When a clinical laboratory has produced biological reference values according to IFCC recommendations, should systematic error be referred to as the conventional true value of the calibrators used during the production of reference values?

Although some of the most relevant elements contributing to uncertainty can potentially be evaluated in clinical laboratories, the effort required to undertake such an endeavor might be so great that it will be difficult to bring into general use the uncertainty of patients’ results.

Fluorogenic Probes to Detect the A~-444C Transversion in the Leukotriene C4 Synthase Promoter

To the Editor:
Adverse hypersensitivity reactions to aspirin and other nonsteroidal anti-inflammatory drugs (1) resemble pseudoallergic reactions with bronchospasm, chronic rhinocongestivity, nasal polyps, urticaria, angioedema, life-threatening bronchospasm, and anaphylactic shock (2). Up to 25% of hospital admissions for acute asthma may be caused by nonsteroidal anti-inflammatory drug ingestion. In aspirin-induced asthma, cysteine-leukotriene release into airways (3, 4) is associated with increased concentrations of leukotriene C4 synthase, the enzyme that forms leukotriene C4 [for review, see Ref. (5)]. A polymorphism in the promoter region of the gene for leukotriene C4 synthase predisposes to disease (4). The A~-444C transversion creates a new MspI restriction site.

PCR-restriction fragment length polymorphism analysis has been used to detect this polymorphism (4). This method is time-consuming and requires multiple manual steps. We used rapid-cycle PCR combined with real-time fluorescence monitoring and fluorescent probe melting point analysis to detect the polymorphism (6) in 1 h.

Genomic DNA from 300 patients was extracted from whole blood or serum (n = 20). To confirm the LightCycler genotyping results, DNA samples (n = 60) were analyzed by PCR-restriction fragment length polymorphism analysis as described (4). The two methods were in complete agreement. PCR was performed in a reaction volume of 10 μL with 1 μL of DNA in the presence of primers (0.5 μmol/L; sense, 5’-TCATTCTGAAGCAAGGC-3’; antisense, 5’-ACACACTTGGTCTGTG-3’) with reaction buffer (LightCycler DNA Master Hybridization Probes 10 × buffer, 1.75 mmol/L; Roche Molecular Biochemicals) and the probes (0.2 μmol/L). The detection probe 5’-ACCTTATCTGTCCCTGTCCCACT-3’ was labeled at the 3’ end with fluorescein; the anchor probe 5’-CCAGGCCTCAGGTAAACTCCGCTCC-3’ was labeled with LC Red 640 at its 5’ end and modified at the 3’ end by phosphorylation to block extension.

The amplification and melting curve analyses were performed in a LightCycler device and included initial denaturation at 94 °C for 120 s, followed by 60 cycles of denaturation (94 °C for 0 s, with a temperature transition rate of 20 °C/s); annealing (57 °C for 10 s; transition rate, 20 °C/s) and extension (72 °C for 13 s; transition rate, 20 °C/s). After amplification, we recorded the melting curve by cooling the reaction mixture to 54 °C for 10 s and then by slowly raising the temperature to 80 °C at 0.1 °C/s. The fluorescence signal (F) was continuously monitored during the temperature ramp and then plotted against the temperature (T) to obtain melting curves for each sample. The melting curves were subsequently converted to derivative melting curves [−(dF/dT)] vs T.

The melting curves of the AA, AC, and CC genotypes of the LTC gene are shown in Fig. 1. The melting peaks of samples homozygous for the A allele were at 64.4 °C (range, 63.7–64.9 °C), whereas in samples homozygous for the C allele, the melting peak was 59.0 °C (range, 58.7–59.4 °C). The heterozygous samples produced both peaks. Repeated analysis (10 times) of three samples with different genotypes (AA, AC, CC) showed no misclassifications.
We compared different DNA amounts and several DNA sources. Variant DNA amounts ranging from 9 to 640 ng of DNA template from either blood or serum gave reproducible genotyping results. The latter indicates the high flexibility of the method and allows for the omission of DNA quantification before genotyping.

We conclude that this assay is a valuable method that allows simple and rapid high-throughput genotyping in the clinical laboratory.

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References