control serum and pooled positive *Toxoplasma* serum were used as negative and positive control, respectively. After the incubation, plates were read against a black background with a lateral light and are read by pattern. A positive reaction exhibits agglutination of the toxoplasma in a mat covering about half of the well base. In the absence of specific antibody a compact button was obtained.

The differential agglutination (AC/HS) test consist of two tests the HS- and the AC-agglutination tests. Sera negative in the screening test were not evaluable in the AC/HS test.

HS agglutination test

The HS agglutination test was performed as described in the conventional direct agglutination test evaluating only the sera that were positive in the screening test. Starting dilutions from 1/2,000 (25 µl of the 1 in 20 dilution + 2.5 ml of PBS containing 0.2 M 2-mercaptoethanol) then followed as twofold sera dilutions. The serum dilution for HS antigen (1:2,000) was assigned an antibody titer of 100 IU/ml (Dannemann et al. 1990; Liesenfeld et al. 2001; Montoya et al. 2007). Sera that were negative in the conventional direct agglutination test were not evaluable in the AC/HS test. Thus, an HS test result of <100 IU/ml was positive with serum dilutions from 1:20 to <1:2,000 (as the starting serum dilution was 1:20 and the dilution of 1:2,000 was determined as titer of 100 IU/ml in HS agglutination test).

AC agglutination test

The AC agglutination test was performed as described in the conventional direct agglutination test using acetone fixed tachyzoites instead of formalin fixed tachyzoites. Twofold dilutions of sera were done starting with 1:100. The serum dilution for AC antigen (1:100) was assigned an antibody titer of 50 IU/ml (Dannemann et al. 1990; Liesenfeld et al. 2001; Montoya et al. 2007). Thus, an AC test titre of <50 IU/ml was considered in sera positive in conventional agglutination test and negative in the AC test.

Reading of the tests

A smooth button or ring at the bottom of the well is recorded as negative (0). A complete carpet covering about

more than the half the round bottom of the well was recorded as positive (+++). Intermediate readings from $\pm$ (doubtful) to ++ are also noted where the carpet covers less than half the wells. Positive reaction (+++): The agglutination of *Toxoplasma* appears as a mat or carpet covering about more than half the round bottom of the well. In the HS-test, the first dilution used (1: 2,000) corresponds to antibody titer of 100 IU/ml, in AC-test; the first dilution (1:100) corresponds to a titer of 50 IU/ml. The final dilution of serum showing positive agglutination is the titer of antibody in serum.

Interpretation of AC/HS test

The AC/HS test was interpreted by comparing antibody titers obtained using the AC antigen with those obtained using the HS antigen. The final result of each AC/HS test was reported as follows: if the titer in the AC test was 400 IU/ml and that in the HS test was 100 IU/ml, the result was reported as 400/100, and interpreted according to the criteria shown in Fig. 1.

Fig. 1 Criteria used for interpretation of the differential agglutination (AC/HS) test results (Liesenfeld et al. 2001; Montoya et al. 2007). *NR* non-reactive, *A* acute pattern, *E* equivocal, *NA* not acute. *NR*: HS titer if >0 but <100; this pattern may be seen in the earliest stages of infection (non-reactive pattern)

Results

IgM antibodies were detected in 80% of sera using FAST-ELISA test on the 5th day after infection, this percent increased to 100% of tested sera starting from 6th day post infection till the 21st day post infection then disappear completely from sera. Specific IgG was detected in 40% of mice in the 13th day post infection and increased to be present in 100% of infected mice starting from the 21th day of infection (Table 1; Fig. 2).

Brain cysts were detectable from the 21st day post infection.

Direct agglutination test started to be positive on the third day post infection in 70% of samples, and then all the samples were positive on the fourth day till the end of the experiment.

