Presence of the Hemochromatosis S65C Mutation Leads to Failure of Amplification in a Multiplex C282Y/H63D PCR

To the Editor:

Hemochromatosis is an autosomal recessive disorder of iron metabolism affecting 0.2%–0.5% of white populations. Approximately 90% of affected individuals are homozygous for the C282Y mutation, but the H63D and S65C mutations are also of interest.

Various molecular diagnostic methods for detection of these mutations have been described, including a multiplex PCR from Stott et al. (1) that simultaneously detects the C282Y and H63D mutations. In this method, PCR-mediated site-directed mutagenesis is used to create a BbrPI restriction site in the wild-type C282Y and H63D products. To detect the C282Y mutation and the H63D mutation, respectively, 1 specific mismatch in the forward 282mut primer and 2 specific mismatches in the reverse 63mut primer are incorporated. The presence of either mutation abolishes the restriction site.

Comparing the method of Stott et al. (1) with a reverse hybridization line-probe assay (LiPA) (2) we found a discrepant result. A compound heterozygous H63D/S65C sample was characterized as H63D heterozygote by the LiPA method, and as H63D homozygote by the method of Stott et al. (1). The S65C mutation introduces an additional 3rd mismatch between primer and target, because the S65C mutation is also located in the binding site of the reverse 63mut primer. We therefore hypothesize that only the H63D mutant allele is amplified in the multiplex PCR in the case of a H63D/S65C compound heterozygous genotype, resulting in a H63D homozygous genotype.

Starczynski et al. (3) found the same discrepant results in compound heterozygous H63D/S65C samples with the method of Stott et al. (1). Starczynski et al. (3) hypothesized, however, that the presence of the S65C-mutation leads to a failure of digestion and not to a failure of amplification because an undigested PCR product was produced in all S65C/H63D compound heterozygous samples. The failure of digestion occurred only in samples from S65C-heterozygous patients who were also heterozygous for H63D. In patients who were heterozygous for S65C and did not have H63D, complete digestion at the BbrPI site did occur. Although the S65C mutation is outside the BbrPI restriction site and is therefore not expected to interfere with the digestion, Starczynski et al. (3) theorized that methylation occurs and prevents digestion.

Recently we found a sample that produced only the C282Y PCR product and failed to produce the H63D PCR product. DNA sequencing results confirmed that this sample was S65C homozygous and H63D wild-type. This S65C homozygous genotype provides evidence that presence of the hemochromatosis S65C mutation leads to failure of amplification and not to failure of digestion.

Because it is assumed that the H63D and S65C mutations do not occur on the same chromosome (4), the risk for overestimation of H63D homozygotes should be taken into account when using the method of Stott et al. (1), which may lead to error in up to 2% of the samples (3). Either an alternative method can be considered, or every sample found to be H63D homozygous or “no-product” should be genotyped separately for the S65C mutation to exclude the presence of this mutation.

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References


A Recommended Improvement for Specifying and Estimating Serum Creatinine Performance

To the Editor:

Myers et al. (1) discuss the importance of creatinine analytical performance in the estimation of the glomerular filtration rate. They correctly specify a model of assay performance but subsequently do not seem to use that model. I suggest a variation of their model that is less subject to misinterpretation. The Myers et al. (1) model is:

\[ Y = \text{bias} + \text{imprecision} \]

where \( Y \) is a result of a field creatinine assay, \( \text{bias} \) is the average bias between \( Y \) and a reference method for creatinine measurement, and imprecision includes imprecision sources from short-term (within-run or repeatability) and long-term (within-laboratory or reproducibility) and random patient interferences [called specimen-specific effects by Myers et al. (1)]. Here we refer to the 1st 2 sources of imprecision as total imprecision and the last source as random patient interferences.

Although the Myers et al. (1) model is correct, the simulation carried out by Myers et al. is subject to misinterpretation. It is confusing to present the 2 error sources, total imprecision, and random interferences as 1 combined error source, because these...