Interference by Anti-Immunoglobulin G Antibodies in Immunoradiometric Assays of Thyrotropin Involving Mouse Monoclonal Antibodies

Mark H. Zweig, Gyorgy Csako, Carol C. Benson, Bruce D. Weintraub, and Barbara B. Kahn

"Sandwich"-type assays are subject to positive interference by the patient's "heterophile" antibodies. If present, these bind to the animal immunoglobulins in the assay reagents, forming artefactual sandwiches indistinguishable from those formed with the analyte itself. Immunoglobulins from non-immunized animals, added to the assay reagents, can diminish this effect by blocking the patient's antibodies. Elsewhere, we studied several patients with anti-mouse immunoglobulin activity, whose sera gave spuriously high results for thyrotropin (TSH) concentrations. Here we have studied this phenomenon by adding, to pooled zero-TSH serum, antibodies to mouse, goat, and horse immunoglobulins and then assaying TSH by several other sandwich-type assays involving mouse monoclonal antibodies. Assays not supplemented with blocking immunoglobulins from mice or other animals were more susceptible to this effect. When large amounts of antibody were added, the antibody excess diminished the interference. However, the presence of blocking immunoglobulins could reverse such antibody excess, actually enhancing, instead of diminishing, the positive interference. Users should be aware that blocking immunoglobulins may diminish but not necessarily eliminate this problem with such assays.

Additional Keyphrases: analytical error · heterophile antibodies · "sandwich"-type assays · thyroid status · peptide hormones

The availability of mouse monoclonal antibodies has increased the effectiveness of two-site ("sandwich") immunoassays and hence their numbers in the marketplace. These assays offer much in terms of convenience, analytical sensitivity, and specificity, and allow a wide working range of analyte concentration. However, the presence of endogenous antibodies in patients' specimens can lead to positive interference, presumably by simulating the behavior of an analyte molecule to link the labeled ("signal") antibody to the solid-phase ("capture") antibody. These artefactual sandwiches, which are not distinguishable from the sandwiches designed to be formed by specific binding of mouse antibody to the analyte, have been observed in both monoclonal and polyclonal sandwich-type immunoassays (1-12). Thompson et al. (6) found evidence of anti-mouse antibodies in 9% of 1008 blood donors screened.

We studied three patients for whom falsely high concentrations of thyrotropin (thyroid-stimulating hormone, TSH), were measured with the Serono "MAIAclone" immunoradiometric assay (IRMA), a three-site sandwich immunoassay (manuscript submitted). The patients' antibodies reacted with the mouse antibodies in the assay, but the interference could be blocked by adding immunoglobulins to "absorb" the patients' antibodies in solution or by absorbing the patients' antibodies onto solid-phase CH-Sepharose 4B coupled to mouse IgG1. In all three patients the actual concentrations of thyrotropin were normal or subnormal; two were being treated with thyroid hormone inappropriately.

Because this interference can be diminished or eliminated by including nonspecific animal immunoglobulins in the reagents, some manufacturers have added immunoglobulins to their assay mixtures, either from the same species as the reagent antibodies or from other species. The Serono assay we used had added sheep and calf serum but not mouse serum. This addition decreased some interferences, but it did not remove all of the interference from the three patients' sera. However, three other TSH assays, which contained blocking mouse immunoglobulin in the kit reagent, did not show any interference with these patients. (The Serono assay has since been modified, eliminating the interference in these three patients.) We have now further examined this interference by using the conventional Serono TSH kit as well as a version in which the sheep and calf sera were omitted. We studied the ability of exogenous anti-IgG antisera to reproduce the interference and the blockage of this interference by added mouse serum. We then also investigated the susceptibility of three other TSH assays—all based on mouse monoclonal antibodies—to this phenomenon.

Materials and Methods

We studied four sandwich-type mouse monoclonal assays for human TSH, all used according to the manufacturers' instructions.

