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References


Techniques for Identifying Heterophile Antibody Interference Are Assay Specific: Study of Seven Analytes on Two Automated Immunoassay Analyzers, Margarette Jane Ellis* and John Hamilton Livesey (Endolab, Christchurch Hospital, Private Bag 4710, Christchurch, New Zealand; * author for correspondence: fax 64-3-3640-818, e-mail Jane.ellis@cdhb.govt.nz)

Interference by human anti-animal immunoglobulins, commonly referred to as heterophile antibodies, in immunoassays 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 artificial 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 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) on the Eclsys analyzer. Analyzer reagents and consumables were obtained from Beckman-Coulter and Roche Diagnostics, respectively. Plasma (EDTA) samples were collected from healthy individuals in a study approved by the Canterbury Ethics Committee, New Zealand. Samples (n = 103–113) were assayed directly and after pretreatment of a 0.5-ml aliquot in blocking tubes (HBT; Scanti-bodies) at room temperature for 1 h. A third aliquot was treated with an equal volume of PEG solution (PEG 6000; 250 g/L in 0.05 mol/L phosphate buffer, pH 7.4, contain-
ing 0.5 g/L Triton X-100), and the supernatant obtained after centrifugation was assayed. All tests for each sample were performed on the same day. Results are expressed as percentage recovery of hormone in the treated compared with the untreated sample aliquots. A dilution factor of 2 was used for PEG recovery calculations. We also tested aliquots from an individual whose plasma had shown extremely high values for PRL, LH, and FSH after HBT treatment by a different methodology (Abbott Architect) and after 1-in-2, 1-in-4, and 1-in-8 dilution of untreated and HBT-treated samples (Access assays).

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 median values differed from 100% for some assays. 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 (8)% 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 an-

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.
tibodies 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-antimouse monoclonal antibody 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 antibodies, the frequency of interference in some immunometric assays is generally positive, likely to be spurious. The 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.

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 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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Hemoglobin H Disease Classification by Isoelectric Focusing: Molecular Verification of 110 Cases from Thailand, Pranee Sutcharitchan,1† Wen Wang,2† Rung Set-tapiboon,3 Supaporn Amornsriwat,4 Arnold S.C. Tan,2 and Samuel S. Chong2,3†

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Hemoglobin (Hb) H disease is a mild to severe form of α-thalassaemia caused by the absence/inactivation of three