Sample Viscosity Can Be a Source of Analytical Error When Discrete Sampler–Dilutors Are Used

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Total protein concentration in the serum of a patient with hyperviscosity syndrome differed as measured by the bilucret procedure in the DuPont aca (80 g/L) and the SMA 12/60 (105 g/L), owing to viscosity-dependent errors with the aca sampling system; the magnitude depended on sample temperature and volume of sample aspirated. This kind of error was not observed with the SMA 12/60 and was far less severe when a Micromedic sampler–dilutor was tested. It could be eliminated in the case of the aca by adding sample to test packs with a syringe rather than with the aca automated sampler–dilutor. We thus recommend use of the syringe method when unusually viscous samples (serum or other body fluids) are analyzed in the aca.

Additional Keyphrases: total serum protein • variation, source of • hyperviscosity syndrome

Roller-pump dilutors reportedly are affected significantly by viscosity, leading to underestimates exceeding 15% when samples from patients with hyperviscosity syndrome are analyzed (1, 2). It has been generally assumed that discrete sampler–dilutor devices are free of sampling errors originating from variations in viscosity. However, we recently noticed a 25 g/L discrepancy between total protein as measured with the aca discrete analyzer (DuPont Instruments-acu Division, Wilmington, DE 19898) and a continuous-flow analyzer (SMA 12/60; Technicon Instruments Corp., Tarrytown, NY 10591) in serum from a patient with hyperviscosity syndrome. This led us to investigate systematically the influence of viscosity on the accuracy of sampling with three different sampler–dilutors and to conclude that some discrete sampler–dilutor devices such as that used with the aca may be severely affected by changes in sample viscosity.

Materials and Methods

Patient studied. The patient studied (N.E.) has been described in detail elsewhere (3). The discrepant protein values were observed during her admission in 1979 for severe anemia and the presence of occult blood in feces. During this admission, her total-protein value was 105 g/L, albumin 19 g/L (both measured on the SMA 12/60), and serum protein electrophoresis revealed a paraprotein. Her serum was subsequently noted to be viscous at 4 °C but fluid at 25 or 37 °C. Immunoglobulin values (g/L) as determined by nephelometry with the Beckman Immunochemistry Analyzer were as follows: IgG 45.1, IgA 28.3, and IgM 25.7. Bone-marrow examination revealed a well-differentiated plasma cell infiltrate, compatible with a diagnosis of Waldenström’s macroglobulinemia. The relative viscosity of this patient’s serum increased during this hospital admission, with values ranging from 2.0 to 9.0 (normal 1.5–1.8). Because of this, plasmapheresis was initiated, leading to a decline in her serum IgM value to 12.0 g/L and in relative viscosity to 2.0.

Serum samples. Serum was sampled from patient N.E. at various times during her 1979 hospitalization and stored at −20 °C until analyzed.

Plasma samples were also obtained at plasmapheresis. After defibrination with added thrombin, the serum was dialyzed against two changes of 100 units of de-ionized water, lyophilized, and then re-dissolved in water.

Glycerol solutions. We analyzed solutions containing constant concentrations of albumin (50 g/L), creatinine (45 mg/L), and uric acid (50 mg/L) but various concentrations of glycerol (50–70 mL/dL) and blank solutions containing only glycerol in the same concentrations.

Analytical procedures. Viscosity relative to water (relative viscosity) was measured at 37 °C with a Cannon-Sanse viscosity tube (Kimax 100; Curtin-Matheson, St. Louis, MO). Measurements of total protein, albumin, uric acid, and creatinine were performed with the aca according to the manufacturer’s directions (regular aca procedure). A modified aca procedure was also used, consisting of adding the appropriate volume of sample into the aca test pack by means of a 0.1-mL syringe (Hamilton Co., Reno, NV 89510). The test pack was placed behind an empty sample cup and then analyzed in the aca. This modification assured accurate delivery of the appropriate volume of sample, regardless of viscosity, to the reagent pack.

