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Thyroglobulin (Tg) Recovery Testing with Quantitative Tg Antibody Measurement for Determining Interference in Serum Tg Assays in Differentiated Thyroid Carcinoma, Adrienne C.M. Persoon,1 Thera P. Links,2 Jürgen Wilde,2† Wim J. Sluiter,1 Bruce H.R. Woffendinbut,1 and Johannes M.W. van den Ouweland2✉ (1 Department of Endocrinology, University Medical Centre Groningen, Groningen, The Netherlands; 2 Nichols Institute Diagnostics, Bad Vilbel, Germany; 3 Canisius-Wilhelmina Medical Centre, Department of Clinical Chemistry, Nijmegen, The Netherlands; ✉ current affiliation: R-biopharm, Darmstadt, Germany; * address correspondence to this author at: Canisius-Wilhelmina Medical Centre, Department of Clinical Chemistry, Weg door Jonkerbos 100, 6500 GS Nijmegen, The Netherlands; fax 31-24-3658671, e-mail j.w.d. ouweland@cww.nl)

**Background:** Thyroglobulin (Tg) measurements are complicated by interference from Tg autoantibodies (TgAbs) or heterophilic antibodies (HAMAs). We used a new automated immunochromiluminoimmunometric assay (ICMA) with Tg recovery (TgR) on the Nichols Advantage® platform to reassess the clinical utility of recovery testing in detecting interference in serum Tg measurement in patients with differentiated thyroid carcinoma.

**Methods:** We used 2 TgAb methods to detect Tg measurement interference with TgR and quantitative TgAb measurement in sera from 127 patients. In a limited number of samples, we used an RIA as comparison method because it appeared to be minimally affected by TgAb.

**Results:** Prevalence of TgAbs was 13% (17 of 127) in either 1 or both TgAb assays. A compromised TgR (<70%) corresponded with TgAb positivity in either TgAb assay for 10 of 11 samples (91%), whereas a normal TgR (≥70%) corresponded with TgAb negativity in both assays for 95 of 101 samples (94%). In 6 TgAb-positive sera with TgR within the reference interval, there were no discrepancies between RIA and ICMA results. We obtained discordant RIA and ICMA results for 6 of 9 TgAb-positive sera with decreased TgR. In 1 TgAb-negative sample, the Tg result was falsely increased because of interference by HAMAs, as shown by an overrecovery of 126%.

**Conclusions:** The Nichols Advantage TgR assay is a valuable complementary method to overcome the technical problem of interference by TgAbs or HAMAs in TgAb assays. Further studies are needed to confirm the potential added value of this TgR assay.

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Serum thyroglobulin (Tg) measurements play a key role in the postsurgical follow-up of patients with differentiated thyroid cancer (DTC) (1), but these measurements are severely hampered by the presence of Tg antibodies (TgAbs), which can cause under- or overestimation of Tg concentration depending on the Tg assay format (2, 3). The National Academy of Clinical Biochemistry guideline (4) recommends the use of sensitive TgAb immunoassays to detect TgAbs in favor of Tg recovery (TgR) testing because TgR testing fails to differentiate TgAb-positive and -negative sera (5, 6). The differences in immunoreactivity between endogenous Tg and Tg added to patient serum, as well as the amount of Tg added and the duration of incubation, all appear critical to the TgR result (5, 7). However, limitations of TgAb testing are also recognized: TgAb concentrations do not correlate with the degree of interference (3, 8); TgAb positivity does not indicate interference per se; substances other than TgAbs can interfere with Tg measurement (9); and TgAb detection is strongly method dependent (10).

A reliable hallmark of TgAb interference is the presence of RIA/immunoassay discordance (4, 10), but intermethod comparisons are impractical because few RIAs are available (10). Therefore, the technical problem of TgAb interference in Tg measurements has not been overcome. The release of a new TgR assay enabled us to reassess the clinical utility of recovery testing in detecting interference in serum Tg measurements by comparing the TgR assay with a quantitative TgAb test and the methodologic benchmark for TgAb interference, RIA/immunometric assay discordance (10), in relation to the clinical status of the patient.

