min measurements previously observed in our laboratory.

In summary, the presence of 5 g/dL (50 g/L) of M-protein results in BCP albumin determinations that are approximately 30% lower than those obtained using capillary SPEP. According to the International Myeloma Staging System, samples with albumin values of <3.5 g/dL (35 g/L) can potentially be classified as stage I in the absence of increased serum β₂-microglobulin. Here, 521 samples had albumin concentrations <3.5 g/dL (35 g/L) by BCP assay, whereas only 374 samples exhibited albumin concentrations <3.5 g/dL (35 g/L) by capillary electrophoresis. Because the Multiple Myeloma International Staging System does not specify a preferred method for albumin determination, clinicians should be aware of these differences in methods when interpreting albumin concentrations in myeloma patient samples (1, 5).

Acknowledgments: The authors would like to thank the University of Arkansas Clinical Laboratories Immunology and Chemistry Sections.

References

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DOI: 10.1373/clinchem.2008.117119

Changes in Solvent Composition in Tandem Mass Spectrometry Multiplex Assay for Lysosomal Storage Disorders Do Not Affect Assay Results

To the Editor:

Lysosomal storage disorders (LSDs) embody a collection of >40 unique genetic diseases that cause the accumulation of macromolecular substrates normally degraded by lysosomal (and in some cases, nonlysosomal) enzymes involved in lysosomal metabolism (1). Although individual LSDs are rare, their combined incidence has been estimated at 1 per 7700 live births (1). Recently, 2 reports that describe the use of a tandem mass spectrometry–based multiplex assay for newborn screening for LSDs have been published in Clinical Chemistry (2, 3). A third report describes the availability of QC dried blood spot materials for monitoring the quality of the multiplex assay for Krabbe (galactocerebrosidase), Gaucher (acid β-glucocerebrosidase), Niemann-Pick types A and B (acid sphingomyelinase), Pompe (acid α-glucosidase), and Fabry (acid α-galactosidase) disorders (4). The multiplex assay has been shown to be robust and is currently in use or under evaluation in many screening and diagnostic laboratories for newborns around the world.

Current protocols call for the use of acetonitrile as a major component of the mobile phase. Acetonitrile is a widely used solvent in newborn-screening laboratories for LSD and other mass spectrometry–based assays (e.g., for amino acids and acylcarnitines). Several chemical suppliers have informed their customers of a worldwide acetonitrile shortage, which may severely impact all tandem mass spectrometry–based assays conducted in newborn-screening laboratories around the world. To
maintain the continuity of current LSD newborn-screening efforts, we evaluated the use of methanol as an alternative solvent to acetonitrile in the multiplex assay described by Zhang et al. (2).

HPLC-grade methanol was purchased from Fisher Scientific and used as received. The mobile phase and reconstitution solvent consisted of 0.2% formic acid in methanol/water (volume ratio, 80:20). The last step of the assay entails reconstituting samples in 200 µL of the mobile phase and introducing the samples into the mass spectrometer via flow injection. Analysis at the CDC was conducted on an API 3200 instrument (Applied Biosystems) as previously described (4). We prepared 16 dried blood spots of LSD QC material exhibiting typical-to-low activities of lysosomal enzymes. Samples were assayed in duplicate according to our protocols, divided into 2 aliquots, and then evaporated in separate 96-well plates before their introduction into the mass spectrometer. One plate was reconstituted with the previously described mobile phase, and the second plate was reconstituted with the methanol-substituted solution. The samples for all 5 disorders were analyzed according to our standard operating procedure, as previously reported (4). The mean (SD) enzyme activities for the acetonitrile-based assay were 2.31 (0.19) μmol·L⁻¹·h⁻¹ for galactocerebrosidase, 6.81 (0.58) μmol·L⁻¹·h⁻¹ for acid β-galactosidase, 3.06 (0.36) μmol·L⁻¹·h⁻¹ for acid β-glucocerebrosidase, 16.5 (1.25) μmol·L⁻¹·h⁻¹ for acid α-glucosidase, and 7.41 (0.44) μmol·L⁻¹·h⁻¹ for acid α-galactosidase (Fig. 1). No statistically significant differences were observed among the samples analyzed for this study, and the overall variation between sample set means was <5%, well within the assay’s imprecision as described by Zhang et al. (2).

These results strongly suggest that HPLC-grade methanol may be substituted for acetonitrile in the LSD multiplex assay without deleterious effects on instrument performance or sample results. Although we found different mobile phases to have very similar ion intensities (data not shown), we did not evaluate different methanol grades and suppliers. We recommend that users refer to Annesley’s report on matrix effects associated with electrospray ionization (5) to understand the potential differences between methanol products from different manufacturers before effecting a change in solvent for the LSD assay. Laboratories interested in changing the assay protocol should conduct a thorough validation study to recognize the effects of their choice of solvent grade and supplier, as well as their specific equipment’s analytical performance. The QC materials developed and distributed by the CDC may be used for this purpose. Any efforts to evaluate the multiplex LSD assay by newborn-screening laboratories worldwide should not be hindered by the unavailability of acetonitrile, which may become a large issue in the near future.

**Author Contributions:** All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

**Authors’ Disclosures of Potential Conflicts of Interest:** Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:

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**Fig. 1. Effects of substituting methanol (MeOH) for acetonitrile (ACN) in the mobile phase on the multiplex tandem mass spectrometry assay for 5 LSD diseases.**

GALC, galactocerebrosidase (Krabbe disease); ABG, acid β-glucocerebrosidase (Gaucher disease); ASM, acid sphingomyelinase (Niemann-Pick types A and B); GAA, acid α-glucosidase (Pompe disease); GLA, acid α-galactosidase (Fabry disease).
Consultant or Advisory Role: None declared.
Stock Ownership: None declared.
Honouraria: None declared.
Research Funding: R.F. Vogt is principal investigator of the CDC Newborn Screening Translation Research Initiative, which receives partial support from Genzyme Corporation through a grant to the CDC Foundation.
Expert Testimony: None declared.
Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

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DOI: 10.1373/clinchem.2008.122176