Assay A, "TSH3 MAIAclone" (Serono Diagnostics, Inc., Norwell, MA), a three-site IRMA, was in routine use in the laboratory and was used the most extensively. The radiolabeled antibody reagent contained sheep and calf serum to reduce nonspecific interactions between patient's serum or other source of mouse immunoglobulin was added other than the specific monoclonal anti-TSH antibodies. Serono generously also supplied us with labeled antibody reagent without added sheep or calf serum. Assays with this reagent are designated as the Serono "special" assay, as opposed to the conventional assay.

Assay B is a two-site mouse monoclonal IRMA: "Tandem-R TSH" (Hybritech, Inc., San Diego, CA 92121).

Assay C is a two-site mouse monoclonal IRMA with a novel sucrose separation procedure: "Sucrosep TSH" (Boots-Celltech Diagnostics Ltd., U.K.).

Assay D is also a two-site IRMA: "Allegro HS-TSH" (Nichols Institute Diagnostics, San Juan Capistrano, CA 92675).

Assays B, C, and D all contain mouse immunoglobulins, added to decrease interference from endogenous anti-mouse antibody activity.

We purchased goat anti-mouse IgG (protein concentration 44.3 g/L) from Meloy Laboratories, Inc., Springfield, VA.

1 Clinical Pathology Department, Clinical Center, National Institutes of Health, Bethesda, MD 20892.

2 Molecular Cellular and Nutritional Endocrinology Branch, NIDDK, National Institutes of Health, Bethesda, MD 20892.

3 Current address: Diabetes Unit, Beth Israel Hospital, 330 Brookline Ave., Boston, MA 02215.

Received November 18, 1986; accepted March 11, 1987.
22151, and goat anti-mouse IgG1 (27.6 g/L), rabbit anti-
horse IgG (affinity isolated, 5 g/L), and rabbit anti-goat IgG
(33.6 g/L) from Sigma Chemical Co., St. Louis, MO 63178.
The Sigma products, obtained lyophilized, were reconstituted
to give the concentrations indicated.

For dilution curves, patients' sera were serially diluted
twofold with zero-TSH pooled patients' serum (<0.1 milli-
int. units/L). Studies in our laboratory indicate that dilutions of
normal specimens (with TSH concentrations within the
range of the standard curve) give a linear assay curve. In all
cases, the sample volumes designated by the manufacturer
were utilized.

The blocking experiment was performed by including
mouse serum (1 μL per tube) in the various mixtures of zero-
TSH pooled patients' serum and goat anti-mouse IgG. The
mixtures were then treated the same as a conventional
sample.

In the figures, TSH results are designated by asterisks if
the number of counts bound in the assay exceeded that of
the highest standard (50 milli-int. units/L). These results (obtained
by extrapolation) are shown to indicate the approxi-
mate position of the peak and the shape of the curve.
Our dilution studies of patients' sera with concentrations of
TSH between 50 and 100 milli-int. units/L indicate that
these concentrations may be underestimated by about 10 or
15%. Thus, these estimates of TSH in specimens with
concentrations >50 milli-int. units/L are close enough to
provide useful information about the shape of the interfer-
ence curves.

Results

Interference by Anti-Mouse Immunoglobulins in Assay A

Previous work with the first patient studied (manuscript
submitted) revealed that addition of either mouse IgG or
IgG1 blocked the interference in Assay A. Later, we were
informed that Assay A was indeed formulated with mouse
IgG1 monoclonal antibodies. Therefore, we added various
amounts of anti-mouse IgG or anti-mouse IgG1 to zero-TSH
pooled patients' sera to simulate the interference observed
in the case of patients with endogenous anti-mouse activity.
The mixtures were treated in the assays as normal samples.

Figure 1 shows that both animal antisera produced spuri-
ously high TSH concentrations, which varied according to
the amount of antibody added. At the higher amounts of
added antibody, a zone of antibody excess with relatively

Fig. 1. TSH concentrations observed with Assay A when goat anti-
mouse IgG (circles) or goat anti-mouse IgG1 (triangles) was added to
zero-TSH pooled human serum.

In Figs. 1–4, asterisks indicate observations that, because they exceed values for
the highest-concentration standard, are approximations.