We collected sera from 127 patients with DTC undergoing thyroid hormone suppression therapy who visited our outpatient clinic between May and September 2003. No evidence of disease was defined as absence of clinical, scinti-
graphic, or radiologic evidence of recurrent or persistent disease, including undetectable Tg with an IRMA [ELSA-hTg; CIS Bio international; functional sensitivity (defined as the lowest concentration for which the interassay CV did not exceed 20%), 1.5 μg/L] during thyroid hormone suppression therapy for at least 1 year (for the clinical characteristics of the patients, see Table 1 in the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol52/issue6).

We performed serum Tg measurements with a fully automated 2-step immunochemiluminometric assay (Tg-ICMA) that used 3 monoclonal antibodies (Advantage®; Nichols Institute Diagnostics). The assay characteristics will be reported elsewhere (11).

In a limited number of samples, we used an RIA (Tg-RIA) as the comparison method because it appeared minimally affected by TgAb (5, 10).

Both the Tg-ICMA and Tg-RIA were calibrated against the CRM 457 reference preparation, but the Tg-ICMA showed a 1.8-fold higher Tg reading, likely reflecting differences in assay specificity for circulating Tg isoforms (11). Tg ICMA/RIA discordance, as a methodologic benchmark for TgAb interference, was defined as being present when there was a difference between the results obtained by both Tg assays after correction for the 1.8-fold difference and each method’s reproducibility (CV). TgAb measurements were performed either by chemiluminescence immunoassay (TgAb-ICMA performed on an Advantage®; Nichols Institute Diagnostics) or by microparticle enzyme immunoassay (TgAb-MEIA performed on an AxSYM®; Abbott Diagnostics). Interassay imprecision profiles, as determined by measuring human DTC serum pools with various TgAb concentrations in 20 runs over a 2-week period with 2 different lots of reagents, were 79%, 19%, and 13% at 0.1, 2, and 75 kilounits/L, respectively, for the TgAb-ICMA and 23%, 11%, and 7% at 31, 155, and 522 kilounits/L, respectively, for the TgAb-MEIA. The cutoff values used for TgAb positivity, based on the lowest TgAb concentrations in serum for which the interassay CV did not exceed 20% (functional sensitivity), were 2 and 45 kilounits/L for the TgAb-ICMA and TgAb-MEIA, respectively. These values were slightly higher than the manufacturers’ cutoff limits of 1 and 34 kilounits/L, respectively. Both TgAb assays are referenced to the WHO Tg autoantibodies First International Reference Preparation (WHO 65/93).

We performed TgR testing with a new recovery assay on the Nichols Advantage platform by adding 10 μg/L of purified human Tg in DTC sera with Tg-ICMA concentrations <80 μg/L. We measured Tg in sera with (Tg2) and without (Tg1) added Tg (aTg) and calculated the percentage recovery from the formula:

\[
\frac{Tg2 - Tg1}{aTg} \times 100
\]

Tg, TgAb, and TgR results and the clinical data for all TgAb-positive patients are summarized in Table 1.

The prevalence of TgAbs was 13% (17 of 127) in either one or both TgAb assays. TgAbs were detected in 12% (16 of 127) of samples with the TgAb-ICMA and in 10% (13 of 127) with the TgAb-MEIA, and 9% (12 of 127) showed positivity in both assays. The intermethod variability for the detection of TgAbs likely resulted from differences in assay sensitivity and specificity despite standardization against WHO 65/93 (10). The prevalence of TgAbs in our DTC patients (10%–12%) seemed to be lower than the prevalences reported by others (20%–30%) (5, 12). Nevertheless, our results correspond with previous data (9) reporting a TgAb prevalence in DTC patients of 29% at initial examination, decreasing to <10% after 3 years of follow-up. The median follow-up in our study was 4 years.

