wild type, although the duplex is destabilized by two mismatches in both cases. This illustrates the deviation from n-n behavior in longer DNA strands and makes the results difficult to interpret. Furthermore, sometimes a product $T_m$ differs by only $0.1\, ^\circ\text{C}$, whereas mean SDs are $0.14\, ^\circ\text{C}$ (samples 15 and 2; samples 6 and 11). This makes it impossible to “easily distinguish different types of AGXT mutations” as claimed by Pirulli et al. (2). This is exemplified in Fig. 1, which does not show melting curves, but the expected probability distribution defined by $88.0\, ^\circ\text{C}$ with an SD of $0.14\, ^\circ\text{C}$ and $88.1\, ^\circ\text{C}$ with an SD of $0.18\, ^\circ\text{C}$ (solid line) and $88.0\, ^\circ\text{C}$ with an SD of $0.14\, ^\circ\text{C}$ and $88.1\, ^\circ\text{C}$ with an SD of $0.18\, ^\circ\text{C}$ (dashed line) [samples 15r and 2 in Table 1 of Ref. (2)].

Our conclusion is that DNA $T_m$ assays based on SYBR Green I melting curves are powerful tools for screening various types of mutations but lack both sequence and mutation specificity. A lack of sensitivity must be expected if stable mismatches occur among stable neighboring bases (high GC content). Results obtained with these methods at highly polymorphic loci must be confirmed by a mutation-specific detection method. Interpretation of results arising from only the inspection of SYBR Green I melting curves requires great caution. In our opinion, such methods should not be used for routine genotyping applications.

References


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Use of Heterophilic Antibody Blocking Agent (HBT) in Reducing False-Positive hCG Results

To the Editor:
The USA hCG Reference Service aids physicians in the interpretation of discordant or irregular human chorionic gonadotropin (hCG) results that do not concur with physical findings. The Service has now observed 24 cases of false-positive hCG results (14–571 IU/L). All have been cases erroneously diagnosed with chorioniccarcinoma or gestational trophoblast disease without demonstrable pregnancy or tumor (1–4). Because of false-positive hCG results, patients have needlessly received chemother-apy, hysterectomy, and/or other surgery. The suspected cause for serum false-positive hCG is the presence of heterophilic antibodies that bind both capture and detection antibodies, effectively bridging them in the same way as the target antigen should in a standard ELISA format.

Until recently, we primarily demonstrated false-positive hCG by the following criteria: (a) more than fivefold variation in results from different hCG assays; (b) positive hCG test results for serum and not urine; and (c) positive values for urinary hCGβ-core fragment in serum (not urine). Recently, however, we have added a test that includes a heterophilic antibody blocking agent [heterophilic blocking tube (HBT) blocking agent; Scantibodies Inc.] to our protocol for the detection of false-positive hCG. The HBT blocking reagent is a unique formulation of immunoglobulins targeted specifically against heterophilic antibodies to neutralize their interference in immunoassays (David Cantor, Office of Development, Scantibodies Laboratories Inc., San Diego CA, personal communication). In this study, serum samples (0.5 mL) were incubated in HBTs for 30 min before use (exactly as described by the manufacturer). They were then assayed in the same manner as untreated serum, in the usual hCG Reference Service hCG immunometric assay.

Here we present the prospective data from the nine most recent false-positive hCG cases where we used the HBT blocking agent and also from five retrospective, or previous, cases (from both stored frozen serum and subsequently provided serum samples) reassayed both with and without the HBT blocking agent (cases 10–14). Table 1 shows hCG values found in serum by the original referring laboratory, the hCG Reference Service hCG immunometric assay, and our assay again with the addition of the HBT blocking agent. In 12 of 14 cases, lower false-positive hCG concentrations were detected by the
Reference Service ELISA than by the original laboratory’s test. Of the 11 samples that gave a positive result with the Reference Service assay, 9 were completely blocked by the addition of the HBT blocking agent and 2 were considerably reduced (>50%) by the HBT blocking agent. In the remaining 3 of the 14 cases, no hCG was detected by the Reference Service assay, and the effect of the HBT blocking agent could not be evaluated.

The finding that the HBT blocking agent blocked all 11 serum samples that gave false-positive results confirms that heterophilic antibodies are the source of the assay interference. The reasons for the occurrence of heterophilic antibodies in healthy individuals are poorly understood (5).

In one large study, the prevalence of heterophilic antibodies, at a concentration sufficient to produce discordant results, was estimated as ~3.4% of the population studied (6). It is clear that heterophilic antibodies present a real problem to immunoassay-generated results, a problem that is made more pertinent especially when serious therapies depend heavily on the results. The HBT blocking agent used here appears to be extremely effective in preventing false-positive hCG results. However, although we have demonstrated the efficiency with our immunometric assay, similar efficiency with other commercial immunometric assays should be individually confirmed.

Table 1. Effect of HBT on hCG immunoassay results.

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<th>Case</th>
<th>Original laboratory hCG test, IU/L</th>
<th>hCG Reference Service hCG assay, IU/L</th>
<th>hCG assay + HBT, IU/L</th>
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References