Antibody interference in thyroid assays: a potential for clinical misinformation

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Measurements of thyrotropin and of total and free thyroxine and triiodothyronine are widely used diagnostic methods for thyroid function evaluation. However, some serum samples will demonstrate a nonspecific binding with assay reagents that can interfere with the measurement of these hormones. Several recent case reports have described the presence of such interferences resulting in reported abnormal concentrations of thyroid hormones inconsistent with the patient's thyroid state. Circulating thyroid hormone autoantibodies, described in thyroid and nonthyroid disorders, are an important class of interference factor and can bind to hormone tracers used in various immunoassays. Two additional categories of interfering antibodies may particularly interfere within two-site immunoassays for thyrotropin. These include heterophile antibodies, especially human anti-mouse antibodies, and rheumatoid factors, which can cause interferences by immunoglobulin aggregation and (or) cross-linking of both capture and signal antibodies. Here we review the nature of these disturbances; their occurrence, prevalence, and detection; and the clinical consequences of the failure to recognize such interference.

The repertoire of clinical tests for thyroid function evaluation includes measurement of thyrotropin (TSH)[1], free thyroxine (FT_4), free thyroxine index, free triiodothyronine (FT_3), thyroxine (T_4), and triiodothyronine (T_3). In the past 20 years, there have been numerous reports of interferences in thyroid hormone immunoassays. In highly sensitive single- or double-antibody immunoassays, the presence of circulating endogenous antibodies directed against different antigens may cause either falsely depressed or falsely increased values of thyroid hormones, depending on the nature of the interfering antibody or the assay design. The importance of interference on clinical laboratory analyses may be estimated by frequency and impact on patient care. Because these abnormal values may influence the clinical decisions, they have important clinical consequence and may lead to unnecessary clinical investigations as well as inappropriate treatments.

The three major possible sources of antibody interference in thyroid hormone immunoassays are autoantibodies, heterophile antibodies, and rheumatoid factors (RF). Autoantibodies can cause an analyte-specific interference in thyroid assays [1, 2], in contrast to heterophile antibodies and rheumatoid factors, which may be responsible for method-specific disturbances in a wide range of immunoassays, including thyroid hormone measurement techniques [1, 3–5]. After considering the nature of endogenous factors that may interfere in thyroid function evaluation, their prevalence, and their detection, we will focus on their clinical consequences, if not recognized, and on the methods to overcome these interferences. This review can be used as a guide to clinical chemists and physicians in cases where thyroid function test results that are inappropriate to a patient's clinical state could be attributable to antibody interference. An excellent general overview of interfering endogenous and exogenous factors that may affect clinical chemistry tests has been presented previously [6].

AUTOANTIBODIES AS INTERFERENCE FACTORS

Many antibody/antigen systems have been described in autoimmune thyroid diseases. The most common include antibodies to thyroglobulin, antibodies to microsomal thyroid peroxidase, antibodies to the TSH receptor [7–10], and antibodies reacting with T_4 and T_3 [2, 11–16]. Thyroid hormone autoantibodies (THAAb) directed specifically against T_3 and T_4 are less common than the other autoantibodies [2, 16, 17], and they are the only reported autoantibodies to interfere in thyroid function tests

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[1] Nonstandard abbreviations: TSH, thyrotropin (thyroid-stimulating hormone); FT_4, free thyroxine; FT_3: free triiodothyronine; T_4, (total) thyroxine; T_3, (total) triiodothyronine; THAAb, thyroid hormone autoantibodies; PEG, polyethylene glycol; HAMA, human anti-mouse antibodies; RF, rheumatoid factors.

[1–2, 6, 13, 17–25]. THAAb have been known since 1956, when Robbins et al. *[11]* first described the presence of T_4-binding gamma globulin in a case of papillary carcinoma of the thyroid gland treated with ^{131}I. Following this first report, the presence of THAAb was described in patients with thyroid and nonthyroid disorders *[14–17]*. These autoantibodies are mostly of the IgG isotype and the autoreactive response is usually polyclonal, with isolated cases of monoclonality. In contrast to anti-T_3 and anti-T_4 antibodies, autoantibodies against TSH are very uncommon and few investigators have proposed the possibility of interference of these antibodies in TSH measurement *[26, 27]*. In addition, most of the reported anti-TSH antibodies were shown to react against bovine but not human TSH *[28–30]*.

THAAB PREVALENCE

Previous studies have reported discordant results on the prevalence of THAAb among various patient subgroups with or without thyroid diseases. As reviewed by Sakata et al. in 1985 *[2]*, most of these THAAb occur in autoimmune thyroid diseases. The prevalence of these autoantibodies has been well documented but there is no clear unanimity. Since 1985, the prevalence of THAAb has been reported to be between 0% and 25% *[2, 12, 15–17, 31, 32]*. Such wide variations of prevalence could reflect differences in patient subgroups studied as well as differences in the detection methods used, such as assay sensitivity and specificity.

Table 1 summarizes the most recent studies on the prevalence of THAAb in patients with thyroidal and nonthyroidal illnesses as well as in healthy subjects. In these studies, detection of THAAb was mainly performed by radioimmunoprecipitation of labeled thyroid hormones or analogs according to commonly used methods *[2, 33]*. In addition, some investigators have concurrently studied other thyroid autoantibodies, particularly anti-microsomal and anti-thyroglobulin antibodies *[13, 16, 31, 32]*. Some studies have evaluated the extent of THAAb interference with specific thyroid assays *[22, 34–37]*.

A comparison of results presented in Table 1 reveals that THAAb prevalence varies with the detection method, the era when the study was performed, and the category of patients studied. A THAAb radioimmunoprecipitation assay using pretreated sera with acid-dextran-coated charcoal gave positive results in 4.8% of untreated patients and in as many as 20% of Graves disease patients *[2, 32]*. Use of a direct THAAb immunoprecipitation assay involving thyroid hormone derivatives (polyaminocarboxy T_3 or T_4) indicated a prevalence of 17.5% in untreated Graves disease patients *[31]*. With both techniques, a high incidence of thyroid autoantibodies was associated with the presence of THAAb. The higher prevalence reported by the last group may also be explainable by the use of labeled thyroid hormone derivatives. Antibody titer is also an important factor to consider in assay interference. Wang et al. *[32]* reported a high

prevalence of THAAb, but most of the positive samples had such low titers of THAAb that T_4 and T_3 measurements were not affected. The results outlined above thus suggest that when more severe thyroid autoimmune diseases are considered, when detection methods are less stringent, and when derivative molecules are used in the detection assay, THAAb prevalence is increased.