Evidence of disease was present in 3 of 17 (18%) TgAb-positive patients. Two patients (patients 12 and 16 in Table 1A) had extensive metastatic disease. In the third patient (patient 8), TgAbs were measured shortly after radioiodine ablation therapy. Tg was detectable by Tg-ICMA in 2 of these 3 patients (Table 1A).

We determined a reference interval for TgR by performing recovery testing in 96 TgAb-negative DTC sera. We obtained a nongaussian distribution (P < 0.05, Shapiro–Wilk normality test) with a slight left tail, with recoveries of 67%–125% (mean, 96%; 95% confidence interval, 70%–120%; see Fig. 1A in the online Data Supplement). TgR <80% might reflect some form of interference, e.g., by autoantibodies not recognized by the TgAb assay or from substances other than TgAbs. To reveal possible interference, we used the Tg-RIA to retest samples from 5 of 7 patients with TgR values of 67.5% to 76.4% and a Tg-ICMA result <0.6 μg/L. The Tg-ICMA and Tg-RIA results were concordant for 4 of 5 sera, providing no evidence for interference in Tg measurements. In 1 TgAb-negative patient with a slightly compromised recovery (67.5%), Tg RIA/ICMA discordance (1.1 μg/L by Tg-RIA vs <0.6 μg/L by Tg-ICMA) supported the recovery result, indicating serum Tg interference.

We suspected overrecovery in 1 sample with a TgR of 126%, a serum Tg-ICMA result of 8.6 μg/L, and Tg that was undetectable by RIA (<1 μg/L) and by IRMA (<1.5 μg/L; ELSA-hTg; CIS Bio international). In this sample, the Tg-ICMA result was falsely increased as a result of interference by heterophilic antibodies (11). We therefore used 70%–120% as the reference interval for TgR.

TgR differed significantly in TgAb-negative (n = 96) and TgAb-positive sera (n = 17; P < 0.0005, Mann–Whitney U-test). The TgR was compromised (<70%) in 91% of samples (10 of 11) that tested positive for TgAbs in both TgAb assays, whereas the TgR was within the reference interval (>70%) for 95% of samples that were negative for TgAbs in the TgAb-ICMA (96 of 101) and 97% of samples (98 of 101) that were negative in the TgAb-MEIA (Table 1B).

Ten of 12 (83%) sera with positivity for TgAbs in both
assays had compromised TgR. Considering the TgAb assays separately, 67% of the sera positive in the TgAb-ICMA (10 of 15) and 77% of the sera positive in the TgAb-MEIA (10 of 13) showed TgR <70% (Table 1). Patients with higher TgAb titers showed more compromised recovery \[r = 0.79\ (P < 0.0003)\] for TgAb-ICMA, \[r = 0.55\ (P < 0.03)\]; see panels B and C, respectively, of Fig. 1 in the online Data Supplement\]. Sera that were positive only in the TgAb-ICMA \(n = 3\) or the TgAb-MEIA \(n = 1\) had TgR within the reference interval (Table 1A) and relatively low antibody titers.

Remeasurement of 6 TgAb-positive sera (patients 4, 8, 13, 14, 15, and 17 in Table 1A) by Tg-RIA to determine whether TgR was falsely unaffected showed no RIA/ICMA discordance, thus favoring the outcome of the TgR results. The results for patient 8, with TgR within the reference interval, illustrate that despite TgAb positivity in both TgAb assays, serum Tg was measurable by the Tg-ICMA and seemed unaffected on the basis of a comparison with the Tg-RIA result (Table 1A). Follow-up studies are needed to determine whether low TgAb positivity in sera with TgR within the reference interval indicates disease activity or represents signal noise, a distinction of major concern in clinical practice. The detection of TgAbs in a patient means the loss of clinical utility of Tg measurements. In addition, the presence of supposed TgAb positivity might prompt unnecessary

