Letter to the Editor

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Impact of heat-inactivation on anti-*Toxoplasma* IgM antibody levels

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To the Editor,

In diagnostic laboratories, heat-inactivation of serum is usually performed to inactivate the complement in serum, in order to avoid non-specific reactions and decrease the background in immunological assays [1, 2]. More recently it has been used to inactivate the human immunodeficiency virus (HIV) to reduce the risk of accidental exposure of laboratory workers [3, 4]. Since the 1980s, the effect of heat-inactivation has been assessed on several components of serum such as, e.g. α antitrypsin, β_2-microglobulin, IgE, fibrinogen… [3, 4]. The effect of heat-inactivation has also been evaluated on serological immunoassays, and the consequence of heating serum has been found to have a positive or a negative impact [5–8]. In heat-inactivated sera, false-positive HIV results were observed when using the Abbott HIV EIA, proving that heating serum should be avoided [5–7], but this HIV assay is not used anymore. On the contrary, heat-inactivation is included as part of the sample preparation procedure for the human papillomavirus (HPV) multiplexed competitive Luminex immunoassay, in order to eliminate heat-labile factor(s) which could give false-positive results [8]. However, for many assays, such as anti-*Toxoplasma* IgM assays, the potential impact of heat-inactivation of serum is not published, nonetheless some *in vitro* diagnostic manufacturers recommend in package insert instructions not to use heat-inactivated specimens.

To avoid misinterpretation of anti-*Toxoplasma* IgM serology and meet the requirements of quality assurance systems, we prospectively assessed the impact of serum heat-inactivation at 56 °C for 30 min on anti-*T. gondii* IgM levels measured by ELISA-immunoassay using VIDAS® Toxo IgM reagents (bioMérieux®, Marcy l'Etoile, France) on 50 sera from a French Parasitology-Mycology Clinical Laboratory (Grenoble, France).

Antibody titers for IgM were determined with an ELISA immunoassay (VIDAS® Toxo IgM, bioMérieux®, Marcy l'Etoile, France) on fresh sera and after heat-inactivation. Anti-*Toxoplasma* IgM titers are expressed in indexes and the cut-off values determined by the manufacturer are: negative <0.55; 0.55≤ equivocal <0.65; ≥0.65 positive. All testing was done in accordance with the manufacturer's guidelines. Toxoplasmosis serological routine analyses were performed in the Parasitology-Mycology Clinical Laboratory of Grenoble using VIDAS Toxo IgM and VIDAS Toxo IgG reagents [9, 10]. Based on the results of the IgM tests, 50 sera were prospectively studied from October 2015 to January 2016. Sera with a volume greater than 500 µL with negative (n=10), equivocal (n=4) and positive (n=36) IgM values were selected and 250 µL of serum were heated at 56 °C during 30 min in a water bath. IgM testing was repeated on the same day or the following day with the same lot of reagents. Univariate analyses were performed to compare IgM results using the Wilcoxon signed rank sum test (Statview).

The 50 sera came from 36 pregnant women, six immunocompromised patients and eight immunocompetent patients. The IgM values measured after heating were significantly lower than the values measured on fresh serum (Wilcoxon test, $Z=-6.126$, $p<0.0001$) (Table 1). Moreover, for all categories of sera, the coefficient of variation on fresh and heated serum was higher than 20% for negative values, equivocal (27.7%) and positive values (21.3%) (Table 1). Among the 50 sera analyzed, the biological interpretation of IgM changed for 15 samples after

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Table 1: IgM indexes of the 50 sera measured before (fresh serum) and after heating for 30 min at 56 °C.

Categories	Mean of indexes in fresh serum	Mean of indexes after heating	Global mean[a]	Mean of SD	CV, %
Negative fresh sera (n = 10)	0.121	0.084	0.102	0.026	28.5
Equivocal fresh sera (n = 4)	0.600	0.427	0.513	0.122	27.7
Positive fresh sera (n = 36)	1.996	1.577	1.786	0.297	21.3
All sera (n = 50)	1.509	1.186	1.347	0.229	23.2

SD, standard deviation; CV, coefficient of variation. [a]Global mean: means of indexes measured in fresh serum and after heating for each category of sera.