In contrast to these investigators who found such high incidences of THAAb, more recent and more extensive studies using polyethylene glycol (PEG) precipitation of the radiolabeled complex have reported prevalences ranging from 1% to 7% in autoimmune thyroid diseases, and between 0% and 1.8% in the normal population *[14, 16, 17]*. The prevalence of 1.8% was obtained by use of a thyroid analog-based method.

Overall, we may consider that the prevalence of THAAb (anti-T_3 and anti-T_4 antibodies) among the overall population is uncommon, but their frequency may be higher in hypothyroid, hyperthyroid, and nonthyroid autoimmune patients, with prevalence up to ~10% *[14, 17]*. The review by Sakata in 1985 *[2]*, which was based on some very early observations, suggested that the prevalence of THAAb might be as much as 40% in autoimmune thyroid disease.

Two additional findings should be taken into consideration when detecting THAAb. First, the interesting observations reported by John et al. *[22, 38]* as well as Sakata et al. *[39]* suggest that anti-microsomal and (or) anti-thyroglobulin antibodies are simultaneously detected in most THAAb-positive samples showing assay interference. As shown in Table 1, all studies that used thyroid autoantibodies detection reported a very high incidence of these antibodies (80–100%) in THAAb-positive samples; this is not, however, an invariable association. Second, the THAAb prevalence seems to be higher with methods that use analog thyroid hormones rather than their respective native components, as discussed in the next section.

METHOD DEPENDENCY OF THAAB INTERFERENCE

In the absence of interfering factors, the labeled tracer and the sample analyte compete for binding sites on the capture antibody. In the presence of THAAb, however, labeled tracer and analyte may bind abnormally to the autoantibody, thus resulting in inaccurate thyroid hormone measurements. Therefore, many factors should be taken into consideration, especially single- vs double-antibody procedure, one- vs two-step assay, analog vs nonanalog tracer, and molecular features of the tracer used. As reviewed by Kohse and Wisser *[1]*, the nature of interference leading to depressed or increased thyroid hormone values depends especially on the separation technique used. In assays using a single-antibody technique, the presence of autoantibodies will result in low hormone concentrations because the tracer (labeled thyroid hormone or its analog) is bound by the autoantibod-

Table 1. Prevalence studies of thyroid hormone autoantibodies.

Study	Study design	THAAb prevalence	Thyroid AAb	THAAb detection methods	Evidence of interference
[2] 1985	89 subjects: 38 Hashimoto 21 Graves disease 30 Graves with antithyroid drug treatment	All anti-T_4+ and anti-T_3−: 3/38 (7.9%) 1/21 (4.8%) 6/30 (20%)	n/a	RIA of radiolabeled thyroid hormones of acid-dextran-coated charcoal-treated sera	n/a
[12] 1988	51 subjects: 45 patients with Graves, idiopathic myxedema, Hashimoto, subacute thyroiditis, and thyroid tumors 6 normal subjects	0/51 anti-T_4+; 1/51 (2%) anti-T_3+ (Graves disease patient)	n/a	Radioimmunoprecipitation of labeled thyroid hormones	The untreated Graves disease patient with anti-T_3 antibodies had a falsely normal T_3 result (2.4 nmol/L) and a clearly increased T_4 result (296 nmol/L), both measured by RIA.
[13] 1988	253 subjects: 116 normals 101 hyperthyroid: 79 Graves disease 22 toxic goiter 36 hypothyroid: 15 Hashimoto 21 idiopathic hypothyroidism	0/116 1/101 (anti-T_4+) 2/36: 1 anti-T_4+; 1 anti-T_3+	All THAAb+ samples were also anti-Tg+	RIA of PEG-precipitated complexes	2/3 THAAb-positive samples had spuriously high FT_4 and (or) FT_3 as measured by Amerlex-M and Coat-A-Count FT_4 and FT_3 RIA.
[32] 1990	112 subjects: 63 Graves disease 49 controls	16/63 (25%) anti-T_3+; high prevalence but very low titers of THAAb; anti-T_4 antibody not measured	All 16 anti-T_3+ subjects were also anti-Tg+	RIA after acid-dextran-coated charcoal treatment of patient's sera	T_4 and T_3 measured by RIA. No interference at low titers of anti-T_3 Ab.
[14] 1992	358 subjects: 58 normals 169 autoimmune thyroiditis 131 thyroid diseases	0% 1–2% 0–1%	n/a	RIA of PEG-precipitated complexes	n/a
[31] 1994	70 subjects: 40 untreated Graves disease 30 normal subjects	7/40 (17.5%): 6 anti-T_4+; 1 anti-T_4/T_3+	34/40 (85%) anti-Tg+; 32/40 (80%) anti-TPO+	Radioimmunoprecipitation	n/a
[16] 1994	880 normal subjects	3/880 (0.3%) with native T_4 or T_3; 7/335 (1.8%) with T_4 or T_3 analogs	7 THAAb+: 1 anti-Tg+; 0 anti-Mic+	RIA of PEG-precipitated IgG complexes	No interference in Amerlex-M FT_4 and in Amerlex-M FT_3 analog assays
[17] 1994	727 subjects: 200 hypothyroid 200 hyperthyroid 200 nonthyroid autoimmune diseases 20 insulin AAb+ 100 normals	7% 1.5% 7.5% 0% 0%	n/a	RIA of PEG-precipitated complexes	21 THAAb+ sera tested by Amerlex-M FT_4 analog RIA all showed spuriously high results. After THAAb removal by PEG precipitation, FT_4 decreased in all 21 samples.

AAb, autoantibody; n/a, not applicable; Mic, microsomal antibody; Tg, thyroglobulin; TPO, thyroid peroxidase.

ies as well as by the capture antibodies. Hence, both the capture antibodies and the autoantibodies are measured, an abnormally high amount of tracer is detected, and the apparent concentration of hormone will be spuriously low. On the other hand, in methods using a double-antibody technique, the tracer is again bound by both the capture antibody and the autoantibody, but the second antibody used in the separation step binds only the capture antibody. Consequently, an abnormally low amount of tracer is detected and the apparent concentration of hormone will be spuriously high.

Many investigators have shown that methods for measuring the concentrations of thyroid hormones (e.g., equilibrium dialysis methods for FT_4) appear to be less susceptible to THAAb interference when the procedures used ensure that there is no contact between serum components and thyroid hormone or its analog tracer [22, 25, 37, 40–43]. Thus, two-step assays in which thyroid hormone is extracted from serum by antibody-coated tubes or by antibody-coated beads, and the extraction is followed by a washing step, appear to be less affected or unaffected by endogenous THAAb. In these methods, all other serum components are eliminated before addition of the hormone tracer. In contrast, one-step assays, in which the assay antibody, the patient's serum, and the labeled tracer are all in contact, appear to be more prone to THAAb interference.

