Interference by human anti-animal immunoglobulins, commonly referred to as heterophile antibodies, in immunologic assays is known to be an important consideration for medical testing laboratories (1–3). Although automated immunometric assays are formulated to reduce these effects, it is unlikely that complete elimination occurs (2), and artifactual results attributable to heterophile antibodies have been reported for some assays (4, 5). Such results often are identified by addition of blocking agents to the samples before assay. A simple sample pretreatment method uses a commercially available blocking tube, HBT. An alternative technique uses polyethylene glycol (PEG) to precipitate immunoglobulin-sized molecules before assay. For both of these techniques, a difference between values for the treated and untreated specimens is interpreted as evidence for heterophile antibody interference.

It is not clear that either of these methods is appropriate for every immunoassay. It is important to know the effect of sample pretreatment on the results of each assay. This has been done recently for HBT and a thyroglobulin assay (4). We examined seven automated analyzer hormone assays, using pretreatments with both HBT and PEG of samples from healthy adults with the aim of determining the expected change in results post treatment for both techniques for each assay.

We investigated luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin (PRL), and growth hormone (GH) on the Access2 analyzer and insulin, parathyroid hormone (PTH), and cross-linked C-terminal telopeptide of type I collagen (β-CTx; β CrossLaps) assays on the Elecsys analyzer. Analyzer reagents and consumables were obtained from Beckman-Coulter and Roche Diagnostics, respectively. Plasma (EDTA) samples were obtained from Beckman-Coulter and Roche Diagnostics, respectively. Plasma (EDTA) samples were obtained from Beckman-Coulter and Roche Diagnostics, respectively. Plasma (EDTA) samples were obtained from Beckman-Coulter and Roche Diagnostics, respectively.

These were also tested for LH, FSH, and PRL by a different methodology (Abbott Architect) and with the Access assay after 1-in-2, 1-in-4, and 1-in-8 dilution of the untreated and HBT-treated samples.

The concentrations in treated samples as percentages of the concentrations in the nontreated samples are shown in Fig. 1. For both HBT (Fig. 1A) and PEG treatment (Fig. 1B), the values differed from 100%. The median (CV) values after HBT treatment were 89 (8)% for PTH, 100 (7)% for β-CTx, 99 (6)% for insulin, 81 (130)% for LH, 73 (38)% for FSH, 92 (24)% for PRL, and 98 (400)% for GH. The median values after PEG treatment were 179 (22)% for PTH, 188 (22)% for β-CTx, 111 (7)% for insulin, 53 (15)% for LH, 95 (15)% for FSH, 100 (14)% for PRL, and 125 (21)% for GH.

These data can be used to establish the expected values applicable to each pretreatment technique. Such reference information can be derived even when there is not “recovery” of 100%; for example, a target value for PTH recovery after HBT treatment would be 89%, and a reference interval could be calculated nonparametrically or by other appropriate techniques. A reasonably tight distribution of recovery values is, however, required.

If a CV of <10% is regarded as acceptable, our data suggest that HBT pretreatment is a suitable method of testing for heterophile antibody interference in the Elecsys PTH, β-CTx, and insulin assays. Similarly, PEG treatment is acceptable for the Elecsys insulin and the Access FSH assays. For both techniques, validation using specimens with true positive or negative interference would be desirable.

These conclusions assume that there was no contribution to the distributions from interfering heterophile antibodies or other substances in these presumably normal specimens. The Access PRL assay after PEG treatment may be suitable despite the CV of 14% because PEG treatment is frequently used to detect macroprolactin interference (6). It is possible that two samples with low recoveries contained macroprolactin. Our experience with this assay is that samples with PEG recovery values <60% contain high-molecular-weight prolactin immunoreactivity after gel filtration.

The considerable variability seen in the Elecsys PTH and β-CTx and the Access GH assays after PEG treatment probably reflects interference by the PEG in the antibody–antigen reactions of these assays. Similarly, although we do not know the mechanism, it appears that HBT treatment can produce a spuriously high recovery of hormone in some assays. The manufacturers state that HBT contains a “unique blocking agent” limited to use for antigen assays to confirm or disqualify an original result in conjunction with other data (such as symptoms and other testing). Our data suggest a further limitation that its use is assay specific, possibly dependent on the assay configuration. We did not observe overrecovery in the three Elecsys assays (all configured with mouse monoclonal antibodies for both capture and detection). It was, however, apparent in the Access assays (LH, FSH, GH, and to a lesser extent, PRL), which contain solid-phase goat
anti-mouse monoclonal complexes for capture and goat antibodies for detection.

We believe that these high HBT recovery values are likely to be spurious. The heterophile antibody interference in most immunometric assays is generally positive, leading to lower concentrations after blocking treatments, not increased values as we have mostly observed. Although 30–40% of the population may have heterophile antibodies through exposure to animals or monoclonal antibody treatment, the frequency of interference in modern immunoassays is reported to be very low (1, 2). Finally, although we did not investigate all of the specimens with HBT-induced increases in hormone concentrations, we demonstrated that the results were spurious in one case. The specimen outlier detected in the prolactin assay (Fig. 1A) and also showing overrecovery in the Access LH, FSH, and GH assays did not appear as an outlier in any Elecsys assays, or in any assay after PEG treatment. The hormone concentrations of the untreated sample were consistent with those of a “normal” 53-year-old male (7.1 IU/L LH, 12.5 IU/L FSH, 99 mIU/L PRL, and 0.02 mIU/L hGH) and were within our laboratory’s reference intervals. In contrast, the post-HBT values were unexpectedly high (108 IU/L LH, 37 IU/L FSH, 325 mIU/L PRL, and 1.6 mIU/L GH). Analysis by another laboratory with the Abbott Architect analyzer confirmed the untreated values (10 IU/L LH, 12 IU/L FSH, and 142 mIU/L PRL) with no increase after HBT treatment. Dilution of the untreated sample showed linearity in the Access LH, FSH, and PRL assays with dilution recoveries in the range 103–126%. In contrast, dilution of a HBT-treated aliquot showed poor linearity (170–486%) in all assays, indicative of nonspecific interference in the assay.

In summary, the validity of the technique used to detect heterophile antibody interference is specific to each assay method. Our data suggest that use of HBT tubes may be a suitable simple technique for the Elecsys PTH, β-CTx, and insulin assays but not the Access LH, FSH, PRL, or GH assays. Other blocking agents may be appropriate for these methods. Pretreatment with PEG is suitable for the

Fig. 1. Percentage of untreated concentrations (“recovery”) of hormones in HBT-treated (A) and PEG-treated (B) samples compared with untreated human plasma.

n = 103 samples for the Access GH and 113 samples for the Access LH, FSH, and PRL and Elecsys PTH, β-CTx, and insulin assays.
Elecsys insulin and Access FSH and, possibly, PRL assays. We conclude that the reagents used to test for heterophile antibody interference can themselves cause variable interference in some assay systems; therefore, the validity and expected ranges should be checked for each assay and heterophile antibody detection method.

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References


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