Fig. 2. TSH concentrations measured in various amounts of three
patients' sera with the "special" version of Assay A.
Sera were diluted with zero-TSH pooled serum.

low apparent TSH concentration occurred for both types of
antisera (see right-hand side of Figure 1). The plots of TSH
per microgram of protein illustrate where antibody excess is
occurring. As one moves from right to left along the curve,
antibody excess is seen to be present as long as observed
TSH per microgram of protein added is below the maximum
(plateau at left).

The antibody excess shown in Figure 1 could occur with
patients' sera if sufficient amounts of endogenous anti-
mouse antibody were present. Figure 2 shows the TSH
observed with use of the "special" assay to eliminate all
suppression, for various amounts of serum from the three
patients who exhibit this interference. The usual sample
volume is 200 μL. Patients 2 and 3 have TSH concentra-
tions that are off the curve at 200 μL (undiluted serum). None
of these exhibits a decrease in apparent TSH as the curve
progresses to the right (greater amount of patient's serum
present). However, the TSH per unit volume of whole
patient's serum present is greatest at 50 μL, not 200 μL, for
patient 1. At 100 and 200 μL, then, patient 1 exhibits a
rather decreased efficiency of interference, suggesting that
there is some antibody excess. This point of maximal
interference cannot be determined for patients 2 and 3,
because the observed TSH concentrations at 200 μL are
off the standard curve and are therefore approximations.

If antibody excess at high concentration results in a
diminished "efficiency" of interference, then the addition of
blocking mouse proteins evidently could actually increase
the interference by decreasing the amount of antibody
excess. To test this, we assayed goat anti-mouse IgG₁ (in a zero-TSH serum pool) with and without the addition of 1 µL of normal mouse serum (Figure 3). The mouse serum did shift the curve to the right, resulting in increased interference at some points. For example, the apparent TSH concentration with 1.25 µL of anti-mouse IgG₁ increased from 17.1 to 47.7 milli-int. units/L.

Interference by Anti-Mouse Immunoglobulins in Assays B, C, and D

In our earlier work, Assays B, C, and D did not exhibit the interference with patients' sera that we observed with Assay A. Therefore, we tested the effect of goat anti-mouse IgG and IgG₁, added to a zero-TSH serum pool, on results of those assays. Even up to 2200 µg of added goat anti-mouse IgG, less than 5 milli-int. units of TSH per liter was observed with Assays C and D (data not shown). Assay B, on the other hand, yielded a maximum value of apparent TSH of 54.5 milli-int. units/L at 220 µg of added protein. By comparison, the highest apparent TSH value for Assay A was 69-78 milli-int. units/L, seen at only 5.5-11 µg of added protein. Assay A, then, is about 20 times more susceptible to the effect of the antibody than is Assay B.

Goat anti-mouse IgG₁ was studied with Assays B and D, and, for comparison, with Assay A. Results are shown in Figure 4A. In Assay A about 10-fold less added anti-mouse IgG₁ will produce interference; thus Assay A is more susceptible to interference than are Assays B or D. The two studies show that, although Assay A is the most susceptible to interference by a given amount of anti-mouse immunoglobulin antibodies, the other assays may still be affected in spite of the presence of blocking mouse-immunoglobulin.

Interference by Anti-Goat and Anti-Horse IgG

Our previous work showed that the interference by the patient's endogenous antibodies could be completely or partly blocked by adding serum or IgG from species other than mouse, including horse, goat, sheep, and cow. Therefore, we examined the possibility that antibodies directed at IgG from animals other than mouse could produce the fictitious TSH in all four assays. We also included the "special" unblocked version of Assay A, which, lacking sheep and calf serum, might reveal interference more readily than the conventional version.