Some newly developed free thyroid hormone assays have used thyroid hormone derivative, coated on a solid-phase, that competes with the sample free hormones. Free T_3 assays may use a diiodothyronine-coated solid-phase, or FT_4 assays may use a T_3-coated solid-phase, to compete with the sample analyte for the antigen-binding site of the assay antibody. These immunoassays were considered to be less affected by THAAb interference. Indeed, Sapin et al. [42] reported no THAAb interference in FT_3 assays that used a diiodothyronine competitor; however, spuriously high FT_4 values were found in sera containing anti-T_3 antibodies that bound to the T_3-coated solid-phase used in the FT_4 assays. The latter observation was also reported by other investigators [43].

To support this observation, Sakata et al. [16] extensively examined the prevalence of THAAb in 880 apparently healthy subjects by using native or analog thyroid hormones as tracers. They found THAAb in 3 of 880 (0.3%) subjects when using native tracers, in contrast to 7 of 335 (1.8%) subjects when they used analog tracers. These results suggest that the use of labeled thyroid hormone analogs detected THAAb more efficiently than did labeled thyroid hormones and that THAAb have a higher affinity for analog molecules. Thus, when patients' sera showing a high incidence of thyroid antibody are considered and when analog tracers are used in the detection method, the estimated prevalence of THAAb could increase.

CLINICAL IMPORTANCE OF THAAB INTERFERENCE

For >30 years now, including recently, many authors have reported thyroid hormone assay interference in sera of patients with autoantibodies against T_4, T_3, both T_4 and T_3, or their analogs [2, 11, 17–24, 37, 44–53]. Most importantly, some of these authors have described a clinical impact, including misdiagnosis, inappropriate diagnostic interventions, and inappropriate treatment over a long period of time, caused by misinterpretation of such interferences. For this reason, even with the best of methods, clinical chemists and physicians should continue to be vigilant to THAAb interference.

As mentioned before, the prevalence of THAAb varies from report to report and may be up to 40% in autoimmune thyroid diseases; however, the presence of these antibodies in patients' samples does not necessarily lead to assay interference. In most cases, samples containing THAAb seem not to interfere in thyroid hormone measurements. Some immunological features, such as autoantibody titer, specificity, and affinity, can determine clinically important interference. The studies performed by John et al. [22, 38] support that only a minor portion of THAAb-positive samples shows thyroid assay interference. When they evaluated the incidence of THAAb interference in patients tested in a 1-year period, only 1 sample from 2460 patients tested showed abnormal thyroid hormone results [22]. They also [38] used radiolabeled analogs of T_4 or T_3 to screen all postpartum women seen over a 2-year period for the presence of THAAb and identified 148 women positive for autoantibodies to these analogs. Measuring the concentrations of circulating FT_4 and FT_3 with analog methods in the 148 THAAb-positive women, they found only 3 patients (2%) who demonstrated antibody interference, i.e., spuriously high values for FT_4, FT_3, or both. Interestingly, their longitudinal data findings indicated that some patients could have changes of interfering antibodies in parallel with changes in concentrations of anti-microsomal autoantibodies. Similar results were also obtained by Sakata et al. in 1994 [16] for serum samples from 880 healthy subjects; none of the THAAb-positive samples showed assay interference because of both low titer and low affinity.

Almost all patients with THAAb were identified because of discrepancies between clinical findings and the laboratory data from thyroid function tests. Without systematically measuring THAAb and therefore evaluating the extent of interference in the respective methods, it is not possible to really know the prevalence of autoantibody interference in thyroid function tests. In most of these cases, fortunately, assay interference was identified before multiple inappropriate investigations or potentially harmful treatment was invoked. However, some asymptomatic and clinically euthyroid patients, who showed abnormal thyroid hormone concentrations, have received unnecessary investigation and inappropriate therapy. The reported cases of patients with THAAb interference who have received inappropriate clinical

Table 2. Case reports of anti-thyroid hormone autoantibody interference.

Study	Clinical picture	Evidence of inappropriate clinical intervention	Thyroid tests	THAAb	Thyroid AAb
[18] 1978	16-year-old boy with chronic lymphocytic thyroiditis and asymptomatic goiter	Thyroid indices were in the hyperthyroid range and L-T_4 was inappropriately discontinued for 3 months. Unnecessary diagnostic testing.	Abnormally high T_4 and T_3 values by RIA. After ETOH extractions, T_4 and T_3 values were restored to normal.	PEG precipitation of labeled T_4 or T_3 gave 88% binding to T_4 and 82% binding to T_3	Anti-Tg+ 1/77 000
[46] 1990	2 patients with Graves disease: *Patient 1:* 21-year-old woman with subtotal thyroidectomy and treated with methimazole *Patient 2:* 37-year-old woman	Dose variations of methimazole according to inappropriate FT_4 results.	Abnormally high FT_4 results by Amerlex-M analog method (normal: 9.7–24.5) Patient 1: 46.5 pmol/L Patient 2: 251 pmol/L T_4 by RIA (normal: 51.5–167.3) Patient 1: 202 nmol/L Patient 2: 381 nmol/L T_3 by RIA (normal: 1.08–2.76) Patient 1: 3.8 nmol/L Patient 2: 9.57 nmol/L TSH by IRMA (normal: 0.3–3.5) Patient 1: <1.0 mU/L Patient 2: 0.1 mU/L	Patient 1 sample showed binding activity of 8.3% to T_4 and 58.7% to T_4 analog; patient 2 sample showed binding activity of 6.8% to T_4 and 35.5% to T_4 analog; both samples showed a binding activity in the IgG fraction; both samples did not bind significantly to T_3 or T_3 analog.	Patient 1: anti-Mic+, anti-Tg− Patient 2: anti-Mic+, anti-Tg+
[22] 1990	2 Graves disease 3 Hashimoto thyroiditis 3 nontoxic goiter	In some patients, inappropriate T_4 replacement therapy or misclassification of thyroid status	Five FT_4 analog and two nonanalog methods; two FT_3 analog and one nonanalog methods; analog methods more prone to interference: overall incidence of interference 0.04% (1/2460 subjects) in analog methods.	5/8 anti-T_4+ 8/8 anti-Amerlex-M T_4+ 8/8 anti-Coat-A-Count T_4+ 7/8 anti-Seria T_4+ 4/8 anti-T_3+ 5/8 anti-Amerlex-M T_3+ 4/8 anti-Seria T_3+	7/8 anti-Tg/Mic+
[54] 1990	33-year-old clinically euthyroid woman, studied over a 2-year period	Misdiagnosis of hyperthyroidism and investigation for TSH-secreting pituitary tumor. Inappropriate treatment with carbimazole. True diagnosis was hypothyroidism and treatment with L-T_4.	Sequential biological status 1) FT_4 31.2 pmol/L (9–23)[a] by Amerlex-M 2) FT_4 54 pmol/L 3) FT_4 94 pmol/L TSH >25 mU/L (<5) T_3 1.6 pmol/L (0.8–2.5) 4) FT_4 120 pmol/L TSH 120 mU/L T_3 1.1 pmol/L	PEG precipitation of labeled T_4 analog: 70% binding of labeled T_4	Anti-Mic+ 1/400 Anti-Tg+ 1/80
[55] 1991	48-year-old euthyroid woman with no history of thyroid disease	Patient underwent 2.5 years of thyroid function testing.	T_3 13.8 nmol/L (0.8–1.8) by Dainabot solid-phase RIA; FT_3 9.8–12.2 pmol/L (2.7–5.9) by Amerlex-M RIA; T_4, FT_4, and TSH normal.	PEG precipitation of Amerlex-M analogs: 60% binding of labeled T_3 analog, using serum sample or purified IgG fraction.	Anti-Tg+ of 33.4% (<10%)
[44] 1991	39-year-old woman with 20-year history of Hashimoto thyroiditis	Initially treated adequately with 200 mg L-T_4 per day. With a high T_3 result, L-T_4 was inappropriately decreased to 100 mg and patient became hypothyroid, with increased TSH.	High T_3 4.3 nmol/L (1.2–3.4) by Organon Teknika method and high FT_3 >39 (4–9) by the Amerlite-M analog method; T_4 and TSH normal.	PEG precipitation showed a 21% binding of labeled T_3 Anti-T_3 antibodies mostly IgG class.	n/a
[41] 1992[b]	9.6-year-old girl with mild signs of hyperthyroidism and goiter	Unnecessary diagnostic testing, including TRH and T_3 suppression tests, pituitary imaging, and further thyroid function tests	FT_3 28 pmol/L (3.4–7.7) by one-step analog assay and 13.3 pmol/L (3.8–8.5) by two-step Lisophase; FT_4 30 pmol/L (9–25) by one-step analog assay and 28.2 pmol/L (9–20) by two-step Lisophase; TSH normal. In summary, one-step assay more prone to interference.	PEG precipitation of serum binding to labeled T_4 or T_3; 38% binding of labeled T_3; no anti-T_4 documented	Anti-Tg+ 800 units/L Anti-Mic+ 256 units/mL

