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

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, β2-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 papilloma-virus (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
Toxoplasma IgM corresponding to IgM levels that remain detectable several months or years after acute infection. The IgM cut-offs (index) defined by the manufacturer are: negative ≤0.55 positive (P); 0.55 < IgM ≤0.75 equivocal (E); 0.75 < IgM < 0.78 equivocal (N); 0.78 ≤ IgM ≤ 0.80 positive (P). CV, coefficient of variation. aGlobal mean: means of indexes measured in fresh serum and after heating for each category of sera.

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

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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**References**