coagulation indicators, except the free thyroxine test on the AxSYM, which showed an increase to 122%. From these data it is tempting to speculate that the Triton X-100 inactivation treatment could be useful for most commercial assays on high-throughput analyzers. However, it may well be that certain assays are more influenced by the presence of 1 mL/L Triton X-100 than others.

These data suggest that it is possible to protect laboratory workers while offering essential analyses to the intensive or emergency care unit in case of a suspected viral infection with corona, Lassa, Ebola, or Marburg viruses. However, there are two limitations to the use of these inactivation procedures. The first limitation is that all of these procedures require that the transfer of plasma into the second tube and the inactivation procedure be performed by trained personnel in a biosafety level 2 safety cabinet under rigorous safety practices. The second limitation is that although there is evidence for an inactivation of these viruses by heat, there is evidence for effectiveness of Triton X-100 treatment only for the lipid-enveloped HIV and Berne viruses (7, 8) and no published investigation of inactivation of the lipid-enveloped corona, Lassa, Ebola, or Marburg viruses. Nevertheless, the presented data can serve as a resource to estimate the effects on an analyte when one of these inactivation procedures is used as a safety measure to protect workers from lipid-enveloped viruses.

We thank Ruth Böswald, Isabelle Peerboorn, Sabine Pfister, and the staff of the Institute of Clinical Chemistry of the University Hospital Zurich for their efforts to analyze the serum samples before and after the inactivation procedures.

References

DOI: 10.1373/clinchem.2004.031666

Misleading High Thyrotropin Results Obtained with a Two-Site Immunometric Assay Involving a Chimeric Antibody, Rémy Sapin,* Arnaud Agin, and François Gasser (Unité Mixte de Recherche 7004, Université Louis Pasteur/Centre National de la Recherche Scientifique, Faculté de Médecine, Strasbourg Cedex, France; * address correspondence to this author at: Institut de Physique Biologique, Faculté de Médecine, F-67085 Strasbourg Cedex, France; fax 33-3-90-24-40-57, e-mail sapin@ipb.u-strasbg.fr)

Serum thyrotropin (TSH) measurements are widely used in the diagnosis of thyroid dysfunction and to monitor l-thyroxine (T₄) replacement therapy in primary hypothyroidism. The accepted TSH reference interval in serum is 0.4–4.0 mIU/L, and a TSH concentration between 0.5 and 2.0 mIU/L is generally considered as the optimum therapeutic target during replacement therapy (1). Because of technical problems such as interference from heterophilic antibodies reacting with assay anti-mouse antibodies, immunometric assays may give falsely increased TSH values (1). Chimeric antibodies have been introduced in some assays with a view to eliminate interference from anti-mouse antibodies (2). We report here for the first time two cases of misleading high TSH results obtained with an immunometric assay involving a chimeric anti-TSH antibody.

The first case (patient 1), a 56-year-old woman on long-term l-T₄ replacement therapy, was referred to the laboratory for biological thyroid follow-up. Her serum TSH concentration had been slightly above the reference interval for 2 years. The TSH value (5.4 mIU/L) measured with the Elecsys immunometric assay performed on the Elecsys 2010 analyzer (Roche Diagnostics) confirmed this finding. This slightly increased TSH value was in agreement with the previous TSH results but disagreed with the free thyroxine (FT₄) concentration (20.5 pmol/L) measured with the previous TSH results but disagreed with the free thyroxine (FT₄) concentration (20.5 pmol/L) measured with the AxSYM, which showed an increase to 122%. From these data it is tempting to speculate that the Triton X-100 inactivation treatment could be useful for most commercial assays on high-throughput analyzers. However, it may well be that certain assays are more influenced by the presence of 1 mL/L Triton X-100 than others.

This unexpected TSH value prompted further TSH measurements with two other methods. The results were 0.51 mIU/L with the ADVIA Centaur TSH-3 assay (Bayer Diagnostics) and 0.27 mIU/L with the Architect TSH assay (Abbott Diagnostics Division). In the one-step Elecsys assay, the first antibody, a mouse biotinylated monoclonal anti-TSH antibody, and the second anti-TSH antibody, a chimeric antibody (human Fc and mouse Fab fragments) labeled with a ruthenium complex, are mixed with serum. After a 9-min incubation phase, streptavidin-coated microparticles are added, and the mixture is left to incubate for 9 min. In the one-step ADVIA Centaur assay, a monoclonal mouse anti-TSH antibody labeled with acridinium phenyl ester and a polyclonal sheep anti-TSH antibody immobilized on magnetic microparticles are incubated for 7.5 min with serum. The Architect TSH
The assay is a two-step assay. During the first 18-min incubation phase, a mouse monoclonal anti-βTSH antibody fixed to microparticles is mixed with serum. After intermediate washing and addition of a second mouse monoclonal anti-αTSH antibody labeled with acridinium (n-sulfonyl) carboxamide, the mixture is incubated for 4 min (3). The discrepancies between the Elecsys TSH result and the results obtained with the two other assays supported the interference hypothesis. This prompted us to undertake further investigations to characterize the interference with the Elecsys assay.