Table 2. Continued.

[56] 1992	30-year-old woman with Hashimoto thyroiditis, and with goiter for 10 years	Inappropriate cessation of L-T_4 treatment, because of increased results for free thyroid hormone; after identification of anti-T_3/T_4 Ab in 09/1991, L-T_4 dosage was restored to 150 μg/day.	01/91 TSH 14.3 mU/L (0.3–5.0) FT_3 10.2 pmol/L (3.3–7.7) FT_4 22 pmol/L (9–22) 04/91 TSH 36.6 FT_3 9.9 pmol/L (3.7–9.2) FT_4 12 pmol/L (10–24) 09/91 TSH 0.7 FT_3 8.3 FT_4 25.2	PEG precipitation of labeled T_4/T_3 analogs showed the presence of both anti-T_4 and anti-T_3 AAb	Anti-Mic+ 1/409 600
[49] 1992	Euthyroid neonate male from mother with Graves disease treated with PTU during and after pregnancy, having high FT_3 and FT_4 results because of THAAb. THAAb were transferred from the mother.	Misdiagnosis of hyperthyroidism in the neonate and inappropriate treatment with antithyroid drugs (Lugol's solution and PTU)	At birth, FT_4 26 pmol/L (10–20) and FT_3 11 pmol/L (0.5–1.7) by Amerlex RIA; TSH 84 mU/L (1.8–21.1) by IRMA. At day 10 (before treatment), FT_4 70 pmol/L, FT_3 >38 pmol/L, suppressed TSH; T_3 and T_4 concentrations, in ETOH-extracted serum, were normal.	PEG precipitation of serum binding to labeled T_3 or T_4 gave: Neonate: 25.6% labeled T_3 binding 13.6% labeled T_4 binding Mother: 22.5% labeled T_3 binding 17.5% labeled T_4 binding	Anti-TSH receptor+ 75% (<10%)

[a] Reference intervals are listed in parentheses.

[b] They report that in a series of 127 patients with suspected inappropriate secretion of TSH, 32.3% were misdiagnosed because of interferences. Among this group, 70.7% had THAAb.

ETOH, ethanol; PTU, propylthiouracil; TRH, thyroliberin (thyrotropin-releasing hormone); other abbreviations as in Table 1.

interventions are listed in Table 2. Thyroid assay interference seems to be more frequently described in autoimmune thyroid disease patients. In addition, most of these anomalous thyroid function results led to inappropriate diagnosis of thyrotoxicosis because of very high concentrations of total or free thyroid hormones. The unnecessary clinical interventions these patients received have varied from changes in their dose of daily hormone replacement therapy to misclassification of thyroid status, as well as additional diagnostic investigations, including thyroid hormone suppression tests and scintigraphy. For some patients, these interventions have taken place over a considerable time *[54–56]*.

Despite their relative rarity, autoantibodies causing interference should be suspected when laboratory data are not compatible with the clinical picture. Under these circumstances, four major approaches can assist in evaluation of assay interference: (*a*) measure TSH by a sensitive immunometric method; (*b*) measure thyroid hormone concentrations after immunoglobulin depletion; (*c*) use a comparative method (however, interference may be seen in more than one method; for suspected interference with FT_4 assays, measure by equilibrium dialysis); and (*d*) test for the presence of THAAb against the hormone or analog tracer used in the assay reagents.

LABORATORY INVESTIGATION OF THAAB INTERFERENCE

Three different approaches are commonly used to overcome THAAb interference. First, interfering antibodies can be removed from serum by ethanol precipitation, so that the subsequent analytical values are free from interference *[19, 57, 58]*. This simple method consists of incubating the serum sample with 9 volumes of 90% ethanol for 30 min at room temperature. The precipitate is centrifuged at 1400*g* for 15 min. The supernatant is then collected, evaporated, reconstituted with the zero calibrator, and reanalyzed. This method cannot, however, be performed for FT_4 and FT_3 measurements, because it precipitates all serum proteins. Second, because autoantibodies are mostly of the IgG isotype and since Protein G binds to the Fc region of all four IgG subclasses, the serum IgG fraction that may interfere in some assays can be reduced or eliminated by affinity binding with Protein G–Sepharose beads (Pharmacia Biotech) *[59, 60]*. Protein A–Sepharose beads have also been used successfully in serum IgG depletion studies *[23]*; however, Protein A binds only three of the four IgG subclasses.

