Heterophilic antibodies are human antibodies that can bind animal antibodies. They can cause problems in immunoassays, particularly immunometric assays, where they can form a bridge between the capture and detection antibodies, leading to a false-positive result in the absence of analyte or, if analyte is also present, to a false increase in measured concentrations. Very rarely, heterophilic antibodies can also lead to false-negative or falsely low results. By adding blocking reagents, assay manufacturers have reduced the incidence of heterophile interferences from the 2–5% observed in unblocked assays but have been unable to completely eliminate the problem (1, 2).

During the last 10 years, tumor marker assays for human chorionic gonadotropin (hCG), prostate-specific antigen (PSA), cancer antigen 125 (CA 125), carcinoembryonic antigen, and calcitonin have all been reported to suffer from this problem, frequently with undesirable clinical outcomes (3–7).

In our own laboratory, we noted that our thyroglobulin assay displayed a higher than acceptable rate of heterophile interference (8). Although we were able to rectify this problem through pretreatment of samples in heterophile blocking tubes (HBT) (8), we became concerned that some of our other tumor marker assays may suffer from similar problems. We therefore decided to systematically evaluate all of our tumor marker assays for evidence of interference from heterophilic antibodies.

Our laboratory performs the following tumor marker assays: calcitonin on the Nichols Advantage, gastrin on the Immulite 2000, CA 125 and cancer antigen 15-3 (CA 15-3) on the Vitros ECi, and a-fetoprotein (AFP), hCG, total PSA, and free PSA on the Beckman Access. All of these assays use at least one mouse monoclonal antibody (Table 1).

We designated the maximum tolerable heterophile interference rate for any of these assays as 0.5% and started collecting samples prospectively. Power calculations suggested that 500 specimens would give us >80% power to detect a doubling of the heterophile interference rate. Because of much lower test volumes for calcitonin and gastrin, we aimed for 200 samples for these tests, somewhat reducing the statistical power for these two assays. All specimens except those with insufficient volume for heterophile interference testing were included in the collection. We used comparison of paired results of untreated samples and samples pretreated with HBT or heterophile-blocking reagent (HBR1 nonmurine, hCG assay only; both from Scantibodies) to determine whether heterophile interferences had occurred. HBT/HBR blocking reagents consist of immunoglobulins that have been selected to bind human heterophilic antibodies, and they block the majority of heterophile interferences (5, 8–10).

Before and during our evaluation of heterophile interference rates, we ensured that HBT treatment did not affect assay performance adversely by comparing the means, medians, and variability of at least 50 paired untreated and HBT-treated samples for each assay. These variables were also continuously monitored during the entire study. Assay performance was comparable to that of untreated samples for all assays except hCG. Eleven of 59 samples showed a marked increase in apparent hCG concentrations after HBT pretreatment. We determined through discussion with Scantibodies that assays configured with an antibody complex (goat anti-mouse antibody–mouse monoclonal antibody in our case) could give spurious results when treated with HBT. HBR1 nonmurine reagent is reputed to overcome this limitation. We therefore validated the use of HBR1 in our hCG assay by performing intra- and interassay precision, recovery (from samples with added analyte), and linearity studies after HBR1 treatment. The performance characteristics with HBR1 were comparable to the native hCG assay, with linearity ranging from 95% to 117%, recovery ranging from 91% to 99%, and inter- and intrain assay CVs <10% across the measurement range. The mean CV for paired untreated and HBR1-treated samples was <4% (95% confidence interval, 0–19%).

For each assay, the mean, median, range, and SD were established for the observed absolute and percentage differences between paired untreated and pretreated samples. Results were plotted in modified Bland–Altman plots with the mean of paired original and post-HBT treatment measurements on the abscissa and the percentage difference between paired original and post-HBT treatment measurements on the ordinate. An example modified Bland–Altman plot can be seen in the figure in the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol51/issue1/. Our formal criteria for deciding whether an outlying data point may be attributable to heterophilic antibody interference were as follows:

(a) A percentage difference between the initial result and HBT/HBR treatment result that exceeded a 3 SD percentage difference was considered a possible heterophile interference.