### Table 1. Clinical data for TgAb-positive patients and assay results.

<table>
<thead>
<tr>
<th>Patient</th>
<th>TgICMA, µg/L</th>
<th>TgRIA, µg/L</th>
<th>TgAb-ICMA, kilounits/L</th>
<th>TgAb-MEIA, kilounits/L</th>
<th>TgR, %</th>
<th>Age (years)/Sex</th>
<th>Histologya</th>
<th>TNMb</th>
<th>Follow-up, years</th>
<th>Disease statec</th>
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<tr>
<td>1</td>
<td>&lt;0.6</td>
<td>&lt;1</td>
<td>4.4</td>
<td>46.9</td>
<td>68</td>
<td>43/F</td>
<td>P</td>
<td>T2N1M0</td>
<td>1</td>
<td>NED</td>
</tr>
<tr>
<td>2</td>
<td>&lt;0.6</td>
<td>4.9</td>
<td>94.6</td>
<td>207.5</td>
<td>25</td>
<td>73/F</td>
<td>P</td>
<td>T3N1M0</td>
<td>1</td>
<td>NED</td>
</tr>
<tr>
<td>3</td>
<td>&lt;0.6</td>
<td>NA</td>
<td>18.6</td>
<td>48.6</td>
<td>63</td>
<td>76/F</td>
<td>P</td>
<td>T2N1M0</td>
<td>27</td>
<td>NED</td>
</tr>
<tr>
<td>4</td>
<td>&lt;0.6</td>
<td>&lt;1</td>
<td>20.0</td>
<td>74.8</td>
<td>74</td>
<td>61/F</td>
<td>P</td>
<td>T3N0M0</td>
<td>2</td>
<td>NED</td>
</tr>
<tr>
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<td>&lt;0.6</td>
<td>&lt;1</td>
<td>17.6</td>
<td>49.5</td>
<td>56</td>
<td>82/F</td>
<td>P</td>
<td>T4N0M0</td>
<td>3</td>
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</tr>
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<td>6</td>
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<td>1.4</td>
<td>89.6</td>
<td>133.0</td>
<td>64</td>
<td>49/M</td>
<td>P</td>
<td>Tn1M0</td>
<td>19</td>
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<tr>
<td>7</td>
<td>&lt;0.6</td>
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<td>119.9</td>
<td>140.6</td>
<td>62</td>
<td>45/F</td>
<td>P</td>
<td>T4N1M0</td>
<td>4</td>
<td>NED</td>
</tr>
<tr>
<td>8</td>
<td>4.5</td>
<td>2.1</td>
<td>2.1</td>
<td>91.7</td>
<td>89</td>
<td>37/F</td>
<td>P</td>
<td>T2N0M1</td>
<td>0</td>
<td>ED</td>
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<tr>
<td>9</td>
<td>&lt;0.6</td>
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<td>34.5</td>
<td>64.0</td>
<td>45</td>
<td>41/F</td>
<td>P</td>
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<td>10</td>
<td>&lt;0.6</td>
<td>6.5</td>
<td>760.4</td>
<td>&gt;1000</td>
<td>40</td>
<td>39/F</td>
<td>P</td>
<td>Tn1M1</td>
<td>29</td>
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<tr>
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<td>&lt;1</td>
<td>44.3</td>
<td>125.4</td>
<td>62</td>
<td>77/F</td>
<td>P</td>
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<td>NED</td>
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<tr>
<td>12</td>
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<td>133.7</td>
<td>464.3</td>
<td>34</td>
<td>76/F</td>
<td>P</td>
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<td>1</td>
<td>ED</td>
</tr>
<tr>
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<td>&lt;0.6</td>
<td>&lt;1</td>
<td>5.1</td>
<td>20.7</td>
<td>85</td>
<td>27/F</td>
<td>P</td>
<td>T2N0M0</td>
<td>11</td>
<td>NED</td>
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<tr>
<td>14</td>
<td>&lt;0.6</td>
<td>&lt;1</td>
<td>5.7</td>
<td>23.7</td>
<td>82</td>
<td>25/F</td>
<td>P</td>
<td>T3N1M0</td>
<td>10</td>
<td>NED</td>
</tr>
<tr>
<td>15</td>
<td>&lt;0.6</td>
<td>&lt;1</td>
<td>3.4</td>
<td>23.8</td>
<td>95</td>
<td>67/F</td>
<td>P</td>
<td>T2N0M0</td>
<td>2</td>
<td>NED</td>
</tr>
<tr>
<td>16</td>
<td>4167</td>
<td>NA</td>
<td>2.3</td>
<td>20.7</td>
<td>NDc</td>
<td>70/F</td>
<td>H</td>
<td>T4N0M1</td>
<td>0</td>
<td>ED</td>
</tr>
<tr>
<td>17</td>
<td>&lt;0.6</td>
<td>&lt;1</td>
<td>&lt;2.0</td>
<td>49.1</td>
<td>106</td>
<td>66/F</td>
<td>P</td>
<td>T4N0M0</td>
<td>27</td>
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<table>
<thead>
<tr>
<th>TgAb assay</th>
<th>TgR &lt;70%, n</th>
<th>TgR ≥70%, n</th>
<th>Total, n</th>
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<tr>
<td>ICMA</td>
<td>10</td>
<td>5</td>
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</tr>
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<td>Negative</td>
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</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>101</td>
<td>112</td>
</tr>
<tr>
<td>MEIA</td>
<td>10</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>98</td>
<td>99</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>101</td>
<td>112</td>
</tr>
</tbody>
</table>