Serum IgG depletion can be performed either by batch or column absorption. Briefly, Protein G–Sepharose beads (equal volumes of beads and serum sample) are equilibrated by washing the gel with Tris-buffered saline, pH 7.4. The remaining buffer is discarded without drying the gel. Protein G–Sepharose beads are further incubated overnight at 4 °C with the serum sample. Beads are then centrifuged and the serum sample is decanted and reassayed for thyroid hormones. A control specimen, treated in the same fashion, should be analyzed in parallel.

Martins et al. [60], in an alternative procedure for removal of IgGs from serum to reduce interference, used an in-house-developed anti-human IgG diluent that was more effective than the Protein G method in eliminating interference. Serum immunoglobulins can also be successfully removed by precipitation with PEG [17, 40, 61, 62].

Third, THAAb in the serum sample may be directly identified by radioimmunoprecipitation [2, 13, 16, 18, 21, 32, 33, 55, 63]. This commonly utilized method is reasonably rapid and effective and specifically identifies the nature of the interference. Radiolabeled thyroid hormone or its analog is incubated with the patient's serum, and a control incubation with a normal human serum is also performed. The immune complexes are then precipitated with a final PEG concentration of 125 g/L (125 mg/mL), and the radioactivity of the precipitate is determined as a proportion of the total added radioactive label. Protein A– or Protein G–Sepharose also may be extremely useful for isolation of these immune complexes: Bound radiolabeled tracer can be isolated with as little as 5 μL of Protein G–Sepharose beads instead of using PEG for immune complex precipitation. In both methods, the results are expressed as the percent binding of radiolabeled hormone (bound/total tracer %). In normal serum, ~5% of the radioactivity is detected, whereas up to 75% can be detected if THAAb are present in the serum sample.

HETEROPHILE ANTIBODIES AS POSSIBLE INTERFERING FACTORS

Heterophile antibodies are known to interfere in a wide spectrum of immunoassays, such as those for α-fetoprotein [64], viral antigens [65, 66], ferritin [67], human chorionic gonadotropin [68], creatine kinase MB isoenzyme [69, 70], and tumor-associated antigens [71, 72]. By definition, heterophile antibodies are antibodies against specific animal immunoglobulins or against immunoglobulins of various animal species, depending on the recognized epitope and on the cross-reactivities between species immunoglobulins [1, 5, 73]. The recent development of two-site immunometric assays with specific antibodies, such as mouse monoclonal antibodies, has enabled higher specificities and sensitivities. Since the introduction of these assays, there have been several reports of abnormal concentrations of TSH resulting from heterophile antibody interference [1, 3–5, 66, 73, 74]. The best-known heterophile antibodies are human anti-mouse antibodies (HAMA), which can react with the mouse monoclonal antibodies that are used in many immunometric assays. To counteract this problem, all commercial assays now include blocking reagents, such as nonspecific and polymerized murine IgG. However, the presence of blocking reagents does not completely eliminate the problem of interference in some specimens and with some kits. The major concerns of heterophile antibody interferences for clinical chemistry are the following: the prevalence of these antibodies, when these interferences might be

present, how they can be detected, and, most importantly, how they can be avoided.

Heterophile antibodies may cross-react with various different species' immunoglobulins [1, 3, 5, 64, 68, 69, 75]. Heterophile antibodies may be induced after infusion of murine monoclonal antibodies for diagnostic and therapeutic purposes in cancer patients [1, 76–81]. They may also be induced through vaccines that contain animal immunoglobulins or by environmental contacts with different animal immunoglobulins, as may occur in farmers and veterinary workers [82–84]. It is not always demonstrable, however, that the individuals in question have been previously immunized. Heterophile antibodies are also found in various autoimmune diseases [73, 76, 85–87]. Table 3 shows the results of recent investigations on the prevalence of heterophile antibodies in different patient subgroups. Patients receiving infusion of murine monoclonal antibodies for therapeutic and diagnostic purposes are the most susceptible population to develop heterophile antibodies, particularly HAMA, which has a prevalence of between 40% and 70% [76, 80, 88–90]. The prevalence depends on the bolus size of antibody injected, on the portion of immunoglobulin used, on the number of doses injected, and on the route of administration. The prevalence of heterophile antibodies in the general population has been reported to be between 0.2% and 15% [69, 85, 91–93]—the range depending mainly on the detection method used, the specificity and sensitivity of the method, and the panel of patients selected for screening.

Heterophile antibodies may cause interferences by two mechanisms [1, 3, 5, 78]. The most common heterophile antibody interference is caused by immunoglobulin aggregation, through binding of the capture antibody to the detection antibody. In thyroid function testings, this interference has been most frequently described in TSH sandwich immunoassays [74, 94–104] (Table 4). Interference may also result from idiotypic antibody interactions. This type of interference is very uncommon, and occurs mainly in patients receiving therapeutic or diagnostic injections of the same monoclonal antibodies that are being used to measure the analyte in the assay. For instance, substantial anti-idiotypic antibody interference was previously reported in tumor marker measurements in cancer patients who already had specific monoclonal antibody injections for imaging purposes [72, 88, 105]. However, no idiotypic antibody interference has been reported in thyroid hormone assays.

The presence of heterophile antibodies in a serum sample can promote binding between the capture antibody and the signal antibody, even in the absence of the analyte. This type of nonspecific binding results in abnormally high values. However, a heterophile antibody that binds only to the capture antibody can affect the conformation of the variable region or sterically block the binding of analyte to this antibody, even if it does not bind directly to the recognition site of the analyte. In this case, values will be abnormally low.

HAMA can bind to both F(ab') and Fc fragments of the murine immunoglobulins, but more frequently to the latter [3, 69, 77–79, 85]. Many reports have found that HAMA are of both IgG and IgM isotypes [3, 66, 73, 78, 106, 107]. Because HAMA are commonly directed against the Fc fragment, the use of F(ab') fragments or human/mouse chimeric antibodies for analytical antibodies has been advocated as a means of decreasing heterophile antibody interference [78, 85, 108]. However, the heterogeneity of the HAMA responses as well as their specificities (anti-F(ab') fragments) indicates that this would not always be effective. Interference by heterophile antibodies can usually be abolished or decreased by addition of either nonimmune serum from the same animal species used to raise the antibody reagents or purified or polymerized homologous nonspecific immunoglobulin [3, 4, 66, 70, 73, 85, 109–112]. According to recent investigations, the most active material appears to be serum or purified immunoglobulins from the same strain of mouse as was used for production of the capture and signal antibodies. When nonimmune homologous mouse immunoglobulins are added in the assay reagents, the HAMA bind to these immunoglobulins and analytical antibodies are free of interference. On the other hand, heterophile antibody interference can also be reduced or abolished by pretreating the serum sample with Sepharose beads coupled to Protein A or Protein G (as described above) [69, 72]. Therefore, Protein A or G pretreatment will eliminate total serum immunoglobulins of the IgG class, whereas nonimmune mouse serum or purified immunoglobulin preincubation will specifically block serum anti-mouse antibodies.