Experimental Protocols

The influence of viscosity on the results for protein or other analytes with the aca was assessed by analyzing samples by the regular and modified aca procedures. The error caused by the sampler–dilutor with the aca was then assessed as follows:

% error = (results by modified aca – results by regular aca/ results by modified aca) × 100

The influence of viscosity on another discrete sampler–dilutor, the Micromedic 24004 with a 0.5-mm delivery tip (Micromedic Systems Inc., Horsham, PA 19044) was assessed as follows. We diluted 150-μL samples threefold with a 150 mmol/L solution of NaCl, with both the Micromedic dilutor and the Hamilton syringe, then measured total protein in all these dilutions by the modified aca procedure. The error was expressed as:

% error = (results after syringe dilution – results after Micromedic dilution/results after syringe dilution) × 100

The influence of viscosity on total protein values was also...
assessed with the SMA 12/60. For these studies, total protein was measured with the SMA 12/60 and also with the modified aca procedure and the error expressed as:

\[
\text{% error} = \frac{(\text{results by modified aca} - \text{results by SMA 12/60})}{\text{results by SMA 12/60}} \times 100
\]

We evaluated the influence of temperature by placing samples at 4, 25, and 37 °C (in a water bath) for at least 10 min just before assay.

The influence of the sampling volume was studied with both the Micromedic sampler–dilutor and the aca. For the Micromedic studies, we used sampling volumes of 50, 100, 150, and 200 µL. For the studies with the aca, we assessed the influence of sampling volumes on various analytical procedures that require different volumes of serum: creatinine (200 µL of sample), total protein (160 µL), uric acid (60 µL), and albumin (20 µL).

**Results**

This investigation was prompted in part by the observation that serum from N.E. was notably viscous at 4 °C but fluid at 25 or 37 °C. We therefore assessed the influence of sample temperature on the accuracy of total protein in this patient’s serum as measured with the aca. When the protein concentration was <90 g/L, no errors were found at any sample temperature (Figure 1). At higher protein concentrations, progressively increasing errors were observed, strikingly so when the sample was at 4 °C.

To further assess the influence of viscosity, we tested the aca, SMA 12/60, and the Micromedic, using solutions with various glycerol concentrations. Figure 2 shows that sampling errors due to viscosity when total protein is measured can be large by the regular aca procedure, are smaller with the Micromedic sample–dilutor, and are undetectable with the SMA 12/60.

Sampling error as a function of sample volume was assessed by use of serum from patient N.E., obtained by plasmapheresis (Figure 3A), and with three different glycerol solutions (Figures 3B, C, and D). Clearly, the amount of sample being aspirated has a major effect on the viscosity-related sampling errors. At a given viscosity, the extent of sampling error was proportional to the volume of sample being aspirated.

**Discussion**

Our data indicate that falsely low values may be obtained when viscous serum samples are used in the aca. Simulation of this error by use of glycerol solutions allows each laboratory or manufacturer to assess viscosity effects in their own instruments. When we used a different discrete sampler–dilutor...
(Micromedic) or a SMA 12/60, these errors were either far lower or not observed. This is because the sample probe of the aca has the smallest diameter and the shortest sampling interval (Table 1).

Such sampling errors were negligible for samples with total protein <90 g/L from our patient, but relative viscosity of serum will vary with type as well as concentration of protein (4). Our data suggest that samples with a relative viscosity >9 are likely to result in sampling error on the aca. However, because viscosity is temperature-dependent, samples with widely differing immunoglobulin content but with the same relative viscosity at 37 °C (the temperature at which relative viscosity is commonly measured) may behave differently at room temperature or 4 °C (4). Although sampling of specimen at 4 °C is unwarranted, our data indicate that the errors are greater at 4 °C—another reason to ensure that samples should not be analyzed while they are still at refrigerator temperature.

High relative viscosity values are observed in 50% of patients with Waldenström's macroglobulinemia (4), in 8% of patients with multiple myeloma (4), and (relatively infrequently) in patients with immunoglobulin–anti-immunoglobulin complexes occasionally associated with rheumatoid arthritis, Sjögren syndrome, or other connective tissue diseases (5, 6–12). Sera as well as other body fluids from patients with these disorders may be viscous (pleural, peritoneal, synovial, etc.) and may be subject to error when analyzed by the regular aca procedure. We recommend the modified aca procedure when analyzing such samples.

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References


