described reference intervals *(4)* for gestational age for groups 2 and 3, except for a low concentration of arginine found in 1 FHB patient. In contrast, these amino acid concentrations were all above the reference values in group 1, except for 1 patient with normal lysine and arginine values.

Chromosomal aneuploidy and cystic fibrosis were systematically excluded in the FHC group. The 6 FHC pregnancies went to term, and the newborns had no apparent complications. For 4 of these cases (including the patient with normal AF lysine and arginine), urine samples were obtained at 1–3 years of age. Cystinuria was confirmed by urine amino acid chromatography (half-cystine, ornithine, lysine and arginine concentrations >10 times the upper limit of the reference interval), thus excluding transient hyperexcretion of cystine in the first months of life as an explanation *(5)*.

Cystinuria is caused by defects in the amino acid transport system rBAT/$b^{0,+}$AT of epithelial cells of the renal proximal tubule and small intestine *(6)*. In the kidney, rBAT/$b^{0,+}$AT is the main transport system for cystine reabsorption. During fetal life, tubular maturation begins after the 14th week. After 20 weeks, the kidneys provide >90% of the AF volume. Thus the AF amino acid chromatography in cystinuria-affected fetuses shows the same profile, as does postnatal urine.

The intestinal hyperechogenicity in the 6 cystinuria-affected fetuses was not located in the small intestine, where the transporter is expressed, but downstream. During fetal life, the fetus continuously swallows AF. In the cystinuria-affected fetus, the renal reabsorption defect leads to an abnormally high concentration of cystine in AF. Because the rBAT/$b^{0,+}$AT transport system is the only high-affinity system for cystine absorption in the small intestine, we propose that a progressive overload of the intestinal cystine transporter capacity occurs. Thus, unabsorbed cystine progressively concentrates and then precipitates in the intestinal tract. The occurrence of FHC after only 26 weeks can be explained by the progressive closure of the anal sphincter that commences after 22 weeks. Indeed, FHC resolved after evacuation of meconium in one of our cases.

These findings suggest that FHC should be studied as a potentially useful diagnostic indicator of cystinuria and could be regarded as a possibility to provide preventive medicine.

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Instrument Comparison for Heterozygote Scanning of Single and Double Heterozygotes: A Correction and Extension of Herrmann et al., Clin Chem 2006;52:494-503

To the Editor:

We have discovered that the DNA sample used in our recent paper *(1)* as a control heterozygote at the sickle cell locus of β-globin (17A→T, also known as HBB c.20A→T by HUGO nomenclature) contained an additional variant. Subsequent sequencing revealed a double heterozygote, HBB c.[9C→T; 20A→T]. The HBB c.9C→T is a silent variant for the 3rd amino acid, histidine. In view of this additional sequence variation, we re-evaluated the heteroduplex scanning capabilities of the instruments, as reported in the original Fig. 3, to ascertain their ability to distinguish melting curves of heteroduplexes caused by single and double heterozygotes from melting curves of homoduplexes. The c. 9C→T is a common variant with an allele frequency of 38%, as determined in review of clinical samples submitted for β-globin sequencing (courtesy of Dr. Elaine Lyon, ARUP Laboratories).

The study was repeated as previously described *(1)*, including both single and double heterozygotes. Eight instruments were evaluated for geno-

