Letters to the Editor

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.

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References

2. Lennon VA, Kryzer TJ, Pittock SJ, Verkman AS, Hinson SR. IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 specific autoantibodies. The intellectual property is licensed to a commercial entity for the development of a simple, antigen-specific assay, to be made available worldwide for patient care. The test will not be exclusive to Mayo Clinic. To date, the inventors have received less than $10,000 in royalties. They do not benefit financially from serological testing performed at Mayo Clinic.

Comparison of Bromcresol Purple and Capillary Protein Electrophoresis for Quantification of Serum Albumin in Multiple Myeloma

To the Editor:

According to the International Staging System (1), serum albumin concentration is a key factor in determining patient prognosis in multiple myeloma. A recent study published in Clinical Chemistry described significant discordance between bromcresol green (BCG)1 albumin dye and agarose gel serum protein electrophoresis (SPEP) for albumin concentration determination in patients with high concentrations of monoclonal (M)-protein (2). Also available, however, are commonly used alternative methods for albumin determination, such as bromcresol purple (BCP) albumin dye and capillary zone SPEP (3). To examine whether the previously observed comparative discordance of measured albumin concentration is limited only to the BCG dye and agarose gel SPEP methods of albumin determination (2), we retrospectively compared measured albumin concentrations as determined by BCP albumin dye and capillary zone SPEP in a large cohort of multiple myeloma patients.

The ratio of measured BCP albumin to SPEP albumin concentrations in patients with a previously identified monoclonal protein was retrospectively calculated for 579 specimens submitted to the University of Arkansas Immunology laboratory for routine serum protein electrophoresis by capillary zone SPEP. All specimens had concurrent albumin determination by a BCP albumin dye method. These consisted of 355 IgG, 122 IgA, 29 IgM, 13 IgD, and 60 free-light-chain myeloma patient specimens. The monoclonal protein light chains in these specimens were approximately evenly divided between κ and λ. Serum albumin was measured by the Beckman–Coulter Synchroin BCP assay, and total serum protein was determined by using the Beckman–Coulter Synchroin bireut method on a Beckman–Coulter LX20 Pro modular system. A Sebia Capillaries 2 was used to determine the relative serum sample albumin and M-protein fractions. The relative albumin and M-spike fractions were multiplied by total protein to obtain final SPEP albumin and M-protein concentrations.

Substantial discrepancies in albumin determination in the presence of M-protein between capillary zone SPEP and the BCP albumin determination methods were observed. Although albumin
concentrations similar to those measured by BCP and capillary SPEP were observed in patients with undetectable concentrations of an M-protein, albumin concentrations reported by capillary SPEP were consistently higher than albumin concentrations determined by BCP in the presence of M-protein. When the ratio of BCP albumin/SPEP albumin vs M-protein was plotted (Fig. 1) the effect appeared to be linear. Deming regression fit of all sample measurements yielded the following equation:

\[
\frac{\text{BCP albumin}}{\text{SPEP albumin}} = 0.041 (\text{M-protein in g/dL}) + 0.93, \quad R^2 = 0.55.
\]

The 95% CI of the slope was 0.036 to 0.045 and was significantly different from a slope of 1 (\(P < 0.001\), calculated with CBstat version 5.1). Negative slopes for BCP/SPEP albumin ratios were also observed for IgG (–0.045 g/dL), IgA (–0.032 g/dL), IgM (–0.040 g/dL), and IgD (–0.032 g/dL) specimens. Too few free light-chain samples with measurable SPEP M-protein concentrations >0.2 g/dL (2 g/L) were present to determine if albumin ratios were affected by the presence of free light chains alone.

The differences between capillary electrophoresis and BCP albumin dye measurements presented here are similar to those previously observed for BCG dye and agarose electrophoresis albumin determinations, demonstrating that this discrepancy is not limited to specific types of brom cresol albumin dyes or electrophoretic albumin measurements. In both studies, increasing amounts of M-protein led to relatively higher albumin measurements by electrophoresis compared to albumin determined with serum dye. Although the mechanism for this effect merits further investigation, the observation of relatively higher albumin results in both capillary electrophoresis (which detects proteins by ultraviolet absorbance) and agarose gel electrophoresis disfavors models in which the observed discrepancy is caused by nonlinear staining of monoclonal protein (2). This study, however, cannot rule out other potential models, including nonlinear detection of strong M-protein bands by ultraviolet absorbance, nonlinear and/or nonequivalent measurement of different protein species in the kinetic biruet total-protein determination assay used (4), or interference by M-protein in the BCP albumin dye assay.

In the absence of M-protein, the BCP albumin measurement was approximately 93% of that obtained by capillary SPEP, whereas BCG yielded higher measurements than those obtained by agarose gel electrophoresis (2). This result may be partially explained by a positive bias of BCG to BCP albu-
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).

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References

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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