Galactosemia Screening by Simultaneous Blood Spot Quantification of Galactose and Galactose 1-Phosphate

To the Editor:

Galactosemia, caused by enzyme deficiencies of galactose metabolism, leads to the accumulation of galactose (Gal) and galactose 1-phosphate (Gal 1-P) in blood. The disease is classified into 3 types. The concentrations of Gal and Gal 1-P in galactose 1-phosphate uridylyltransferase (GALT) or galactokinase (GALK) deficiency are quite different (1). Therefore, simultaneous monitoring of Gal and Gal 1-P in blood is useful to distinguish infants with GALT deficiency (type I) from those with GALK deficiency (type II).

Of the various methods for determining Gal or Gal 1-P in blood spots, none are suitable for simultaneous and direct measurement because of the requirements of special reagents or multistep and time-consuming procedures (2, 3).

We developed a novel method based on high-performance anion-exchange chromatography (HPAEC)–pulsed amperometric detection (PAD) that simultaneously measures Gal and Gal 1-P concentrations in patient blood spots.

We prepared blood spot standards using healthy male adult blood and stock solutions of Gal and Gal 1-P. We collected blood spot specimens (70 controls and 12 patients) from Korean newborns within 3 days after birth. All of the samples were identified by quantifying Gal concentrations using the enzymatic colorimetric method (ECM) (3).

Two 3.2 mm–diameter filter paper discs and the internal standard (5 μL of 600 μmol/L fucose)
were extracted with 95 μL of 1% (wt/vol) trichloroacetic acid (TCA) on a shaker for 30 min. For deproteinization, we added 10 μL of 10% (wt/vol) TCA, and the samples were vortex-mixed and centrifuged for 10 min at 12 400g. The supernatants were filtered and injected into the HPLC system. Separation was performed on a Carbopac PA1 column (Dionex), with a gradient of 10 mmol/L NaOH (eluent A) and 10 mmol/L NaOH + 25 mmol/L Na2CO3 (eluent B), as follows: isocratic elution with A:B (100:0) for 3 min, linear gradient elution from A:B (100:0) to (20: 80) for 3 min, isocratic elution with A:B (20:80) for 6 min, back to A:B (100:0) for 0.1 min, and finally equilibration for 14 min; the flow rate was 1.0 mL/min. We monitored the separated analytes using PAD (ICS-3000; Dionex). Using these HPLC conditions, there were no chromatographic interferences. Glucose and glucose 1-phosphate, which can cause false-positive results, were completely separated from Gal and Gal 1-P.

Typical chromatograms for a control and a galactosemia patient are shown in Fig. 1. The transient drift of baseline was caused by the large changes in eluent composition. Our HPLC gradient system was robust, however; we consistently obtained uniform retention times for each chromatogram, and the baseline was quite stable near the Gal and Gal 1-P retention times.

A calibration curve generated using blood spot standards showed linearity from 20 to 500 mg/L. For the intraday analysis of blood spot standards (n = 9), the mean recovery ranged from 97.5% to 100.7% (CV ≤3.0%). For interday analysis (n = 9), the mean recovery ranged from 96.51% to 102.50% (CV ≤7.8%).

Fig. 1 shows the distribution of Gal and Gal 1-P concentrations in blood spots from newborn controls and galactosemia patients. Dashed lines indicate the Gal and Gal 1-P cutoff values (80 and 135 mg/L, respectively) (4, 5). With the ECM method, the Gal concentrations in all normal samples were below the cutoff values. In the HPAEC-PAD method, most blood spot results from normal individuals were also within the cutoff; however, the Gal or Gal 1-P concentrations for 4 individuals were outside the cutoff square, suggesting that these 4 infants would require follow-up. The results also suggest that the ECM, which monitors only a single biomarker (Gal), fails to pick up potentialgalactosemia in approximately 5.7% of cases. On the other hand, the Gal concentrations in all 12 galactosemia patient samples exceeded the cutoff (Fig. 1D). Interestingly, Gal 1-P concentrations in 4 patients were 96.4–132.8 mg/L in the Gal 1-P cutoff range. The Gal 1-P concentrations in 8 patients were abnormal (159.4–330.0 mg/L). All 8 samples showed a correlation between the concentrations of Gal and Gal 1-P. We estimated that 72 samples could be analyzed in 24 h using an autosampler. Therefore, our method will be best suited for precise galactosemia diagnosis in a biochemical diagnostic laboratory. We are now applying this method to quantify UDP-galactose in blood to examine type III galactosemia caused by UDP galactose epimerase deficiency.