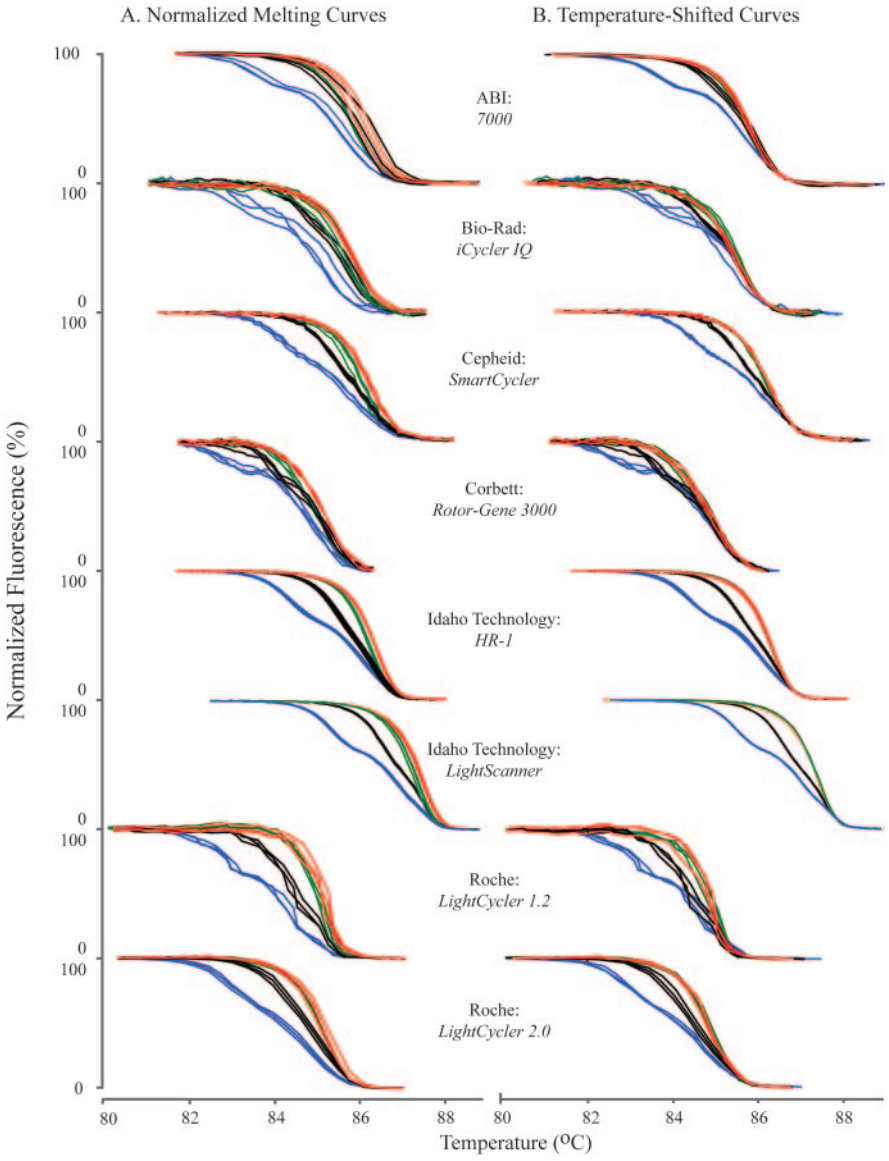

Fig. 1. Melting curves of 110-bp HBB amplicon, including c.20A→T (sickle cell) and c.9C→T (silent variant).

Each genotype was melted and displayed in triplicate on 8 different instruments. Melting curves for the homozygous wild-type are shown in *green*, the homozygous mutant (c.20A→T) in *red*, the single heterozygous mutant (c.[20A→T]) in *black*, and the double heterozygote mutant (c.[9C→T; 20A→T] in *blue*. (*A*), normalized melting curves for genotyping; (*B*), temperature-shifted curves for heterozygote scanning.

typing and heteroduplex scanning resolution (ABI's 7000, Bio-Rad's iCycler, Cepheid's SmartCycler, Corbett's Rotor-Gene 3000, Idaho Technology's HR-1 and LightScanner, and Roche's LightCycler 1.2 and LightCycler 2.0). The resulting normalized melting curves for genotyping and temperature-shifted curves of the 4 genotypes [wild-type, homozygous variant (c.20A→T), single heterozygote (c.20A→T), and double heterozygote (c.[9C→T; 20A→T])] are shown in Fig. 1. The melting curves of the homozygous genotypes are similar to those reported earlier. Melting curves of heterozygous genotypes segregated according to the number of mismatches present, with the double heterozygote resulting in more low-temperature melting than the single heterozygote. After temperature shifting, the heterozygotes were readily distinguishable by curve shape *(2–4)*.

The repeat analysis confirms our earlier results and further shows the capability of DNA melting analysis and its dependency on instrument resolution. Although all melting curves from heterozygotes are distinguishable, some resolve from the homozygotes with greater clarity than others after temperature correction (Fig. 1B), with the fundamental shape of each genotype differing from instrument to instrument. As seen by comparing the single heterozygous sample to the wild-type, the high-temperature regions merge on some instruments but are clearly distinct on other instruments. Heterozygous and homozygous samples segregate either by subtle changes in the melting curve slope or by the formation of multiple distinct melting features separated by inflection points, as was also seen in melting curves from the double heterozygote, for which some instruments resolved 2 melting regions and others 3. The continuing development of instruments with finer temperature control and fluorescence acquisition will lead to increased detail derived from heteroduplex and homoduplex contributions to the overall melting curve, providing even greater ability to identify unique sequence variants.