A falsely increased TSH result as a consequence of anti-TSH antibody was ruled out. Precipitation with polyethylene glycol of radiolabeled bovine TSH, which was the only form available, yielded a normal result (<8%). In our experience samples containing anti-human TSH antibodies are positive with radiolabeled bovine TSH (4). The interference hypothesis was supported by evidence of nonparallel behavior between the suspected serum and the assay calibrators. Two- to eightfold dilutions of the serum with the Elecsys diluent showed measured values decreasing from 72% to 16% of the expected value. A similar decrease was obtained when the serum was diluted with a TSH-free serum (TSH concentration <0.01 mIU/L). Finally, the interference from heterophilic antibodies was evidenced by treating the serum in heterophilic blocking tubes (HBTs) from Scantibodies Laboratory. These tubes contain a prediluted lyophilized active heterophilic antibody-blocking agent designed to neutralize heterophilic antibody interference in immunoassays. Heterophilic antibodies are antibodies exhibiting weak binding and polyspecificity that react with heterogenous antigens (5). In accordance with the manufacturer’s instructions, 500 μL of patient serum was added to a tube and incubated for 1 h at room temperature before TSH measurement. After this treatment TSH measured with the Elecsys assay was 0.62 mIU/L (compared with 5.4 mIU/L before treatment). This treatment had no marked effect on other measurements performed with the Elecsys platform (Table 1).

In two-site immunometric assays, heterophilic antibodies in patient serum can interfere by bridging between the capture antibody and the labeled antibody. Interference from heterophilic antibodies is usually eliminated by adding to the assay reagents nonspecific animal immunoglobulins (or serum) from the species used to produce the test antibodies and/or from other species, such as ox, when the reagent antibodies are mouse antibodies (5). In an attempt to avoid such interference, Roche Diagnostics have used in their TSH assay “special interference-eliminating” reagents and a chimeric antibody (2). A chimeric antibody is a human antibody in which the variable regions (Fab) have been replaced with the corresponding parts of a nonhuman antibody of the desired specificity (mouse anti-TSH antibody in this case). In this way, interference from anti-mouse antibodies directed against the Fc antibody fragment should be eliminated (6). Contrary to the Elecsys TSH assay, Elecsys human chorionic gonadotropin, follicle-stimulating hormone, luteinizing hormone, and prolactin assays are two-site immunometric assays that use mouse monoclonal antibodies rather than chimeric antibodies.

Interference from heterophilic antibodies has been described previously with other TSH assays (7-10). However, to the best of our knowledge this is the first report on such interference in a TSH immunometric assay involving a chimeric antibody. In this patient’s serum, interference arose from anti-animal antibodies. Anti-animal antibodies are specific antibodies produced against animal immunoglobulins (5). Clinical examination and interrogation of the patient revealed that she did not present any marked autoimmune or infectious context, but that for professional reasons she had for 40 years been in close contact with oxen and pigs. The interfering antibodies can be classified as noniatrogenic anti-animal antibodies (6). However, the addition of mouse or bovine serum (10% and 37%, respectively) to the patient’s serum did not produce a decrease in the Elecsys TSH result. Therefore, the interfering antibodies might be idiotypic antibodies that bind to a unique idiotype present on the Elecsys anti-TSH antibodies but not on the Centaur and Architect antibodies. According to information obtained from Bayer Diagnostics and Abbott Diagnostics, their TSH reagents do not contain the Scantibodies HBT blocking agent but do contain other components to minimize interference from heterophilic antibodies and anti-animal antibodies. Anti-chimeric antibodies have been found in patients treated with chimeric antibodies, now used for therapy or imaging purposes (11, 12). To the best of our knowledge, this was not the case here because our patient had not received chimeric antibodies.

