Influence of Methodology on the Detection and Diagnosis of Congenital Analbuminemia, Andrew W. Lyon, PhD, Paul Meinert, MD, Garth A. Bruce, MD, Victor A. Laxdal, MD, and Mark L. Salkie, MD (Departments of 1 Pathology and 3 Pediatrics, College of Medicine, University of Saskatchewan, and Departments of 2 Laboratory Medicine and 4 Pediatrics, Saskatoon District Health, Saskatoon, Saskatchewan, Canada S7N OW8; * address correspondence to this author at: Department of Laboratory Medicine, Royal University Hospital, 103 Hospital Dr., Saskatoon, SK, Canada S7N OW8; fax 306-655-2223, e-mail andrew.lyon@usask.ca; poster presented at the 42nd Annual Conference of the Canadian Society of Clinical Chemists and the Canadian Association of Medical Biochemists, June 14–18, 1998, Ottawa, Canada)

Congenital analbuminemia is a rare recessive inherited disorder characterized by an absence or very low concentrations of serum albumin (1,2). The diagnosis of analbuminemia is suggested by persistent hypoproteinemia with adequate hepatic function and a lack of evidence for either a renal or gastrointestinal protein loss. The diagnosis is typically confirmed by serum protein electrophoresis that depicts an absence of an albumin band (3). Rapid diagnosis of analbuminemia is appealing because patients can be subjected to extensive and prolonged testing to ascertain the cause of their apparent protein-losing disorder. Unfortunately, the diagnosis of analbuminemia may be delayed by clinically misleading laboratory results generated by tests of analytes that are usually bound to albumin, e.g., calcium (4) and thyroxine (5), and tests sensitive to the concentration of albumin in the matrix, e.g., some free thyroxine immunoassays (6,7). In particular, rapid diagnosis of analbuminemia would be delayed if the method for serum albumin falsely reports the presence of albumin.

The most commonly used serum albumin assays in clinical laboratories rely on dye-binding techniques (8). Characteristically, these methods accurately measure the concentration of serum albumin near the limits of the reference range. In conditions such as nephrotic syndrome, the serum albumin and the albumin:globulin ratio are low. Under these conditions, results of the bromcresol green (BCG) methods can be falsely inflated (9–15) and bromcresol purple-binding methods prone to falsely lowered results (16), although reagent manufacturers have adopted many variations of the dye-binding albumin methodology in an attempt to assure accuracy. Dye-binding albumin methods would likely be used to initially assess albumin status in undiagnosed patients with analbuminemia. Dye-binding albumin methods have falsely indicated the presence of albumin in analbuminemia in a rat model (17) and in a case report (18); however, few analytical details were reported. Here we compare the ability of two BCG methods to detect the absence of serum albumin in two new cases of congenital analbuminemia and comment on the role of methodology in previous studies.

Patient DN was admitted as a newborn with hypoglycemia and apnea; investigations revealed apparently low serum albumin (15 g/L). His serum albumin concentration remained low despite 24-h urine protein excretion rates within reference values. He was readmitted on three additional occasions in his first 6 months of life with respiratory distress and was eventually started on treatment for chronic asthma. There was no identifiable cause for the patient’s low serum albumin and no evidence of a protein-losing enteropathy, based on a 99Tc-labeled albumin scan.

Patient KB was admitted at 2 days of age for treatment of cellulitis and was subsequently readmitted three times over the next 6 months for respiratory distress involving upper respiratory tract infections and pneumonia. The reported serum albumin remained low (10–15 g/L), and there was no identifiable source of protein loss.

Serum protein electrophoresis performed at 10 weeks (patient DN) and 12 weeks (patient KB) revealed an absence of an albumin band (0–3 g/L), and the diagnosis of congenital analbuminemia was considered. Both neonates had no symptoms directly attributable to the lack of albumin, and both presented with recurrent respiratory tract symptoms related to bronchial asthma or pneumonia, which may be a coincidental observation (19–21).

In each case serum albumin >10 g/L was reported from an Ektachem 700XR analyzer at an early stage of clinical investigation. Reported albumin concentrations were variable, with no corresponding clinical changes but with method-dependent variability (Table 1). By immunoassay, albumin was <0.01 g/L (assay detection limit) for patient DN. BCG assays are prone to positive bias (9–15); however, the two BCG assays produced different results with analbumiminemic sera (Table 1). Moreover, the Ektachem 700XR analyzer did not detect that the concentration of albumin was substantially below the lower limit of assay linearity. These observations prompted an evaluation of the dynamic range for each albumin assay.
The manufacturer’s lower limit of albumin assay linearity was confirmed to be 10 g/L with saline-diluted serum on the Ektachem 700XR. Saline-diluted serum retains a constant albumin:globulin ratio, and alteration of this ratio is known to affect the Ektachem 700XR method (9–12). Pure human albumin solutions (Bayer Corp.) revealed a nonlinear calibration curve below 20 g/L, with a positive intercept of 4.6 g/L estimated by polynomial regression. False albumin results (6–10 g/L) were also produced by 10–50 g/L purified human IgG (Bayer Corp.). Both positive biases would be anticipated in the analysis of sera from analbuminemic patients with the Ektachem 700XR analyzer.