Kahn et al. [109] in 1988 performed blocking and immunoabsorption studies on the serum of patients with TSH concentrations abnormally increased because of HAMA. When increasing amounts of mouse serum were added to the patient's sample or when the samples were pretreated with CH-Sepharose 4B coupled to mouse immunoglobulins, TSH concentrations were decreased to normal values. Kahn et al. also demonstrated by blocking experiments with different immunoglobulin subclasses that the HAMA specificity was particularly directed against the IgG1 kappa immunoglobulins. Reinsberg [113], recently evaluating the efficacy of three different commercial sources of blocking reagents to reduce or eliminate interference with a CA-125 immunoassay by HAMA produced in monoclonal antibody-treated patients, showed that preincubation with polyclonal mouse IgG or polymerized mouse IgG did not completely abolish interferences. In contrast, an immunoglobulin-inhibiting reagent, a formulation of immunoglobulin targeted against HAMA, seemed to be an effective agent for eliminating HAMA interferences.

A practical approach to attempt to block or reduce the effect of HAMA interference is to preincubate the patient's serum sample for 1 h at room temperature with increasing amounts, between 10 and 100 mL/L (μL/mL), of nonimmune mouse serum. After this absorption procedure, the assay is performed as usual, taking into account the dilution factor used. Commercially available HAMA-blocking reagents may be easily and effectively used to counteract heterophile antibody interferences in the clinical laboratory, including those evaluated by Reinsberg [113], as well as Heterophile Blocking Reagent, Heterophilic Blocking Tube, and Non-Specific Antibody Blocking Tube distributed by Scantibodies Laboratory Inc. In addition, some commercial kits detect HAMA-positive patient samples (HAMA-ELISA medac, from MEDAC; ImmuSTRIP, from Immunomedics; ETI-HAMAK immunoenzymometric assay, from Sorin Biomedica; and IDeaL HAMA ELISA, from ALPCO), although some investigators have reported notable variability among kits [90, 114].

Heterophile antibody interference is considered to be solved by modifications of the current assays, such as addition of nonimmune sera or purified immunoglobulins as well as various blocking agents to the assay reagents. Hence the very high nonspecific serum binding values observed previously are now unlikely. However, as shown in Table 4, many reports have found that some assays may still give nonspecific results, mostly because of high titers of heterophile antibodies in some patients' samples. Wood et al. [110] described the case of a patient with an abnormal serum TSH result caused by a circulating anti-mouse antibody. This clinically euthyroid patient was found to have a normal value for serum T_4 and an above-normal TSH, as measured by a fluoroimmunoassay. Thyroid hormone therapy failed to suppress the TSH concentration. Addition of mouse IgG to the assay (or to the serum sample), however, reduced the patient's TSH value to within its reference range. These observations are consistent with a spurious increase of TSH caused by the presence of HAMA.

More recently, Laurberg studied the presence of nonspecific binding in 6 different TSH immunoassays, using 63 sera from patients with untreated hyperthyroidism [74]. All assays were sandwich immunoassays, with a capture antibody and a signal antibody. None of the assays studied gave the same value for serum TSH in most of the sera, and spuriously high TSH values were reported for some sera, depending on the assay used. Addition of large amounts of mouse serum reduced interference for some sera, thus supporting the presence of HAMA interference.

Finally, Fiad et al. [75] reported the case of a euthyroid patient who gave abnormally high values for all FT_4, T_4, T_3, and TSH measurements when tested with enhanced chemiluminescence assays. Reassay of the patient's serum after immunoglobulin precipitation with 500 g/L PEG or addition of anti-immunoglobulin antibodies gave values for the thyroid hormones that were within the reference ranges, suggesting that the serum contained heterophile antibodies interfering in all thyroid function tests. To our knowledge, this is the only report of artifactual increases

Table 3. Prevalence studies of human anti-mouse antibodies.

Study	Study design	HAMA detection method	HAMA prevalence	Evidence of interference
[73] 1986	188 normal subjects; 261 hCG-free samples; 219 thyroid function samples; 6 RF samples; 95 hCG-positive samples	Modified two-site IRMA interference assay using an hCG monoclonal antibody	By the modified interference assay, ~40% of serum samples showed significant binding to murine monoclonal antibodies	When testing 190 hCG-free samples with a two-site IRMA for hCG, 15% showed a confirmed false-positive result.
[69] 1986	Serum samples from 1008 blood donors	Interference assay using a "reverse two-step" version of the monoclonal based CK-MB two-site assay	91/1008 (9.1%) of serum samples had HAMA and gave spuriously high CK-MB values	Spurious CK-MB values decreased when nonimmune mouse serum (10 mL/L) was included in the assay reagents.
[117] 1987	All IgM RF-positive subjects: 24 blood donors; 13 ovarian cancer; 5 mixed essential cryoglobulinemia; 4 rheumatoid arthritis (RA); 4 lung cancer	ELISA using solid-phase murine monoclonal antibodies	IgM HAMA were detected in all subjects except those with mixed essential cryoglobulinemia	n/a
[93] 1989	70 healthy subjects; 45 Sjögren syndrome (SS); 54 systemic lupus erythematosus (SLE); 54 RA	ELISA to detect human IgM reacting with IgG from various species, including mouse	IgM anti-mouse IgG were detected in: 1% of healthy subjects; 7% of SS; 6% of SLE; 31% of RA	n/a
[92] 1992	60 normal subjects 119 Graves disease 60 Hashimoto thyroiditis 15 thyroid tumors	ELISA	5/60 (8.3%) 7/119 (5.9%) 3/60 (5%) 1/15 (6.7%)	No interference was shown with HAMA-positive samples in using TSH and thyroid hormone assays.
[85] 1992	2600 serum samples from a blood bank	Interference assay combining two monoclonal antibodies with different specificities (CEA and TSH monoclonal antibodies)	81/2600 (3.1%) had significant HAMA titers; HAMA recognized both F(ab) and Fc fragments, mainly the latter	When testing the 81 HAMA+ samples for CK-MB, 18 (22%) gave false-positive results.
[88] 1994	32 cancer patients receiving labeled CA-125 monoclonal antibodies for immunoscintigraphy	Three different HAMA assays: Enzygnost HAMA micro-ELISA measuring anti-isotypic and anti-idiotypic HAMA; ImmunSTRIP anti-isotypic HAMA assay; TruQuant HAMA RIA measuring anti-isotypic and anti-idiotypic HAMA	11/32 (34%) developed HAMA after infusion of CA-125 monoclonal antibodies: 3/7 after the first administration, 6/13 after the second administration, 2/2 after the third administration	10/32 developed extremely high CA-125 levels as measured with the CIS IRMA assay, in parallel to a significant HAMA increase.
[76] 1994	61 cancer patients receiving a single diagnostic injection of radiolabeled monoclonal antibodies	Specific ELISA using injected monoclonal antibody-coated plates and anti-human IgG or IgM as tracer	25/61 (41%) developed HAMA within 14 days after infusion: 3/61 (5%) IgM HAMA, 5/61 (8%) had IgG HAMA, 17/61 (28%) both IgM/IgG HAMA	n/a
[80] 1994	12 patients receiving anti-IL6 monoclonal antibody (IgG1): 6 multiple myeloma, 6 metastatic renal cell carcinoma	Specific ELISA using two mouse anti-IL6 monoclonal antibodies	9/12 (75%) developed HAMA 7–15 days after the beginning of the treatment: 9/9 IgG HAMA, 4/9 both IgM/IgG HAMA	n/a