Table 2: IgM indexes before and after heating the 15 sera with discordance in biological interpretation.

Indexes of IgM measured with VIDAS TXM				
Fresh sera	**After heating**	**Mean**	**Standard deviation**	**CV, %**
0.55 (E)	0.19 (N)	0.37	0.255	68.8[a]
0.60 (E)	0.54 (N)	0.57	0.042	7.4
0.62 (E)	0.45 (N)	0.535	0.120	22.5[a]
0.63 (E)	0.53 (N)	0.580	0.071	12.2
0.71 (P)	0.55 (E)	0.630	0.113	18.0
0.75 (P)	0.60 (E)	0.675	0.106	15.7
0.96 (P)	0.63 (E)	0.795	0.233	29.4[a]
1.02 (P)	0.60 (E)	0.81	0.297	36.7[a]
1.06 (P)	0.61 (E)	0.835	0.318	38.1[a]
0.65 (P)	0.53 (N)	0.590	0.085	14.4
0.69 (P)	0.54 (N)	0.615	0.106	17.2
0.71 (P)	0.51 (N)	0.610	0.141	23.2[a]
0.72 (P)	0.27 (N)	0.495	0.318	64.3[a]
0.78 (P)	0.51 (N)	0.645	0.191	29.6[a]
0.79 (P)	0.53 (N)	0.660	0.184	27.9[a]

The IgM cut-offs (index) defined by the manufacturer are: negative <0.55 (N); 0.55≤ equivocal <0.65 (E); ≥0.65 positive (P). CV, coefficient of variation. [a]CV (IgM indexes in fresh serum compared to indexes in heated serum) >20%. Grey clear, equivocal IgM values; dark grey, positive IgM values.

heating. Four equivocal sera became negative, and among the 11 positive fresh sera, five positive sera became equivocal and six positive sera became negative. The IgM indexes of these 15 sera with discrepant biological interpretation are reported in Table 2. More precisely, for the six sera with a major discrepancy we noted that coefficients of variation were higher than 20% for four sera (23.2%, 27.9%, 29.6%, 64.3%). If we did not consider the equivocal results, the percentage of false-negative results observed after heating was 16.7% (6/36), corresponding to four sera from pregnant women, one serum from an immunocompetent patient and one from an immunocompromised patient. After considering the full set of serological results for these six cases, we concluded that the IgM measured were residual anti-*Toxoplasma* IgM corresponding to IgM levels that remain detectable several months or years after acute infection.

The decrease in IgM rates after heating was significant under our conditions, leading to false-negative results and incorrect biological interpretations in some cases. The CV of rates before and after heating was 23.2% while the intrinsic variability commonly accepted by the assurance quality system is between 15% and 20% [10, 11]. For the negative samples, the CV was not as relevant, as the *T. gondii* serological status was not affected. Furthermore, the reproducibility of IgM titration calculated by the manufacturer (bioMérieux) and by our laboratory during routine analysis was 5.3% and 5.0%, respectively, for 163 IgM values around 1.0 and was 13.7% on 242 sera measured after being stored at –20 °C from 1 month to 10 years [10]. The impact of heating on IgM indexes or levels in serum is of major importance while freezing has only a limited effect: on the 36 IgM positive sera, the serological interpretation was modified for 16.7% (6/36) after heating whereas it was different for only 1.2% (3/242) after storage at –20 °C [10]. The main hypothesis for the decrease in IgM levels after heating sera is the production of aggregates composed of IgA, IgM, IgG and albumin resulting from heat-induced aggregation of serum proteins [12]. In our study, the anti-*Toxoplasma* IgM would not have been captured by the solid phase, as they were masked by the aggregates. Therefore, statistically significant differences are observed among all immunoglobulin classes [4].

In conclusion, the decrease of anti-*Toxoplasma* IgM levels observed after heating sera could lead to serological misinterpretation, and therefore sera inactivated by heating at 56 °C for 30 min should not be used for IgM testing using the VIDAS® Toxo IgM assay.

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