The Hitachi 717 albumin assay demonstrated a linear response with saline-diluted serum to 4 g/L (lowest concentration tested), which is below the manufacturer’s stated lower limit of 10 g/L. In the absence of globulins, pure human albumin solutions demonstrated a linear calibration curve with a near-zero intercept (0.03 g/L); the assay demonstrated no reaction with purified human IgG. A false albumin result (8 g/L) for analbuminemic patient DN from the Hitachi 717 analyzer was suppressed because it was below the dynamic range of the assay (Table 1).

The reaction times of these two BCG assays are 96 s (Hitachi 717) and 145 s (Ektachem 700XR); the shorter reaction time can confer greater albumin specificity (22). The lack of sample dilution in the multilayer dry film technology of the Ektachem 700XR may also promote the reaction of globulins with BCG.

The serum electrophoresis results indicated non-zero concentrations of albumin in each case (Table 1). This can be attributed to a small densitometry peak caused by the staining of non-albumin proteins that migrate near albumin. Although non-zero results were obtained with both agarose gel (patient DN) and cellulose acetate (patient KB) electrophoresis, other analbuminemia studies have occasionally reported albumin concentrations of 0 g/L with electrophoresis. Variability of albumin quantification by electrophoresis may be partially attributed to falsely low measurements caused by protein absorption to the matrix (e.g., paper).

Past literature reviews have focused on the clinical attributes of analbuminemic patients (1, 2), with few comments on the analytical methods used to confirm the diagnoses. Case studies report albumin concentrations of 0–12 g/L among patients that by definition lack serum albumin, although most results were 0.03–3 g/L (1, 2, 18–21, 23–41). We evaluated the influence of methodology on past case reports and our own cases (Fig. 1). Cases reports were included in this analysis if albumin methods were described (2, 3, 5, 18–41); others were excluded (42–44). Reports of albumin with modified mobility by electrophoresis were also excluded (45, 46). Fig. 1 shows that most variation in the apparent albumin concentration in analbuminemia reports is accounted for by the albumin methodology (one-way ANOVA, P <0.001). Both dye binding and the Howe (47) sulfate precipitation methods are particularly prone to overestimation of serum albumin. Many of the albumin immunoassays reported non-zero concentrations in analbuminemic patients. This may be accounted for by both the imprecision of assays and by weak reactions with serum albumin fragments produced by incomplete transcription and translation of the albumin gene (48).

In summary, some conventional BCG methods are prone to overestimations of albumin that may delay diagnosis of congenital analbuminemia. When dynamic ranges of BCG methods are assigned using saline-diluted serum (with a constant albumin:globulin ratio), there is no assurance that very low concentrations or the absence of albumin can be detected when the albumin:globulin ratio

### Table 1. Analysis of serum albumin and total protein on single specimens from the analbuminemia patients using different methods.

<table>
<thead>
<tr>
<th>Method</th>
<th>Patient DN</th>
<th>Patient KB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ektachem 700XR&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>Hitachi 717&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1</td>
</tr>
<tr>
<td>Serum electrophoresis&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>Radial immunodiffusion albumin immunoassay&lt;sup&gt;e&lt;/sup&gt;</td>
<td>&lt;0.01&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Total protein, g/L</td>
<td>45</td>
<td>36</td>
</tr>
</tbody>
</table>

<sup>a</sup> Reagents: Vitros®, Ortho Clinical Diagnostics.
<sup>b</sup> Reagents: Boehringer Mannheim Canada.
<sup>c</sup> The measured value of 8 g/L was below the assay linearity limit and was suppressed by the instrument.
<sup>d</sup> SP-17 Titan agarose gel electrophoresis system, Helena Laboratories.
<sup>e</sup> Kallestad™ Endoplate™ single radial immunodiffusion method, Sandif Pasteur Diagnostics.
<sup>f</sup> Estimated by linear regression and interpolation as 0.003 g/L.

Fig. 1. The distribution of reported serum albumin concentrations in patients diagnosed with analbuminemia (1, 2, 18–21, 23–41).

The data include one entry per method per patient from reports that provided a description of the albumin methods. Method types are as follows: (A) brom cresol green-binding albumin methods; (B) sodium sulfate precipitation method of Howe (47); (C) serum electrophoretic albumin methods; and (D) immunoassay albumin methods.

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[Image of Table 1 and Fig. 1]
The risk of reporting inaccurate albumin results is present not only in analbuminemia but also in hypoalbuminemic states such as nephrotic syndrome and burn injuries, using these methods (8, 9). The concentration of albumin reported by clinical laboratories for specimens from analbuminemic patients is method-dependent. Of the four categories of albumin methods, dye-binding methods demonstrate the greatest overestimation of albumin concentrations and can produce misleading reports that indicate the presence of albumin. Immunoassays of serum albumin are likely the most accurate (8). Electrophoretic quantification is widely available and often produces near-zero albumin results that are consistent with the diagnosis of analbuminemia. The challenge for clinicians and clinical laboratorians is to realize that initial dye-binding albumin test results can be falsely inflated and that a more definitive albumin method must be used to evaluate congenital analbuminemia.

References