(b) Any result deviating from the mean by more than a 5 SD percentage difference or any sample whose value decreased by more than 80% after HBT/HBR treatment was considered definite heterophile interference.

All possible and definite heterophile interferences were further evaluated for their potential clinical significance, with all cases that were likely to lead to different patient management being regarded as clinically significant. The results are summarized in Table 1. We observed no significant systematic bias attributable to HBT/HBR treat-
For each of the eight assays, we observed at least one result outside the ±3 SD percentage difference range. The rates of possible heterophile interferences ranged from 0.2% (AFP) to 3.7% (calcitonin), with an overall mean of 1.4% and median of 1%. However, in most instances the outlying results fell just outside the ±3 SD percentage boundaries, and the observed changes after HBT/HBR treatment were not consistently downward, as is typical for samples affected by heterophile interference (Fig. 1). The possible heterophile interference rates significantly exceeded the a priori prevalence threshold of 0.5% only for calcitonin (7 of 192; 3.7%; \( P < 0.037 \), two-tailed Fisher exact test) and hCG (13 of 510; 2.5%; \( P < 0.023 \), two-tailed \( \chi^2 \) test, 1 degree of freedom, Yates-corrected). Changes greater than a 5 SD percentage difference or a decrease in value >80% after HBT/HBR treatment, indicative of definite heterophile interference, were observed in only two cases (one each for hCG and free PSA). One of these, which decreased the patient’s hCG concentration from 5.5 IU/L to 1 IU/L, was considered potentially clinically relevant.

The assays examined do not suffer from major problems with heterophile interference. With the exception of the calcitonin and the hCG assay, the observed rates of possible heterophile interference were consistent with the a priori-determined acceptable range. However, even for these two assays, the majority of results outside our predefined cutoff lay just outside these limits and did not display the typical >80% decrease in value after HBT/HBR treatment that is indicative of definite heterophile interferences. It is likely that the majority of these slight outliers reflect a minimal increase in assay variability attributable to the HBT/HBR treatment.

The manufacturers of the assays used in our laboratory have been largely successful in minimizing the incidence

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**Table 1. Summary of heterophile interference testing.**

<table>
<thead>
<tr>
<th>Analytes (units)</th>
<th>Assay configuration</th>
<th>n</th>
<th>Result range of tested sera</th>
<th>Mean value untreated/mean value pretreated</th>
<th>Median value untreated/median value pretreated</th>
<th>Sera with &gt;3 SD% difference untreated/pretreated</th>
<th>Sera with &gt;3 SD% difference untreated/pretreated</th>
<th>( n \ (% )</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFP (g/L)</td>
<td>Two-site; one step; two MMABs</td>
<td>505</td>
<td>0.5–9600</td>
<td>3.4/3.1</td>
<td>0.2/0.2</td>
<td>1 (0.2)</td>
<td>1 (0.2)</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>Calcitonin (ng/L)</td>
<td>Two-site; one step; two MMABs</td>
<td>192</td>
<td>3 to 28</td>
<td>138.4/138.3</td>
<td>11/11</td>
<td>5 (3.7)</td>
<td>5 (3.7)</td>
<td>5 (3.7)</td>
</tr>
<tr>
<td>CA 125 (kilounits/L)</td>
<td>Two-site; one step; two MMABs</td>
<td>508</td>
<td>1.3–9775</td>
<td>138.4/138.3</td>
<td>11/11</td>
<td>5 (3.7)</td>
<td>5 (3.7)</td>
<td>5 (3.7)</td>
</tr>
<tr>
<td>CA 15-3 (kilounits/L)</td>
<td>Two-site; one step; two MMABs</td>
<td>203</td>
<td>8–4000</td>
<td>138.4/138.3</td>
<td>11/11</td>
<td>5 (3.7)</td>
<td>5 (3.7)</td>
<td>5 (3.7)</td>
</tr>
<tr>
<td>Gastrin (ng/L)</td>
<td>Two-site; one step; two MMABs and one PGAB</td>
<td>203</td>
<td>&lt;20 to 711</td>
<td>146.2/144.9</td>
<td>99.8/99.1</td>
<td>2 (1)</td>
<td>2 (1)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>hCG (IU/L)</td>
<td>Two-site; one-step, one GAM-MMAB complex and one PRAB</td>
<td>510</td>
<td>0.5 to 464 000</td>
<td>146.2/144.9</td>
<td>99.8/99.1</td>
<td>2 (1)</td>
<td>2 (1)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Free PSA (g/L)</td>
<td>Two-site; one step, two MMABs</td>
<td>500</td>
<td>0.1–11.5</td>
<td>0.9/0.8</td>
<td>0.7/0.7</td>
<td>5 (1)</td>
<td>5 (1)</td>
<td>5 (1)</td>
</tr>
<tr>
<td>Total PSA (g/L)</td>
<td>Two-site; one step; two MMABs</td>
<td>500</td>
<td>0.1–29</td>
<td>0.9/0.8</td>
<td>0.7/0.7</td>
<td>5 (1)</td>
<td>5 (1)</td>
<td>5 (1)</td>
</tr>
</tbody>
</table>