The AC titer was at low level very early in infection, started to increase to reach high level ($\geq 1,600$ IU/ml) on the seventh day post infection, went in a plateau till the 21st day post infection then it declined to lower level, on the other hand the HS titer remained low till 13th day post

(a)

HS test Result (IU/ml)	Interpretation with the following AC test result (IU/ml)							
	<50	50	100	200	400	800	1,600	>1,600
<100	NR	E	E	A	A	A	A	A
100	NA	E	E	A	A	A	A	A
200	NA	E	E	A	A	A	A	A
400	NA	E	E	A	A	A	A	A
800	NA	NA	E	E	A	A	A	A
1,600	NA	NA	NA	E	E	A	A	A
3,200	NA	NA	NA	NA	E	E	A	A
>3,200	NA	NA	NA	NA	NA	E	E	A

(b)

HS IU/mL)	Interpretation with the following AC result (IU/mL)							
	<50	50	100	200	400	800	1600	>1600
<100	Equivocal pattern			Acute pattern				
100								
200								
400								
800	Nonacute pattern							
1600								
3200								
>3200								

Table 1 *Toxoplasma* serology and the *Toxoplasma* differential agglutination (AC/HS) patterns for mice following induction of *Toxoplasma* infection

Time of serum samples (days)	Number of positive (%)		Direct agglutination test	Differential agglutination (AC/HS) test AC/HS pattern			
	IgM-FAST-ELISA	IgG-FAST-ELISA		Acute	Non-acute	Equivocal	Non-reactive
1	0 (0%)	0 (0%)	0 (0%)	The AC/HS test was not done because the direct agglutination test was negative			
2	0 (0%)	0 (0%)	0 (0%)				
3	0 (0%)	0 (0%)	7 (70%)	0 (0%)	0 (0%)	0 (0%)	7 (70%)
4	0 (0%)	0 (0%)	10 (100%)	2 (20%)	0 (0%)	8 (80%)	0 (0%)
5	8 (80%)	0 (0%)	10 (100%)	10 (100%)	0 (0%)	0 (0%)	0 (0%)
6	10 (100%)	0 (0%)	10 (100%)	10 (100%)	0 (0%)	0 (0%)	0 (0%)
7	10 (100%)	0 (0%)	10 (100%)	10 (100%)	0 (0%)	0 (0%)	0 (0%)
9	10 (100%)	0 (0%)	10 (100%)	10 (100%)	0 (0%)	0 (0%)	0 (0%)
11	10 (100%)	0 (0%)	10 (100%)	10 (100%)	0 (0%)	0 (0%)	0 (0%)
13	10 (100%)	4 (40%)	10 (100%)	10 (100%)	0 (0%)	0 (0%)	0 (0%)
15	10 (100%)	4 (40%)	10 (100%)	10 (100%)	0 (0%)	0 (0%)	0 (0%)
17	10 (100%)	6 (60%)	10 (100%)	10 (100%)	0 (0%)	0 (0%)	0 (0%)
19	10 (100%)	7 (70%)	10 (100%)	10 (100%)	0 (0%)	0 (0%)	0 (0%)
21 ^a	10 (100%)	10 (100%)	10 (100%)	0 (0%)	0 (0%)	10 (100%)	0 (0%)
28 ^a	1 (10%)	10 (100%)	10 (100%)	0 (0%)	10 (100%)	0 (0%)	0 (0%)
35 ^a & later	0 (0%)	10 (100%)	10 (100%)	0 (0%)	10 (100%)	0 (0%)	0 (0%)

^a Time of appearance of *Toxoplasma* cyst in brain of mice

Fig. 2 Individual kinetics of antibody responses to the AC-fixed tachyzoite and HS-fixed tachyzoite antigens and of the IgM and IgG FAST-ELISA titers for mice with toxoplasmosis

infection and increased up (>3,200 IU/ml) on 17th day post infection to form a plateau till 28th day post infection then decline (Fig. 2).

In AC/HS test, acute pattern (200/100) was noticed in 20% of sera on the 4th day post infection and in 100% of sera starting from 5th day post infection till the 19th day

post infection. Non-acute pattern was noticed from the 28th day post-infection in 100% of sera and stayed till the end of the experiment. Equivocal pattern was seen on the 4th and 21st days post infection only (Table 1; Fig. 3a).