Figure 4B shows that the "special" version of Assay A showed a marked increase in fictitious TSH with anti-goat IgG as compared with the conventional version of Assay A, indicating a suppressive effect of the animal sera present in the latter. Assays B, C (data not shown), and D—all three containing blocking mouse immunoglobulins—showed very much less effect from the antiserum. Note, though, that if a patient's specimen contained as much antibody activity as corresponds to the highest point shown in Figure 4B the measured TSH would still be increased artificially by 5.4 milli-int. units/L in Assay D, the least affected of the four assays shown—but the reference interval for TSH is about 0.5 to 4.5 milli-int. units/L, so 5.4 represents a large difference in a result and probably would affect management decisions for most patients.

Horse serum and horse IgG were particularly effective in blocking the interference observed with two of the three original patients. Therefore, we added anti-horse IgG to a zero-TSH serum pool and measured apparent TSH by the five procedures. No appreciable fictitious TSH was observed with Assays B, C, or D. However, both versions of Assay A were affected, particularly the "special" version, which lacks sheep and calf serum. The highest observed TSH concentration was only 2 milli-int. units/L with the conventional Assay A, but 79 milli-int. units/L with the "special" version.

When 5 µL of mouse serum was incubated for 1 h at room temperature with the zero-TSH serum pool containing enough rabbit anti-horse IgG to produce a fictitious TSH of about 21 milli-int. units/L in the "special" Assay A, the fictitious TSH was completely suppressed. This indicates that the antiserum to horse IgG is producing its effect by cross-reacting with determinants on the mouse immunoglobulins.

Other Studies of the "Special" Assay A

We demonstrated above that the "special" version of Assay A was more susceptible to interference by anti-goat and anti-horse IgG than was the conventional assay. When the original three patients' sera were assayed with the "special" assay, undiluted and at various dilutions, all apparent TSH concentrations were higher than the corre-

Fig. 3. TSH concentrations measured with Assay A, when goat anti-mouse IgG₁, was added to zero-TSH pooled sera, with and without 1 µL of mouse serum.

Fig. 4. TSH concentrations measured after adding goat anti-mouse IgG₁ (A) or rabbit anti-goat IgG (B) to zero-TSH pooled human serum containing blocking mouse immunoglobulins—showed very much less effect from the antiserum. Note, though, that if a patient's specimen contained as much antibody activity as corresponds to the highest point shown in Figure 4B the measured TSH would still be increased artificially by 5.4 milli-int. units/L in Assay D, the least affected of the four assays shown—but the reference interval for TSH is about 0.5 to 4.5 milli-int. units/L, so 5.4 represents a large difference in a result and probably would affect management decisions for most patients.
sponding results with the conventional assay. Apparently
the sheep and calf serum present in the conventional Assay
A were only partly suppressing the interference. We looked
at this further by measuring TSH in three other patients’
sera with undetectable TSH concentrations (<0.1 milli-int.
unit/L) by the conventional Assay A. Two of three showed
acceptable TSH concentrations, 1.7 and 22.6, and the third
showed 0.2 milli-int. unit/L with the “special” Assay A.
Furthermore, when we made two pools of serum from
patients with undetectable TSH by the conventional Assay
A and assayed them with the “special” Assay A, they had
TSH concentrations of 1.5 and 2.6 milli-int. units/L. Evi-
dently the sheep and calf serum do suppress interference
and, appearing in unselected patients’ sera and in two
serum pools, this interference may be common.