\( a \) Pretreated in HBT tubes (all analytes except hCG) or with HBR1 (hCG only).

\( b \) MMAB, monoclonal mouse antibody; PGAB, polyclonal goat antibody; GAM, goat anti-mouse antibody; PRAB, polyclonal rabbit antibody.

\( c \) One sample with >3 SD% difference of untreated vs pretreated, clinically significant in the case of hCG.

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**Fig. 1.** Samples with >3 SD percentage difference after HBT pretreatment.

Changes in analyte concentrations after HBT pretreatment (all analytes except hCG) and HBR1 treatment (hCG only) for all 43 samples that displayed a >3 SD percentage difference after HBT/HBR treatment. Untreated values are depicted on the left of each panel, and concentrations after treatment are on the right. The lines of several samples overlap.
of heterophile interference in tumor marker assays. This is a particular achievement considering that cancer patients often display tumor-induced activation of their immune system or may suffer from infections. Both of these conditions can lead to polyclonal antibody production. Given sufficient incubation time, these would be bound by blocking reagents, but in modern automated assays, reactions are rarely allowed to reach equilibrium and there may be insufficient time to achieve complete blocking. In addition, modern assays are often configured with several mouse monoclonal antibodies rather than a mouse monoclonal antibody and a polyclonal antibody from another species. With the increasing use of mouse monoclonal antibodies in diagnostic imaging and medical therapy for malignancies, and the resulting immunization of the recipients, the potential for interference increases significantly (1,7) for assays that use multiple mouse monoclonal antibodies. Consequently, we cannot assume that assays other than those included in our study perform equally well for the same analytes. For example, one particular hCG assay continues to experience a considerable number of clinical problems attributable to heterophile interference, representing the majority of such problems reported (11).

There were, however, even in our study two results that followed the typical heterophile interference pattern. In one case, a free PSA result, this would not have led to a change in patient management. By contrast, in the second case, a falsely increased hCG, clinical management might have changed depending on the clinical circumstances. hCG assays, mainly from one particular manufacturer, continue to be the single most important source of erroneous test results attributable to heterophile antibody interference (11,12). Clinical correlation is therefore particularly important when interpreting hCG results.

In most suspected cases of heterophile interference, HBT treatment is a convenient way to verify and correct the problem. However, our experience shows that for assays other than those included in our study performed equally well for the same analytes. For example, one particular hCG assay continues to experience a considerable number of clinical problems attributable to heterophile interference, representing the majority of such problems reported (11).

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