We reanalyzed 250 patients for H63D and S65C, using an alternative PCR method (2) with the same forward primer but a different reverse primer and producing a 174-bp product. The H63D genotype was determined by use of bcl-1 restriction enzyme digestion, which produced two fragments of 104 and 70 bp in the mutated allele and an undigested product in the wild-type allele. The S65C genotype was determined by use of the same PCR for H63D but with a Hinf-1 restriction enzyme digestion producing two products of 113 and 61 bp in the wild-type allele but an undigested product with the mutated allele.

According to the multiplex PCR (1), 4% were homozygous H63D and 19% were heterozygous; 4.8% were heterozygous S65C and none were homozygous. By the alternative PCR method (2), six cases that had been genotyped as homozygous for H63D by the multiplex PCR method (1) were found to be compound heterozygotes for H63D and S65C. This was confirmed by DNA sequencing.

The presence of the S65C mutated allele interferes with detection of the H63D genotype by the multiplex method of Stott et al. (1). This erroneously leads to ~1–2% of tested individuals being labeled as homozygous for H63D when they are in fact heterozygous for H63D. The presence of the S65C allele appears to lead to a failure to produce the expected digestion products. In all six such patients, an undigested PCR product was produced, indicating that it was not a failure of PCR but a failure of digestion. In four other patients who were heterozygous for S65C and did not have H63D complete digestion at the BprPI site did occur. This failure of digestion occurred only in those S65C-heterozygous patients who were heterozygous for H63D.

The S65C SNP is outside of the restriction site and would not be expected to interfere with digestion by BprPI according to available information on the restriction enzyme site. One possible explanation is that methylation occurs to prevent restriction enzyme digestion in this region. Such methylation, however, would have to be highly specific for this DNA sequence.

A recent UK NEQAS survey indicated that laboratories in the United Kingdom are using many different PCR methods for detecting the common hemochromatosis polymorphisms, and several use the multiplex PCR method (1). Unfortunately, this multiplex method may lead to error in up to 2% of samples. Our current practice is to test for all three mutations.

References

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Aberrant Thyroid Testing Results in a Clinically Euthyroid Patient Who Had Received a Tumor Vaccine

To the Editor:

We describe here the first case of immunoassay interference from