Serum Protein Electrophoresis: Reptilase Treatment Is Superior to Ethanol Precipitation for Specific Removal of Fibrinogen from Heparinized Plasma Samples

To the Editor:
Qui et al. (1) propose a method to remove fibrinogen from serum specimens by precipitation with absolute ethanol for use in protein electrophoresis (PE). The authors recommend this method as highly selective and inexpensive. We read the article with great interest, as a possible alternative to the method incorporating reptilase (2) that is routinely used in our laboratory, which uses plasma for PE when no serum sample from a patient is available.

For method evaluation, we used 20 samples from laboratory staff with normal concentrations of fibrinogen, as well as 20 samples from patients with plasma fibrinogen of 7.4–12.9 g/L. As suggested by Qiu et al. (1), we used ethanol at a final concentration of 100 mL/L. The samples were incubated for 15 min on ice and centrifuged at 1100 \( g \) for 5 min at 4 \( ^\circ\mathrm{C} \). The supernatant was used for PE. The pellet containing fibrinogen was resuspended in 0.2 mL of saline (9 g/L \( \mathrm{NaCl} \)) and stored for further analysis. For comparison, we used reptilase (STA Reptilase; Diagnostica Stago, Roche Diagnostics) at a dilution of 1:10 in patient plasma. After incubation at 37 \( ^\circ\mathrm{C} \) for 5 min and centrifugation for 5 min at 20 590 \( g \), the supernatant was used for PE. For PE we used a cellulose acetate membrane, Ponceau S staining, and densitometry of the electrophoretic band pattern on the Olympus HITE 320 System (Olympus Diagnostica GmbH) with curve evaluation and quantitative analysis by the HITE 320 Software System (3, 4).

We compared PE from serum to PE from plasma treated with ethanol or reptilase, respectively. In addition, the resuspended pellet from the ethanol precipitation was analyzed by PE. Representative results are shown in Fig. 1.

In contrast to serum samples and reptilase-treated plasma, ethanol-treated samples showed a residual irregularity in the \( \gamma \)-globulin fraction (Fig. 1, A–C), which would be suspected to represent a monoclonal gammopathy when seen in a serum sample.

Quantitative analysis of the serum protein fractions showed no significant differences between serum (Fig. 1A) and reptilase-treated plasma (Fig. 1C), but ethanol-treated plasma (Fig. 1B) showed a mean increase of the \( \alpha2 \) fraction by \( \sim 20\% \), an increase of the \( \gamma \) fraction of \( \sim 10\% \), and a decrease of the \( \beta \) fraction of \( \sim 30\% \). All differences were highly significant (\( P < 0.01 \)). These differences were detected in healthy persons as well as in patients with hyperfibrinogenemia. The results remained unaffected when we used variations of the ethanol precipitation method as described by Qiu et al. (1) (not shown).

PE analysis of the resuspended pellet of the ethanol precipitation
showed nonselective precipitation of serum proteins by ethanol. This may in part explain the quantitative changes in PE after ethanol precipitation compared with PE from serum or reptilase-treated plasma (Fig. 1D). Comparison of the α2/β ratios for serum, ethanol-treated plasma, and the protein pellet after ethanol precipitation, however, suggests changed electrophoretic mobilities of some proteins, possibly as a result of denaturation by ethanol. This might also be causative for the irregularity in the γ fraction of ethanol-treated plasma, as might be residual fibrinogen.

We conclude that the precipitation of fibrinogen from plasma samples with ethanol is neither specific nor selective. The use of ethanol causes changes in the plasma protein distribution and leads to a residual irregularity in the γ fraction that cannot be reliably differentiated from a monoclonal gammopathy. Thus, we recommend the use of reptilase as a more specific and inexpensive pretreatment of plasma for use in PE.

In our laboratory, we therefore proceed as follows: When a suspect peak in the γ fraction is observed in a sample, the sample is treated with reptilase and again subjected to PE. If the peak disappears and no atypical pattern occurs, the electrophoretic pattern of the reptilase-treated plasma is included in the report and the comment is added that reptilase treatment was performed before PE. In cases of residual irregularities in the γ fraction (as seen after PE of ethanol-treated plasma), we would suggest in our comment to repeat the analysis with a serum sample. If this were not possible, we would recommend an immunofixation analysis to be completely sure that no monoclonal gammopathy is missed.

We gratefully acknowledge the support for this work by grants to Y.I. and R.H. from the Ministry for High Education of Syria.

References

Youssef Ibrahim
Martin Volkmann
Racha Hassoun
Walter Fiehn
Heidi Rossmann*

Zentrallabor
Universitätsklinikum
Ruprecht-Karls Universität Heidelberg
Heidelberg, Germany

*Address correspondence to this author at: Zentrallabor des Universitätsklinikums, Ruprecht-Karls Universität Heidelberg, Bergheimerstrasse 58, 69115 Heidelberg, Germany. Fax 49-6221-56-4612; e-mail heidi Rossmann@med.uni-heidelberg.de.

DOI: 10.1373/clinchem.2004.031518

Drs. Levinson and Elin respond:

To the Editor:

In their letter, Ibrahim et al. indicated that they noticed that small amounts of other proteins precipitated with ethanol. We also noted this and pointed out in our article that “although small amounts of albumin, α-globulin, and β-globulin may precipitate with the fibrinogen”, significant changes in the concentrations of polyclonal IgG, IgA, and IgM did not occur. Most importantly, little change was seen in the patterns of monoclonal immunoglobulins that we investigated.

Small changes in albumin and various other globulins would not interfere with the interpretation that the putative band was indeed fibrinogen. We have not noticed a residual irregularity in the γ-globulin fraction as described by Ibrahim et al. In any case, this would not change the final interpretation that the suspect band in the β-γ region was fibrinogen.

In fact, we outline the usual scenario in which, after immunologic techniques fail to identify a monoclonal immunoglobulin, presumptive identification of fibrinogen is made by this straightforward, inexpensive approach. Moreover, we cautioned in our article that “The electrophoretic pattern of the ethanol-treated sample is not suitable for quantitative measurement of a M-protein because of the dilution with ethanol”. Furthermore, the studies by Ibrahim et al. were performed only with plasma samples and not with serum controls. Because serum is the specimen used for electrophoresis, it is unclear to us whether the irregularities they describe are seen under these conditions. This may explain why we have not noticed these irregularities in regular clinical laboratory practice.

For the above reasons, we continue to recommend the ethanol precipitation method.

Stanley S. Levinson
Ronald J. Elin*

University of Louisville
Department of Pathology and Laboratory Medicine
Louisville, KY 40202

*Author for correspondence.

DOI: 10.1373/clinchem.2004.032276

The Interfering Component in Cardiac Troponin I Immunoassays

To the Editor:

In a recent Letter to the Editor, Pan-teghini (1) commented on our report of a blood component that interferes with immunoassays measuring cardiac troponin I (cTnl) if antibodies against epitopes in the central part of the molecule are used (2). We agree that the selection of the standard material used for recovery experiments can be of critical importance. The tissue-derived ternary troponin