Ethanol Precipitation Is Not Reliable for Selectively Removing Nonmonoclonal Peaks Seen in the Fibrinogen Region on Capillary Zone Electrophoresis of Serum Proteins

To the Editor:
The enhanced resolution of capillary zone electrophoresis (CZE) provides superior sensitivity for the detection of monoclonal (M) proteins in serum samples compared with agarose gel electrophoresis (AGE). This comes at the cost, however, of a lower specificity, calculated as 78% in our hands for the Paragon CZE 2000® with software version 1.602 (Beckman) (1). Abnormalities in the electropherogram that cause confusion are most frequently located in the anodal part of the γ-globulin fraction. Notably, fibrinogen, which remains in incompletely clotted serum samples, migrates at the junction between the β- and γ-fractions. Even when following the strict procedure of keeping serum samples for a minimum of 4 h at room temperature before electrophoresis, we found a suspected fibrinogen or possible M-protein peak in 39 (8.6%) of 454 consecutive routine samples in our laboratory.

Recently, a simple method consisting of cold-ethanol precipitation was described for the removal of fibrinogen from serum specimens before agarose protein electrophoresis (2). We evaluated the use of this method in the routine analysis of serum samples by CZE in which a peak at the fibrinogen location could cause confusion with the detection of small M-proteins.

Absolute ethanol was added to the specimens at a final concentration of 100 mL/L. Precipitation was induced by cooling for 15 min in an ice bath, after which samples were centrifuged at 1100g and the supernatant was removed for subsequent CZE analysis. Preliminary experiments demonstrated that this method completely removed the fibrinogen peak from plasma samples on CZE as well as on AGE (Fig. 1A). Clear monoclonal peaks (9–31 g/L) in the γ-globulin area were not affected (data not shown). These data are consistent with the findings reported by Qiu et al. (2). However, fibrinogen was still detectable by immunofixation on postprecipitation plasma samples (Fig. 1A). It should be noted that the β2 peaks are clearly diminished after the procedure (Fig. 1). This could be a consequence of C3-complement turnover, but changed electrophoretic mobilities of some proteins as a result of denaturation by ethanol has also been suggested (3).

When we used ethanol precipitation to treat seven serum samples with suspected fibrinogen peaks, we found that these peaks disappeared on CZE in only three of the samples. To determine whether the peaks in the fibrinogen region that did not disappear represented M-proteins, we analyzed serum samples that were submitted for both electrophoresis and immunofixation and that showed a peak in the fibrinogen area after CZE. Electropherograms on CZE before and after ethanol precipitation were judged by three independent observers. Of the 12 samples thus analyzed, 7 displayed a peak in the fibrinogen area after ethanol precipitation: 4 samples with a M-protein on immunofixation (located in the β-γ area), but also 3 samples with no M-protein detectable (for an example, see Fig. 1C). Of the five samples in which the peak in the fibrinogen area disappeared, three had no M-protein on immunofixation but two were positive, again in the β-γ area (for an example, see Fig. 1B). It should be noted that these two samples were judged still to contain a small peak in the fibrinogen area by one observer; the other results were completely consistent over the three observers. Removal by cold-ethanol precipitation of a M-protein that behaves as a cryoglobulin could also explain some of these observations. Thus, some CZE samples with a peak in the fibrinogen area were still suspect for harboring a M-protein after ethanol precipitation, although they contained no M-protein by immunofixation. Conversely, two of three observers judged two samples to be devoid of fibrinogen after precipitation (because the abnormal peak in the fibrinogen area had disappeared), even in the presence of a M-protein in this area on immunofixation.

We performed immunofixation for the detection of fibrinogen on some samples with suspect fibrinogen peaks: fibrinogen was present in one sample before and after ethanol precipitation on CZE (Fig. 1B). The other four samples were devoid of fibrinogen by immunofixation, and none of them had unambiguous M-proteins, despite the presence of an altered morphology at the β-γ region (Fig. 1C). Ibrahim et al. (3) recently concluded that the ethanol precipitation method is neither specific nor selective for fibrinogen.

In conclusion, the cold-ethanol-precipitation method described by Qiu et al. (2) was effective in removing fibrinogen from plasma samples as judged by CZE and AGE, without interfering with the detection of major M-proteins. When used on truly problematic serum samples, however, in which the presence of a peak in the fibrinogen area can be caused either by fibrinogen or by a small M-protein, this method was unreliable in our hands to make this distinction. We therefore recommend that all samples that are questionable.
after CZE be subjected to immunofixation to rule out the presence of a M-protein.

References

To the Editor:

Blood collection for total homocysteine (tHcy) measurements is usually done in tubes containing EDTA as anticoagulant. At room temperature there is an increase in tHcy in whole blood (1, 2); therefore, tubes must be put on melting ice immediately and centrifuged within 1 or 2 h. Large epidemiologic studies, however, re-