The overall kinetics of the AC/HS test results for infected mice observed in Fig. 3a followed a “clockwise”

Fig. 3 Kinetics and time dependence of AC/HS test patterns in mice with experimental toxoplasmosis.

a In each box the top number is the time from induction of toxoplasmosis in mice to the sampling of the serum in days. The bottom number is the number of mice who had that AC/HS test pattern.

b Diagrammatic representation of the approximate “clockwise” evolution of the AC/HS pattern in mice with experimental toxoplasmosis. D Day, NR Non-Reactive (white), Non-Acute Pattern (light gray), Acute Pattern (dark gray), Equivocal Pattern (medium gray)

(a)

HS test results (IU/ml)	AC test results (IU/ml)							
	<50	50	100	200	400	800	1600	>1600
<100	D 3 7	D 49 10	D 4 6	D 4 2				
100	D 42 10				D 5, 6 4, 2	D 5 2		
200						D 5, 6 4, 4	D 7 1	
400						D 6 2	D 7, 9 2, 7	D 7, 9 7, 3
800	D 35 1							D 11, 13 8, 9
1600	D 35 2	D 35 5						D 11, 13, 15 2, 1, 10
3200			D 35 2					D 19 8
>3200				D 28 10		D 21 2	D 17, 21 7, 8	D 17, 19 3, 2

(b)

HS test results (IU/ml)	AC test results (IU/ml)							
	<50	50	100	200	400	800	1600	>1600
<100								
100								
200								
400								
800								
1600								
3200								
>3200								

pattern of evolution on the interpretation chart, as diagrammatically represented in Fig. 3b.

Discussion

The clinical manifestations of *T. gondii* infection in humans may be protean and non-specific, thus toxoplasmosis must be carefully considered in the differential diagnosis of a large variety of clinical presentations. The correct diagnostic tests must be performed and appropriately interpreted in light of the patient's clinical presentation. Previous search works to specify a diagnostic test capable of determining the accurate time of getting infected have been hampered by lack of knowing the real time of infection. Numerous animal models, including primates, rabbits, guinea pigs, hamsters, mice and rats, have been used in recent years to study the pathology of the infection, efficacy of vaccines and new drugs for the treatment of congenital transmission, chorioretinitis and encephalitis

caused by *Toxoplasma gondii*. Of these, mice have been found to be the most susceptible to *T. gondii* and are particularly interesting model for studying the pathology and immunology of toxoplasmosis (Chinchilla et al. 1994; Fux et al. 2000; Fioretti 2004). The simplicity and availability of using mice for in vivo inducing toxoplasmosis and hence knowing the exact time of infection allowing following up the kinetics and time dependence of the used serological test to diagnose toxoplasmosis may be beneficial in determining a practical discriminative test that can diagnose acutely acquired infection using a single serum sample.

In the present study, the use of avirulent strain gives rise to chronic form of the disease with the formation of tissue cysts lasting for the long life of the animal (Nguyen et al. 1996; Dubey et al. 1998; Fux et al. 2000).

Following the kinetics and time dependence of immune response of mice to *T. gondii* infection in the present study, IgM and IgG antibodies were in agreement with was previously documented by Thiermann and Stagno (1972). IgG antibodies reached their maximum level and persist for a

long time while IgM antibodies disappeared from the serum (Partanen et al. 1984; Godard et al. 1990; Montoya and Remington 2000).

T. gondii brain cysts were detected in the brains of the infected mice starting from 21th day post infection and persisted till the end of the experiment. Cysts are known to be associated with chronic infection and development of immunity in the infected host. As previously reported, cysts begin to be found as early as the sixth day after infection (Nguyen et al. 1996) up to 30 days (Suzuki et al. 1993; Fux et al. 2000) in mice and persists as long as the life span of the host (Dubey 1977; Montoya and Remington 2000; Larry and John 2000).