Discussion

Elsewhere (manuscript submitted), we demonstrated that
three patients’ sera contained antibodies to mouse IgG1,
which resulted in factitiously high TSH concentrations with
Assay A. Here we show that this interference can be
simulated by adding exogenous anti-mouse immunoglobu-
lin (IgG1) antibodies and that the interference diminishes
when the antibody is in excess. We have also shown that
three other mouse monoclonal assays for TSH are subject to
some interference by the addition of exogenous antibody,
even though the kits contain mouse serum added to block
this interference. This merely reflects the fact that the
added mouse serum has a limited capacity to tie up anti-
mouse antibodies and that if enough antibodies are present-
ed, interference will still occur. Obviously it is the ratio of
blocking mouse immunoglobulin to interfering anti-mouse
immunoglobulin antibody present that is important in de-
termining the effect. If a patient’s serum were to exhibit
antibody excess such as seen with added animal antibody
(Figure 1), then we would expect to see the apparent TSH
concentration increase as the serum is diluted. This is
comparable to moving from the right-hand portion of Figure
1 towards the left, that is, towards less antibody excess and
higher TSH. The observed TSH concentration will actually
increase with dilution until the amount of antibody passes
to the left of the peak and out of antibody excess. This
phenomenon can be used to identify those sera that contain
sufficient heterophile antibody to be in the zone of antibody
excess, the right side of the peak (solid symbols) of Figure 1.
Dilution of these sera will result in increased apparent TSH
concentration. For the same reason, the presence of blocking
mouse serum could enhance the interference in a case where
the patient’s anti-mouse antibody is in such excess that the
interference was “self-quenched.” The small amounts of
added mouse immunoglobulin shift the reaction from anti-
body excess towards equilibrium, thereby increasing the
measured TSH, rather than blocking the phenomenon (Fig-
ure 3).

Our limited studies of two “zero”-TSH serum pools and
three patients with undetectable TSH concentration suggest
that the sheep and calf serum present in the conventional
Assay A do indeed suppress some anti-mouse IgG activity.
Assay A now contains added mouse immunoglobulins,
which successfully block the interference in all three of our
original patients’ sera.

Clinical laboratories should be aware of the possibility of
this interference from endogenous “heterophile” antibodies
in all mouse monoclonal IMMAs, even in kits containing
added mouse immunoglobulin. Mouse immunoglobulin is
added to kits with the expectation that the frequency and
(or) amount of interference would be diminished. However,
factitious concentrations of an analyte may still be observed
for several reasons. Firstly, a patient could have an unusu-
ally high anti-mouse immunoglobulin activity, exceeding
the capacity of the blocking immunoglobulin present in the
kit. Secondly, if a kit is formulated with a particular
subclass of mouse monoclonal immunoglobulin that is not
adequately represented in the blocking material selected,
false-positive interference could occur when the limited
blocking capacity is exceeded. Thirdly, if a serum sample
has a high enough interfering activity to be in the zone of
antibody excess with “self-quenching” of the interference
(see right portion of curve with solid circles in Figure 3),
the blocking immunoglobulin could actually increase the
observed factitious analyte concentration (triangles, Figure 3).

Thus, addition of mouse immunoglobulins to sandwich-
type assay kits does not necessarily eliminate the inter-
ference. Assays for chorionic gonadotropin, TSH, carcinoem-
bryonic antigen, α-fetoprotein, and other analytes normally
in low concentrations are especially susceptible, because
small interferences in the observed concentrations may
result in large proportional changes and thus may affect
clinical interpretation. This is particularly important in the
case of TSH because of recent suggestions that the new
high-sensitivity IMMAs alone may “prove to be the single
most cost-effective screening test for thyroid disease” (13). If
TSH is used to screen for hyperthyroidism, accuracy below
1.0 milli-int. unit/L is critical, and interferences of as little
as a few tenths of a unit may be important.

Because each assay is configured and performs differen-
tly, we cannot make specific recommendations as to what
amounts or source of blocking agents should be included in
mouse monoclonal IMMAs. Each manufacturer must address
this issue for each assay individually. As we show here,
experiments by adding proper heterologous antibodies to
human serum may be helpful for studying the possible
interference due to endogenous antibodies.

We much appreciate Dr. Paolo Beck-Peccoz’s generosity in mea-
suring TSH concentrations with the Boote-Celltech assay. We
thank Hybritech Inc. and Nichols Institute Diagnostics for gener-
sely supplying TSH kits for this study. We are particularly
grateful to Serono Diagnostics for providing special reagents
and advice throughout this study.

References


4. Howanitz PJ, Howanitz JH, Lambersen HV, Ennis KM. Inci-
dence and mechanism of spurious increases in serum thyrotropin.