The second case (patient 2), a woman in the 9th week of pregnancy, was referred to the laboratory for biological diagnosis of thyroid dysfunction. The very markedly increased Elecsys TSH value (53 mIU/L) contrasted with an increased Elecsys FT4 value (34.2 pmol/L). TSH assayed with two other immunoassays was very low: <0.05 mIU/L with a manual immunoradiometric assay and 0.01 mIU/L with the Vitros ECI (Ortho-Clinical Diagnostics) assay, which uses two mouse monoclonal anti-TSH antibodies. According to information obtained from Ortho-Clinical Diagnostics, Vitros ECI TSH reagents do not

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**Table 1. Effect of HBT treatment on hormonal measurements performed with the Elecsys platform in serum from patient 1.**

<table>
<thead>
<tr>
<th></th>
<th>TSH, mIU/L</th>
<th>FT₄, pmol/L</th>
<th>FT₃, pmol/L</th>
<th>hCG, IU/L</th>
<th>LH, IU/L</th>
<th>FSH, IU/L</th>
<th>PRL, mIU/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>5.4</td>
<td>20.5</td>
<td>4.0</td>
<td>1.1</td>
<td>43</td>
<td>52</td>
<td>188</td>
</tr>
<tr>
<td>After treatment</td>
<td>0.62</td>
<td>21.8</td>
<td>4.3</td>
<td>1.1</td>
<td>44</td>
<td>57</td>
<td>196</td>
</tr>
</tbody>
</table>

*a* FT₃, free triodothyronine; hCG, human chorionic gonadotropin; LH, luteinizing hormone; FSH, follicle-stimulating hormone; PRL, prolactin.
contain the Scantibodies HBT blocking agent but do contain other components to minimize interference from heterophilic antibodies and anti-animal antibodies. The patient serum did not contain anti-TSH antibodies. A 10-fold dilution of the patient’s sample with the Elecsys diluent yielded a TSH result of 60 mIU/L, and 10-fold dilutions with two waste sera that had very low TSH concentrations (<0.01 mIU/L) yielded discrepant results: 33 mIU/L with the first low TSH serum and 66 mIU/L with the second (results corrected for the dilution factor). We had no explanation for the different results obtained with the two sera without TSH. Treating the serum in HBT tubes had no effect on the Elecsys FT4 result (33.3 pmol/L after treatment), but decreased the Elecsys TSH value (30.3 mIU/L after treatment). The TSH result after this treatment remained high and disagreed with the results of the other methods. As already noted (13), HBT treatment does not guarantee a correct result. The addition of 100 mL/L mouse serum had a moderate effect on Elecsys TSH result (measured value, 84% of the expected value), and the further addition of 100 mL/L bovine serum did not decrease the TSH result. The reduction in measured TSH concentration after treatment in HBT tubes and after addition of mouse serum was consistent with interference from heterophilic antibodies. For this patient no complementary clinical data could be obtained. Having run out of serum, we were unable to undertake other investigations regarding either of these two patients.

In conclusion, interference from heterophilic antibodies in TSH assays has become exceptional but still exists (14–16). As expected (5), and clearly exemplified for the first time in TSH assays by these two cases, interference may occur even with immunoassays involving chimeric antibodies. The use of nonspecific blocking agents efficiently protects current immunoassays against interference from isotopic antibodies but probably protects less efficiently against idiotype antibodies (5). Perfect protection against all interfering antibodies remains a goal difficult to reach, and possible interference should be considered, at the time of thyroid diagnosis or during the follow-up of primary hypothyroidism treatment, when the TSH concentration is not compatible with the clinical history or other thyroid function tests.

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DOI: 10.1373/clinchem.2004.031443

Nonglycosylated Ferritin Predominates in the Circulation of Women with Preeclampsia but Not Intrauterine Growth Restriction, Carl A. Hubel,1* Lisa M. Bodnar,1 Ariel Many,2 Gail Harger,2 Roberta B. Ness,1,2 and James M. Roberts1,2 (Magee-Womens Research Institute and Department of Obstetrics and Gynecology and Reproductive Sciences, University of Pittsburgh School of Medicine, Pittsburgh, PA; Department of Epidemiology, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA; and Lis Maternity Hospital, Sourasky Tel Aviv Medical Center, Tel Aviv University, Tel Aviv, Israel; *address correspondence to this author at: Magee-Womens Research Institute, 204 Craft Ave., Pittsburgh, PA 15213; fax 412-641-1-503, e-mail rsicah@mwri.magee.edu)

Three to five percent of pregnancies are complicated by preeclampsia, a multisystemic disorder characterized by hypertension and proteinuria that occurs after 20 weeks of gestation. Although widespread inflammation and endothelial dysfunction appear to be central maternal abnor-