Journal of Microbiological Methods 134 (2017) 38–45

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Long-term sera storage does not significantly modify the interpretation of toxoplasmosis serologies

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ARTICLE INFO

Article history:
Received 24 October 2016
Received in revised form 3 January 2017
Accepted 4 January 2017
Available online 16 January 2017

Keywords:
Toxoplasma gondii
Toxoplasmosis
Serum
Stability
Storage
Biobank
Freezing
Parasitology

ABSTRACT

Background: Serological investigation of *Toxoplasma gondii* can answer many questions about toxoplasmosis in human pathology. Along these lines, studies on serum storage in biobanks need to be performed especially in terms of determining the impact of storage on relevance of sera analysis after freezing. This study assessed the impact of long-term sera storage on the stability of anti-*Toxoplasma* immunoglobulins.
Material and methods: The stability of anti-*Toxoplasma* IgG and IgM was studied in 244 and 242 sera respectively, stored at −20 °C from one month to ten years. ELISA-immunoassay (Vidas®, bioMérieux) was used for initial and post-storage analyses. Linear models for repeated measures and subgroup analyses were performed to assess the effect of storage duration and sample characteristics on immunoglobulins stability.
Results: Until ten years, the variability attributed to storage (maximum 8.07% for IgG, 13.17% for IgM) was below the variations inherent to the serological technique and allowed by quality assurance systems (15%). Subgroup analysis reported no variation attributed to sera storage. Serological interpretation was modified for 3 sera (1.2%) tested for IgM, all stored more than seven years.
Conclusion: Anti-*Toxoplasma* immunoglobulins can reliably be measured for at least up to six years of storage with no modification of interpretation of toxoplasmosis serologies.

1. Introduction

Toxoplasmosis is a parasitic disease caused by *Toxoplasma gondii*, an intracellular protozoan parasite belonging to the Apicomplexan family. Toxoplasmosis is a global health hazard as the worldwide seroprevalence is around 50% (Montoya and Liesenfeld, 2004). Infection with *T. gondii* can be acquired by the ingestion of *T. gondii* cysts in undercooked meat or oocysts from the environment (Montoya and Liesenfeld, 2004). Three main clinical entities in humans are generally distinguished: primary infection with *T. gondii* in immunocompetent individuals, which is generally asymptomatic and benign; mother-to-child transmission, which can cause serious fetal malformations; and reactivation in immunocompromised patients, which can lead to death (Montoya and Liesenfeld, 2004). Serological tests are used to diagnose toxoplasmosis in most clinical contexts (Dard et al., 2016; Robert-Gangneux and Dardé, 2012).

Usually, anti-*T. gondii* antibodies are rapidly analyzed after blood sampling. If immediate analysis is not possible, manufacturer package insert allows storage of sera at 2 to 8 °C before testing, usually <7 days (Bennet, 1980. Carpenter, 1997; National Committee for Clinical Laboratory Standards, 2001). If longer storage is needed after routine laboratory analysis, the leftover sera - usually under 1.5 mL - can be stored frozen in biobanks. As sera should be thawed and utilized only once after thawing according to the manufacturer guidelines, the use of single usage aliquots is recommended with repartition of the leftover of sera in several aliquots. Within the framework of French legislation and to ensure high quality health care, sera collected for toxoplasmosis serology are consistently stored at −20 °C in biobanks for at least one year (https://www.legifrance.gouv.fr/eli/arrete/2005/9/20/SANS0523407A/jo/texte). The main goal of sera conservation is to permit further analysis later in case of medical needs. This storage process enables us to analyze sequential sera in the same run to assess stability of immunoglobulin. After the legal one year, the frozen sera can be used for various retrospective studies including sero-epidemiological investigations, quality control and methods comparison studies (Murat et al., 2013a, 2013b).

A number of studies have examined how storage conditions affect the stability of various biochemical components (Gao et al., 2007; Hsing et al., 1989; Kale et al., 2012; Tanner et al., 2008; Donnelly et al., 1995; Cuhadar et al., 2012; Boyanton and Blick, 2002; Cuhadar et al., 2013). However, very little information exists on the stability of

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http://dx.doi.org/10.1016/j.mimet.2017.01.003
0167-7012/

immunoglobulins in human sera and what constitutes optimal sera storage conditions (Ellis et al., 2004; Pappin et al., 1995; Rosa-Fraile et al., 2004). Indeed, few studies have investigated the stability of anti-*Toxoplasma* antibodies after short-term freezing but none after long-term storage. Moreover, these studies were based on limited samples and did not consider sera characteristics, related clinical and serological settings. Given the need of the current quality assurance system for robust data and more knowledge about immunoglobulin stability of sera frozen in biobanks, we evaluated the biological relevance of anti-*Toxoplasma* IgG and IgM analysis in sera stored at −20 °C for several years in biobanks.

2. Materials and methods

2.1. Study design

Sera were retrospectively selected from the biobank collection of the Parasitology-Mycology Clinical Laboratory in Grenoble Alpes University Hospital in France. This biobank is registered with the French Ministry of Health number DC-2008-582. The selected sera were stored for toxoplasmosis serological routine analysis between January 1, 2005 and December 31, 2014. All the analyses were performed in the Parasitology-Mycology Clinical Laboratory of Grenoble Alpes University Hospital with Vidas® Toxo IgM and Vidas® Toxo IgG reagents (bioMérieux, France).

