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 $\alpha 2/\beta$ 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.

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References

1. Qiu L, Levinson SS, Keeling KL, Elin RJ. Convenient and effective method for removing fibrinogen from serum specimens before protein electrophoresis. Clin Chem 2003;49:868–72.
2. Heimann D, Wolf V, Keller H. The use of reptilase for electrophoresis of heparinized plasma. J Clin Chem Clin Biochem 1979;17:369–72.
3. Fink PC. A fully automated serum electrophoresis system. J Clin Chem Clin Biochem 1981;19:379–86.
4. Schiatti R, Limberti A, Ignesti C. [Evaluation of an automatic system for the electrophoretic fracturing of serum proteins and a comparison with the manual method]. Quad Sclavo Diagn 1986;22:97–103.

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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.

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The Interfering Component in Cardiac Troponin I Immunoassays

To the Editor:

In a recent Letter to the Editor, Panteghini *(1)* commented on our report of a blood component that interferes with immunoassays measuring cardiac troponin I (cTnI) 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