a Bold numbers indicate measurable serum Tg, TgAb positivity, or compromised TgR.
b Cutoff for positivity, 2 kilounits/L.
c Cutoff for positivity, 45 kilounits/L.
d Histology: P, papillary; H, Hürthle cell.
e TNM classification based on criteria listed in Ref. (14).
f Disease state at time of sampling, see text. NED, no evidence of disease; ED, evidence of disease.
g NA, not assessed.
h ND, recovery not determined because of high serum Tg concentration.
imaging and promote unnecessary patient concern. TgR measurements might be helpful in these particular cases.

We also performed Tg-RIA measurements in 9 of 10 sera that were positive for TgAbs in both assays and showed compromised TgR. In 3 cases (patients 1, 5, and 11), intermethod comparison provided no further evidence for interference in the serum Tg measurement, whereas in 6 cases (patients 2, 6, 7, 9, 10, and 12), RIA/ICMA discordance confirmed Tg interference. Remarkably, only 1 of these patients (patient 12) had known disease activity: the other patients with considerably high TgAb titers and compromised TgR had no evidence of disease and had been in follow-up for up to 29 years (range, 1–29 years). Several studies have shown that persistence of TgAb positivity during long term follow-up may be representative of persistent disease (5,13), whereas serum TgAb concentrations decrease or disappear in disease-free patients. Accordingly, TgAb positivity in conjunction with compromised TgR and ICMA/RIA discordance in these patients should alert the clinician. Serial TgAb measurements as surrogate tumor markers (10) and follow-up will show whether these patients really have persistent or recurrent disease.

In this study, we showed that testing for TgR by the Nichols Advantage TgR assay has value complementary to that of quantitative TgAb measurement in the detection of interference in Tg measurements, in particular in sera with low TgAb titers. Furthermore, this method can detect interference from TgAbs not detected by direct TgAb measurement or from other interfering substances, such as heterophilic antibodies. Further studies are needed to confirm the potential added value of this TgR assay. We would like to emphasize that the results observed with the Nichols Advantage TgR assay cannot be transposed to other TgR assays (5,6).

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