The *Toxoplasma* agglutination test is very simple, rapid and does not require special technique to prepare the antigen neither special equipments nor apparatus to be carried on (Suzuki et al. 2001). The use of sensitive antigenic preparation and adding 2-Mercaptoethanol improves its specificity and sensitivity, to parallel those of the dye test (Desmonts and Remington 1980). Only membrane bound antigens of trophozoites are responsible for reaction with antibodies in the agglutination test. The fixation of tachyzoite forms of *T. gondii* with either formalin or acetone causes a significant difference in their affinity for antibodies from patients with recently acquired toxoplasmosis compared with subjects with past-infection (Thulliez et al. 1986). Acute-stage antigens (AC antigen) present on the surface of tachyzoites were exposed by acetone fixation and agglutinated IgG of the acute stage of infection and chronic-stage antigens (HS antigen) were exposed by formalin fixation and agglutinated IgG of the chronic stage of infection. Since AC antigen preparation consisted of acetone-treated tachyzoites, acetone may have removed certain antigens from the cell membrane or altered the antigens such that they no longer cross reacted with antigens of bradyzoites.

In the present work, the kinetics and evolution of the AC- and HS-tests could be followed individually (Fig. 2). Both tests had low values at the early time of infection (HS <100 IU/ml and AC, 50 IU/ml) then both values increased to attain plateau level. In the present study, the AC titer attained high level early in the infection being caused by the proliferating tachyzoites (Montoya and Remington 1995; Suzuki et al. 2001; Montoya et al. 2007). On the other hand, the HS-test still rises up late, as previously reported in humans where high HS-titers are characterizing chronic infections (Suzuki et al. 1988; Dannemann et al. 1990; Montoya et al. 2007). A clockwise evolution of the values of the AC/HS tests was reported in mice of the present work as was reported to occur in humans (Montoya et al. 2007).

The differential agglutination test (AC/HS) compares the titers obtained with acetone-fixed tachyzoites

(AC-antigen) with those obtained with formalin-fixed tachyzoites (HS-antigen) (Suzuki et al. 1988, 2001). The results are expressed as having equivocal, acute or non-acute patterns depending on the relation between the titers obtained in the AC-test and the HS-test and their location of the AC/HS fraction on a chart (Fig. 1) developed and published by Suzuki et al. (1988) and Dannemann et al. (1990). In the present work, acute patterns of the AC/HS test (Table 1; Figs. 2, 3a, b) were observed early in infection before the appearance of tissue cysts and serum IgG with the presence of IgM in serum. This means that the existence of acute patterns coincided typically with the recent phase of infection in mice concurrently with the absence of brain cysts from brain of mice. In humans, Montoya et al. (2007) although had suspected other aetiology than *Toxoplasma* for lymphglandular enlargement (LE) when they found acute pattern of AC/HS test after 12 months of appearance of LE, they could not specify the exact time of conversion from acute to non-acute patterns.

In the present work, equivocal patterns of AC/HS test were obtained very early in infection, in absence of both IgM and IgG (4th day post infection) and also, later in presence of both IgG and IgM (21th day post infection). The latter condition represents a conflicting situation to diagnose acute or chronic infection. In the present study, equivocal pattern in presence of IgM and IgG coincided with the appearance of brain cysts in mice denoting the development of immunity; thus, equivocal pattern in the AC/HS test in the presence of both IgM and IgG can be considered useful in ruling out acute infection in mice. Non-acute patterns (Table 1; Fig. 3a, b) were obtained in the present work late in the infection on 28th day post infection coinciding with the disappearance of IgM and persistence of IgG denoting the entrance in latent form of infection.

The results of the present study collectively draw a clear conclusion that the differential agglutination test (AC/HS) can be considered a useful tool to differentiate between acute and late experimental toxoplasmosis using both the equivocal and the non-acute pattern to exclude acutely acquired infection.

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