5. Clark PM, Raggatt PR, Price CP. Antibodies interfering in immuno-

6. Thompson RJ, Jackson AP, Langlois N. Circulating antibodies to mouse monoclonal immunoglobulins in normal subjects—in-
dence, species specificity, and effects on a two-site assay for creatine

8. Prince AM, Brothman B, Jass D, Inkram H. Specificity of the
direct solid-phase radioimmunoassay for detection of hepatitis-B
9. Hunter WM, Budd PS. Circulating antibodies to ovine and
bovine immunoglobulin in healthy subjects: a hazard for immuno-
assays using monoclonal antibodies. In: Hunter WM, Corrie JET,
ed., Immunoassays for clinical chemistry, 2nd ed. New York and
Monoclonal antibody immunoradiometric assay for an antigenic
determinant (CA125) associated with human epithelial ovarian
12. Spours JT. Limitations of the radioimmunoassay for hepatitis
13. Ross DS, New sensitive immunoradiometric assays for thyro-

CLIN. CHEM. 33/6, 844–845 (1987)

Determination of Free 3-Methoxy-4-hydroxyphenylethylenglycol in Plasma and in
Cerebrospinal Fluid by Liquid Chromatography with Coulometric Detection
V. Rizzo and G. V. Meld d’Erl

A procedure is described for assay of this metabolite of
norepinephrine in plasma and in cerebrospinal fluid.

3-Methoxy-4-hydroxyphenylethylenglycol (MHPG) is the
major metabolite of norepinephrine in the central nervous
system (1). Recently, "high-performance" liquid chromato-
graphy with amperometric detection has been developed to
measure MHPG in plasma (2–3), urine (4), and cerebrospi-
nal fluid (5–6).

The coulometric electrochemical detector with sequential
electrodes provides a tool for MHPG determination in the
low picogram range with excellent specificity.

At the appropriate voltage setting, 100% of an electroac-
tive substance will react on passing through the cells, while
in an amperometric system only 1–5% will react.

This paper describes an enhancement of liquid-chromato-
graphic methodology for quantification of free MHPG in
either plasma or cerebrospinal fluid: the method requires
minimal sample pre-treatment and combines reversed-
phase liquid chromatography with coulometric electrochem-
ical detection.

Materials and Methods

The liquid-chromatographic system consisted of a Model
5700 pump (ESA, Inc., Bedford, MA), a Model 7125 injection
valve fitted with a 50-μL loop (Rheodyne Inc., Berkeley,
CA), and a 70 × 4.6 mm column of 3-μm Ultrasphere XL
ODS (Beckman Instruments, San Ramon, CA). The Model
5100 ESA coulometric detector was equipped with two ESA
cells (Models 5011 and 5021) containing the electrodes,
through which flow the mobile phase and the sample.

The potential of the conditioning cell was set at +0.40 V
to oxidize most of the MHPG present in the sample. By
setting the potential of the first detector (T1) of the analyti-
cal cell at −0.05 V, the response of the second detector is
decreased by only 10%, but this eliminates many possible
interfering substances. The potential of the second detector
(T2) was set at −0.45 V to obtain the highest signal from
the MHPG reduction together with the lowest noise; this results
in good baseline stability.

The mobile phase consisted of 25 mmol/L sodium acetate
adjusted to pH 5.0 with 3 mol/L acetic acid, containing, per
liter, 100 mg of EDTA and 150 mg of heptanesulfonic acid. It
was filtered through a 0.2-μm filter and degassed under
reduced pressure before use.

All samples (plasma and cerebrospinal fluid) were ultra-
filtered through a PM 10 membrane (Amicon Corp., Dan-
vers, MA) and then 20 μL was injected into the chromato-
graphic system. Samples were eluted isocratically and quan-
tified by comparing the sample peak height with that for the
standard solution.

Results

After a certain number of plasma samples had been
injected, a shift in the current/voltage curve for the MHPG
was observed. This is caused by a layer of lipidic materials
building up on the reference-electrode surface, causing s

![Figure 1](image-url)

**Fig. 1.** Typical chromatogram of (A) a (5 ng/mL) aqueous standard
solution, (B) a plasma sample (2.12 ng/mL), (C) a CSF sample (3.12
ng/mL).