Aspects of melting analysis are covered by issued and pending patents owned by the University of Utah and licensed to Idaho Technology. C.T.W. holds equity interest in Idaho Technology.

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Detection of Monoclonal Proteins by Capillary Zone Electrophoresis: Comparison of 2 Multichannel Automated Systems

To the Editor:

Capillary zone electrophoresis (CZE) is an alternative method for separation of serum proteins *(1)*. Two dedicated and automated multichannel instruments are available, the Paragon 2000 (Beckman-Coulter) and the Capillarys (Sebia). The sensitivity and specificity of Paragon 2000 for detection of monoclonal proteins have been reported to be 95% *(2–4)* and 78% *(4)*, respectively. The low reported specificity reflects the frequent occurrence of slight abnormalities in the electropherogram at the anodal part of the γ-globulin fraction (fibrinogen region) *(4)*.

Only 2 studies *(5, 6)* evaluated the Capillarys for detection of monoclonal proteins and reported a high sensitivity, and prospective studies in a routine clinical setting are lacking.

We studied 597 consecutive samples submitted to the laboratory to test for the presence of a monoclonal protein or to reevaluate a known monoclonal protein. All samples were analyzed by the Capillarys (software version 5.2.1) and Paragon 2000 (software version 1.6.02) systems and by immunofixation, which was used as the reference standard. Immunofixation was performed on the Hydrasys Automate (Sebia) according to the manufacturer's instructions, with the use of Hydragel 4 immunofixation gels.

Immunofixation revealed the presence of a distinct monoclonal protein in 246 of the 597 samples. The distribution of the types of monoclonal proteins is given in Table 1. The sensitivity of Capillarys for detecting a monoclonal protein was 90% [95% confidence interval (CI), 85%–93%]. This sensitivity was comparable to that of the Paragon, which was 91.5% (95% CI, 87%–95%; $P > 0.5$ by χ^2).

In 267 samples, immunofixation revealed no abnormalities. The specificity of the Capillarys was calculated to be 87% (95% CI, 83%–91%), higher than the specificity of the Paragon, which was 58% (95% CI, 52%–66%; $P < 0.0001$ by χ^2). The lower specificity of the Paragon 2000 was attributable to the previously described *(4)* disturbed morphology at the fibrinogen position. If such disturbed morphology was not considered abnormal, then the specificity of the Paragon was 80% (95% CI, 75%–88%; $P \leq 0.03$ for comparison with Capillarys by χ^2).

In 84 samples, immunofixation was difficult to interpret (presence of M-protein not excluded, M-protein very faint or not identifiable, differentiation between monoclonal and polyclonal pattern questionable). The results for the Paragon 2000 and Capillarys for these samples are available from the authors.

Quantification of the monoclonal protein by delimitation was possible in 87 samples. There was a good correlation between the Capillarys and Paragon ($r = 0.99$; Pearson). Bland-Altman analysis revealed an intercept of 0.81 (95% CI, 0.558–1.096) and a slope of 0.96 (95% CI, 0.95–0.98).

As shown above, each system may fail to detect monoclonal proteins. For example, the Paragon 2000 failed to detect a monoclonal protein in a sample in which immunofixation revealed an IgMκ monoclonal protein. This monoclonal protein was clearly detected by the Capillarys. Conversely, the Capillarys failed to detect a significant IgMκ monoclonal protein and a IgGκ monoclonal protein. In both samples the CZE electropherograms showed a minor disturbance in morphology of the γ-region but not the obvious monoclonal peak that was observed with agarose gel electrophoresis. A remarkable finding on the Capillarys analysis, observed in both samples, was an unusually long separation between the albumin fraction and the α1-globulin fraction.

Table 1. CZE analysis of 246 consecutive samples positive by immunofixation.

		CZE, Abnormal Fraction Present, n	
Immunoglobulin class	**n**	**Paragon**	**Capillarys**
IgA	34	31	30
IgD	2	2	0
IgG	151	148	145
IgM	25	23	21
κ	20	12	13
λ	14	9	11
Total	246	225	220

We thank Sebia for providing the reagents to perform this study.

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