Clinical Chemistry 54:4
770 (2008)

Citation Classic

Quantifying Protein in Cerebrospinal Fluid and Urine: Success Achieved

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Featured Article: Pesce MA, Strande CS. A new micromethod for determination of protein in cerebrospinal fluid and urine. Clin Chem 1973;11:1265–7.[2]

This paper described a dye-binding micromethod for measuring protein concentrations in cerebrospinal fluid and urine. The method involved coprecipitation of the proteins with a Ponceau S dye trichloroacetic acid solution, dissolving the protein precipitate in dilute sodium hydroxide, and measuring the absorbance at 560 nm. This method was rapid, easy to use, and required a specimen volume of 5–50 μL.

I began my career as a clinical chemist in 1971 in the pediatric microchemistry laboratory at Columbia Presbyterian Medical Center. This laboratory served the newborn, infant, and child population of Babies Hospital, which is now The Children's Hospital of New York. Manual procedures were performed in the pediatric microchemistry laboratory because the analytical methods used in the clinical chemistry laboratory required large specimen volumes and were unsuitable for use in a pediatric population. In the early 1980s, when microchemistry procedures could be automated, the pediatric microchemistry laboratory was incorporated into the clinical chemistry laboratory.

My first project was to develop a rapid and accurate micromethod for measuring protein concentrations in cerebrospinal fluid and urine. At that time, most protein methods required large specimen volumes, were time-consuming, and could not quantify low concentrations of protein. The Lowry procedure used in this laboratory for measuring protein concentrations in cerebrospinal fluid was subject to interference from drugs such as salicylates and tetracyclines, as well as from numerous color-producing nonprotein substances. I noted that electrophoretic methods used in the 1970s for separating serum protein fractions used the dye Ponceau S to stain the proteins. The success of these methods suggested that a dye-binding procedure using Ponceau S could be developed to measure protein concentrations in cerebrospinal fluid and urine.

Having recently received my PhD in synthetic organic chemistry, I was not accustomed to using microchemical techniques, and knew that pipetting microliter volumes would be a challenge. My doubts were confirmed when my initial optimization and imprecision experiments with this dye-binding method were unsuccessful because of inaccurate pipetting of specimens and reagents. Once I mastered the art of pipetting small specimen volumes using glass Lang Levy pipettes, the development and validation of this dye-binding procedure continued as planned. I optimized the Ponceau S dye and trichloroacetic acid concentrations used to precipitate both albumin and γ globulins, and validated the assay through linearity, precision, recovery, correlation, and specificity experiments.

I asked for, but never was assigned, a technologist to help me with this project. I later realized that my experience at the bench helped me in future method development projects in the clinical laboratory. Sadly, in today's hospital environment, most of the laboratory director's time is consumed by administrative tasks, accreditation and state regulatory requirements, and budgetary obligations. Thus "method development" usually means that a vendor supplies reagent kits to the laboratory and asks the laboratory to validate the in-house performance of the method.

My report of the Ponceau S dye-binding method was the first paper that I published as a clinical chemist, and it has been frequently cited and used because the procedure is technically simple and can be performed in any laboratory that has a centrifuge and a spectrophotometer. I am pleased that others *(1, 2)* have modified this dye-binding method to improve its use in the clinical laboratory.

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[2] This paper has been cited more than 275 times since publication.

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Received June 29, 2007; accepted December 27, 2007.
Previously published online at DOI: 10.1373/clinchem.2007.094177

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