2.2. Preanalytical phase

All the sera were obtained from blood samples collected in 5 or 7 mL polyethylene terephthalate (PET) top BD Vacutainer® tubes with clot activator, either with or without serum-gel separator. Within 72 h of the blood draw, the sera were separated by centrifugation at 2000*g* for 15 min at room temperature and initial anti-*Toxoplasma* IgG and IgM determinations were performed on the same day. After initial testing, the remaining sera were first stored at 4 °C until the end of each week to allow additional analysis if needed. Remaining sera were then transferred into 2 mL graduated polypropylene cryotubes (VWR, Radnor, USA) and frozen at −20 °C in our laboratory biobank for long-term storage.

2.3. Sample selection and characteristics

Thirty to 38 sera samples were selected for each year - from 2005 to 2014 - for IgG and/or IgM analysis (Table S1 in Supplementary data). The selected sera vary in terms of volume stored in biobanks, immunoglobulin levels and clinical and immune status of the corresponding patient. All the sera came from different patients (only one sample per patient). Mainly sera with equivocal and positive immunoglobulin levels on initial analysis were selected (Table 1). Only one serum with negative IgM was included for IgM analysis as its value was very close to the equivocal threshold. Sera with IgG values upper limit of the linear range (IgG > 300 IU/mL) were excluded for post-storage IgG titration. For each serum, corresponding patient and sample characteristics - age, gender, clinical setting category, *Toxoplasma* immune status and date of sampling, volume of frozen sample, date of initial analysis, and date of second analysis - were documented (Table 1).

Table 1
Descriptive of repartition and groups of sera using quantitative and qualitative variables.

	Groups	IgG (N_{total} = 244)	IgM (N_{total} = 242)
Quantitative variables: mean (±std)			
Storage duration (months)	NA	60.9 (±35.7)	61.2 (±35.2)
Initial aliquot volume (mL)	NA	0.8 (±0.5)	0.8 (±0.4)
Age (years)	NA	34.7 (±19.1)	35.5 (±13.8)
Qualitative variables: N (%)			
All sera		244 (100)	242 (100)
Storage duration	<1 year	25 (10.2)	25 (10.3)
	1 to 2 years	27 (11.1)	23 (9.5)
(Time of conservation	2 to 3 years	25 (10.2)	26 (10.7)
between the initial and	3 to 4 years	25 (10.2)	25 (10.3)
the last analysis)	4 to 5 years	23 (9.4)	25 (10.3)
	5 to 6 years	27 (11.1)	29 (12.0)
	6 to 7 years	23 (9.4)	22 (9.1)
	7 to 8 years	25 (10.2)	25 (10.3)
	8 to 9 years	23 (9.4)	18 (7.4)
	9 to 10 years	21 (8.6)	24 (9.9)
Description of groups and subgroups			
Groups	*Subgroups*		
Sex	F	166 (68.0)	188 (77.7)
	M	78 (32.0)	54 (22.3)
Clinical settings categories[a]	Pregnant women serological follow-up	104 (42.6)	137 (56.6)
	Immunocompetent patients investigation	58 (23.8)	45 (18.6)
	Immunocompromised patients follow-up	45 (18.4)	44 (18.2)
	Infants suspected of congenital toxoplasmosis	27 (11.1)	0 (0)
	Undetermined	10 (4.1)	16 (6.6)
Toxoplasma immune status[b]	Past immunity (>6 months)	108 (44.3)	94 (38.8)
	Recent *T. gondii* infection (<6 months)	54 (22.1)	80 (33.1)
	Non immunized	0 (0)	8 (3.3)
	Undetermined	82 (33.6)	60 (24.8)
Initial Ig rate category	Negative	0 (0)	1 (0.4)
	Equivocal	37 (15.2)	39 (16.1)
	Positive	207 (84.8)	202 (83.5)
Volume of frozen aliquots	V = 0.10–0.7 mL	123 (50.4)	118 (48.8)
	V = 0.8–2 mL	121 (49.6)	124 (51.2)
Description of set of reagent			
Set of reagents	A	147 (60.2)	96 (39.7)
	B	97 (39.8)	146 (60.3)

The quantitative and qualitative variables were respectively expressed by using mean (± standard deviation) and N (%).

- Quantitative variables: descriptive of the sera according the mean age, initial volume of sampling and storage duration.
- Qualitative variables: repartition of the sera according their storage duration, their initial and final immunoglobulin rates and the characteristics of the related patient with their sex, clinical settings categories and *Toxoplasma* immune status.

[a] 10 IgG sera and 16 IgM sera non-classified in clinical settings categories due to lack of clinical information in our health records.
[b] 82 IgG sera and 58 IgM sera non-classified in *Toxoplasma* immune status either because taken from immunocompromised patients or infants suspected of congenital toxoplasmosis, either due to lack of clinical information in our health records.

2.4. Description of sera repartition and categories

2.4.1. Sera selected for IgG analysis

Two hundred and forty-four sera that had been stored for up to 10 years were included for anti-*Toxoplasma* IgG analysis (Table 1). Mean storage duration was 60.9 months (±35.7). Mean sample volume was 0.8 mL (±0.5). Sera came from patients with a mean age of 34.7 years (±19.1) and mainly from women (68%). The clinical-categories were: pregnancy monitoring (104, 42.6%), immunocompetent patients (58, 23.8%), immunocompromised patients (45, 18.4%) and suspected congenital toxoplasmosis (27, 11.1%). Ten sera (4.1%) were not classified due to lack of information. As for the *Toxoplasma* immune status, 108 patients (44.3%) had a past infection, 54 (22.1%) a recent *T. gondii* infection, whereas 82 (33.6%) could not be classified, as they belonged to immunocompromised patients and suspected congenital toxoplasmosis groups (Table 1).