**References**


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**Letters to the Editor**

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**References**

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Interlaboratory Variation in 25-Hydroxyvitamin D3 and 25-Hydroxyvitamin D2 Is Significantly Improved If Common Calibration Material Is Used

To the Editor:

Measurements of 25-hydroxyvitamin D2 (D2) and 25-hydroxyvitamin D3 (D3) are used to detect vitamin D deficiency and monitor vitamin D supplementation. Interlaboratory variation within the same method groups makes it impossible to apply standardized reference intervals or published clinical cutoff values. Laboratories therefore must develop site-specific reference intervals.

Advances in the routine use of liquid chromatography–tandem mass spectrometry (LC-MS/MS) in clinical chemistry laboratories have enabled the development of methods for measuring D2 and D3 individually (1). Despite the superior performance of LC-MS/MS methods compared with HPLC and immunoassay approaches, an examination of the UK Vitamin D External Quality Assessment Scheme (DEQAS) data for the LC-MS/MS user group indicates that interlaboratory variation exists within this method group.

Calibration of D2 and D3 assays has been a continuing problem because there is no certified reference material available to test method accuracy.

A recent Letter to the Editor in this journal pointed out that calibration is not the only concern with vitamin D assays. Laboratories also do not have common standard operating procedures and use a wide variety of instrumentation (2).

We used commercially available calibration material (Chromsystems, Munich, Germany) to investigate whether calibrator harmonization could improve agreement between laboratories. We sent out 4 calibrators with manufacturer-assigned values along with 16 plasma pools spiked with different D2 and D3 concentrations. The plasma pools were prepared from spare samples of citrated donor plasma and were spiked with a stock solution of D2 and D3 that had been prepared in methanol and serially diluted to cover the analytical range. Aliquots were prepared and stored frozen until distribution by mail. The calibrators were prepared by serially diluting the lyophilized Chromsystems calibrator (lot 1607) into a PBS solution (137 mmol/L NaCl, 10 mmol/L phosphate buffer, 2.7 mmol/L KCl, pH 7.4) containing 10 g/L BSA. The assigned concentrations of the calibrators were 0, 7.17, 10.75, and 64.5 μg/L for D2 and 0, 7.5, 11.25, and 67.5 μg/L for D3.

Six UK laboratories that were running or developing an LC-MS/MS assay for vitamin D agreed to participate in the study. They were sent 200-μL aliquots of the plasma pools and 500 μL of the calibrators along with a brief method questionnaire. Participants were asked to quantify the D2 and D3 concentrations in the plasma pools and calibrators with their routine method and then recalculate the D2 and D3 concentrations in the plasma pools with the calibrators provided.

All 6 laboratories returned results. One laboratory returned results that had been calibrated with 3 different “in house” calibrators. The laboratories that took part in the study used similar sample-preparation procedures, which were based on the procedure outlined by Maunsell et al. (1). A variety of LC-MS/MS instruments and multiple reaction-monitoring transitions were used in the study.

Fig. 1 shows the intervals, interquartile ranges, and means for the 16 plasma pools before and after D2 and D3 harmonization. The overall D2 interval changed from <2–88 μg/L to <2–61 μg/L, and the D3 interval changed from <6–84 μg/L to <6–50 μg/L.

The mean between-laboratory CV for D2 (calculated from the CVs for the individual pools), decreased from 30% with laboratories’ own calibrators to 9% when harmonized calibrators were used (P = 0.006). For D3, the mean between-laboratory CV decreased from 18% with the laboratories’ own calibrators to 7% with harmonized calibrators (P < 0.0001).

Our results demonstrate that the interlaboratory CV improved after the use of a common calibrator. The CV also improved to a value similar to that expected for intraassay imprecision in an individual laboratory. One could...