Table 3. Continued.

[108] 1995	503 healthy individuals	Interference in immunoassays for CEA, using anti-CEA monoclonal antibody-coated plates with monoclonal or chimeric (human/mouse) anti-CEA antibodies as tracer	29% (146/503) gave a false-positive rate with the parental anti-CEA tracer; 2.8% (14/503) gave a false-positive rate with the chimeric anti-CEA tracer	Adding 10 mL/L normal mouse serum or purified mouse IgG to the parental anti-CEA system reduced the false-positive rate from 29% to 6.2% or 4.8%, respectively.
[89] 1995	93 suspected ovarian cancer patients receiving labeled OV-TL3 Fab' (n = 20) or F(ab')$_2$ (n = 73) monoclonal antibody fragments	In-house homologous sandwich-type immunoassay system using ^{125}I-labeled OV-TL3 F(ab')$_2$ fragments	8/20 (40%) developed Fab'-HAMA after injection of Fab' fragments; 14/73 (19%) after injection of F(ab')$_2$ fragments. Most patients developed HAMA within 3 weeks post-injection of F(ab')$_2$ fragments, and were still positive after 1 year.	n/a
[91] 1995	200 normal subjects 40 kidney transplant recipients treated with rat anti-CD45 monoclonal antibodies 10 RF positives	Two assays specific for rat anti-CD45 monoclonal antibody (YTH 24.5 and YTH 54.12)	10% (20/200) 5% (2/40) within 14 days, returning to negative after 1 to 3 months posttransplant 10% (1/10)	n/a

hCG, human chorionic gonadotropin; CK-MB, creatine kinase MB isoenzyme; CEA, carcinoembryonic antigen.

of thyroid hormone measurements attributable to the presence of HAMA in the patient's sample.

OVERCOMING RHEUMATOID FACTORS

RF also may behave like HAMA and exhibit nonspecific binding to the analytical antibodies [1, 5, 115]. Serum RF are known to be IgM-isotype antibodies, with a specificity against the Fc fragment of human IgG. Their highest prevalence is ~70% in rheumatoid arthritis patients [116]. They may be also present, with a much lower prevalence, in other autoimmune diseases as well as in elderly, otherwise normal, individuals. Because RF consist predominantly of IgM antibodies that are directed against the Fc fragment (CH$_2$ and CH$_3$ domains) of human IgGs and because species' immunoglobulins may have highly conserved epitopes within their Fc portion, some have investigated the cross-reactivities between HAMA and RF activities. Courtenay-Luck et al. [117] demonstrated that preexisting HAMA, mainly of the IgM isotype, and polyclonal RF bind both human and murine immunoglobulins, and that binding of HAMA or RF to mouse IgG may be blocked by preabsorption of the patient's sample with mouse or human IgG, respectively. Hamilton et al. [93, 115] also reported that RF do not bind only human IgG but may also cross-react with other species' immunoglobulins, e.g., rabbit, sheep, goat, and mouse IgG, the lowest serum binding being displayed against mouse IgG. Consequently, RF-positive sera, like heterophile antibodies, can interfere in immunoassays, especially two-site methods. Some investigators, however, recently described for the first time interferences with measurements of serum FT$_4$ caused by RF, resulting in misleading increases of FT$_4$ concentrations with current immunoassays in 5 clinically euthyroid elderly patients [118]. The interference by RF seems to be much less frequent than that by HAMA because RF has much less affinity for murine than human immunoglobulins. The nonspecific binding by RF can be overcome in the same manner as for heterophile antibodies, by using blocking reagents such as nonimmune homologous immunoglobulin.

PRACTICAL PROBLEM SOLVING

The following steps summarize a practical approach for detecting these artifacts:

1. The use of thyroid testing algorithms means that in many cases a single rather than multiple thyroid function tests may be performed at one time. When, however, more than one test is done, the results should be verified in combination for each patient before reporting. If a discrepant result is found, particularly an increased TSH together with an increased FT$_4$, FT$_3$, T$_4$, or T$_3$, then antibody interference should be suspected.

2. The most important strategy is the routine communication between laboratory professional and clinician. In this way, a discrepancy between clinical findings and laboratory findings can be followed up, with interference being evaluated as a possible cause.

Table 4. Case reports of heterophile antibody interference.

Study	Clinical picture	Interfering antibodies	Thyroid tests	Evidence of inappropriate clinical intervention
[100] 1980	Euthyroid patient with coronary artery disease and hypercholesterolemia	Heterophile antibodies reacting with rabbit IgG used in the assay kit	TSH of 846 μU/mL (mU/L) by an IRMA using both labeled and solid-phase rabbit antibodies. Addition of rabbit serum to the assay reagent resulted in normal TSH value (3.9 μU/mL).	Unnecessary additional clinical chemistry investigation
[103] 1981	14 euthyroid neonates of 10 261 subjects (0.1%) gave abnormal TSH results during national screening program for congenital hypothyroidism	Heterophile antibodies reacting with immunoglobulins from different species; these antibodies disappeared from circulation within 2 months in the infants and within 4–6 months in the mothers	TSH of all subjects spuriously high (>40 μU/mL), with NN-TSH and P-TSH kits; TSH values became normal after addition of rabbit serum to the patients' specimens.	Some children and (or) mothers had: TRH stimulation tests; T_3 suppression tests; iodine measurements in milk of lactating mothers; serial thyroid function evaluations
[83] 1981	7 euthyroid infants had high TSH results during screening program for congenital hypothyroidism	Transplacental passage of heterophile antibodies from mothers who previously received injections of a microbial vaccine cultured on a medium enriched with a rabbit tissue homogenate	TSH spuriously high (40–125 μU/mL) with a double-antibody RIA; all patients' TSH values were decreased to normal by addition of rabbit serum or purified IgG.	Misdiagnosis of neonatal hypothyroidism and unnecessary clinical assessments; 4 subjects were inappropriately treated with L-T_4 during 4 months.
[84] 1981	False hyperthyrotropinemia in a 54-year-old clinically euthyroid woman	Heterophile antibodies against rabbit immunoglobulins secondary to an unusual treatment with rabbit serum proteins and human tissue extracts	TSH spuriously high with a double-antibody RIA using rabbit antibodies; TSH decreased from 2500 to 25 μU/mL after addition of rabbit serum.	Unnecessary diagnostic testing, including thyroid uptake of [131]I
[95] 1987	Two cases of euthyroid patients with factitious increase of TSH: 30-year-old veterinarian woman 64-year-old woman	IgG class human anti-mouse antibodies	TSH measurements with the Hybritech monoclonal two-step Tandem-R assay: Case 1: 15.0 mU/L (0.5–6) Case 2: 19.6 mU/L TSH returned to normal after addition of mouse serum to both patients' samples	Case 1: Inappropriate treatment with increasing doses of L-T_4, and unnecessary diagnostic testing, including TRH stimulation, ^{131}I uptake, and tomographic scan Case 2: Unnecessary ^{131}I uptake test, TRH stimulation test and clinical chemistry reassessment
[101] 1988	33-year-old woman investigated for suspected hypothyroidism	HAMA	TSH 8.3 mU/L (0.3–5) with Amerwell IRMA, in contrast to 0.4 mU/L (0.3–5) with Simultrac IRMA. After 6 weeks of T_4 treatment, TSH remained high at 9.4 mU/L. TSH progressively decreased after addition of increasing amounts of mouse serum to the patient's sample.	According to the TSH results, the patient was put on T_4 150 μg daily to see if production of TSH would be suppressed
[109] 1988	Three patients showing spuriously high TSH: 62-year-old slightly hyperthyroid woman with Sjögren syndrome; 62-year-old euthyroid man; 36-year-old euthyroid woman	HAMA specific for mouse IgG1	All three patients showed falsely high TSH (30.5, 74, and >50 mU/L) with the three-site solid-phase Serono MAIAclone IRMA; when increasing amounts of mouse serum or purified mouse or horse IgG were added to the patients' sera, TSH became undetectable.	Two were treated for hypothyroidism inappropriately and one underwent unnecessary diagnostic testing
[104] 1988	13.5-year-old boy with 9-month history of fatigue and difficulty in concentrating; no clinical signs of thyroid disease	HAMA	TSH ~46.5 mU/L (0.4–4.6) with Serono MAIAclone three-site solid-phase IRMA, decreasing to 4.3 mIU/L after addition of mouse IgG to patient's serum	Misdiagnosis leading to inappropriate T_4 replacement therapy and unnecessary diagnostic testing
[110] 1991	34-year-old clinically euthyroid woman with normal physical examination	HAMA	TSH of 8.5 mU/L (0.2–3.5) with Delfia assay, which contains mouse IgG in the assay reagents; addition of increasing amounts of mouse IgG (0–20 μg) to the patient's serum decreased apparent TSH concentration to undetectable.	Misdiagnosis of subclinical hypothyroidism leading to inappropriate treatment with increasing doses of L-T_4 and unnecessary diagnostic testing
[75] 1994[a]	57-year-old euthyroid man with a history of liver fibrosis, portal hypertension, and hypersplenism; a farmer, he also had history of exposure to sheep, mice, and cattle	Heterophile antibodies reacting with sheep and mouse antibodies	TSH 12.8 mU/L (0.15–3.2), FT$_4$ 29.4 pmol/L (9.0–20.6), T_3 5.7 nmol/L (1–2.6) with Amerlite assays using mouse or sheep antibodies; T_4 and T_3 decreased after PEG precipitation or addition of sheep serum, whereas TSH decreased after addition of mouse anti-human IgM antibodies.	Misdiagnosis of TSH-dependent hyperthyroidism or thyroid hormone resistance syndrome and unnecessary diagnostic investigations, including thyroid function, TRH stimulation, ^{125}I-uptake, MRI, and tomography

[a] First report of artifactual increase in results obtained by T_4, T_3, and TSH assays for a serum containing heterophile antibodies.

TRH, thyroliberin; MRI, magnetic resonance imaging.

3. The laboratory should repeat the suspected test to confirm the finding. If the finding is still present, then (*a*) document both the clinical findings (disease state and treatment) and specimen-related information (sample and storage conditions, and results of any other assays, especially immunoassays, done on the same specimen); (*b*) reevaluate by using another, comparable method. In addition, for T_3, T_4, and TSH, nonlinearity with sample dilution may suggest interference; this is not recommended for the free hormone assays, however, where dilution nonlinearity is expected. Other antibody interference investigations might be carried out as described earlier; if these are performed infrequently, however, we recommend use of a specialized evaluation center such as the Centre for Research and Evaluation in Diagnostics (http://www.crc.cuse.usherb.ca/cred or fax 819-564-5445), or refer to the *Directory of Rare Analyses* (DORA) from AACC.

In summary, two major antibody categories are responsible for thyroid hormone assay interference. In the first category, autoantibodies against thyroid hormones, especially anti-T_4 and anti-T_3 antibodies, can give abnormal values in thyroid function evaluation. These endogenous factors particularly interfere in T_4, FT_4, T_3, and FT_3 methods; analog methods are more susceptible to this type of interference. Thyroid hormone antibody interferences are difficult to predict and can occur even with frequently used and well-characterized methods. Antibody prevalence depends on the detection method; it is low in healthy subjects but maybe as high as 10% in patients with autoimmune disease—although only a minority of such samples demonstrate substantial thyroid assay interference. Heterophile antibodies, on the other hand, which include HAMA and RF, interfere by a common mechanism and may give spuriously high values in two-site immunoassays. As regards thyroid function evaluation, this type of interference has mainly been shown in TSH measurements by immunometric assays but has also been described in a competitive FT_4 assay. In contrast to autoantibody interferences of the category described above, heterophile antibodies can usually be blocked, e.g., by adding excess nonimmune immunoglobulin generally obtained from the same species as the reagent antibody. Most modern assays use sufficient amounts of blocking reagents to inhibit the majority of this interference; nevertheless, some samples with high titers may still express clinically important assay interference. Case examples of unnecessary patient interventions attributable to misinterpretation of thyroid function test interference continue to be reported in the literature. Both laboratory professionals and clinicians must be vigilant to the possibility of antibody interference in thyroid function assays. Results that appear to be internally inconsistent or incompatible with the clinical presentation should invoke suspicion of the presence of an endogenous artifact and lead to appropriate in vitro investigative action.

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