Convenient and Effective Method for Removing Fibrinogen from Serum Specimens before Protein Electrophoresis

LING L. QIU,1 STANLEY S. LEVINSO,1,2 KRISTEN L. KEEING,1 and RONALD J. ELIN1*

Background: Fibrinogen in serum specimens can be misinterpreted on protein electrophoresis as a monoclonal protein. We evaluated selective precipitation of fibrinogen with ethanol.

Methods: Pooled human plasma was mixed with absolute ethanol or saline (final concentrations of 40, 80, 100, 120, and 160 mL/L) and incubated at 4 °C overnight or placed in an ice bath for 15 min. After centrifugation, the supernatants and resuspended pellets were used for protein electrophoresis and quantitative measurements of protein and fibrinogen.

Results: The fibrinogen band was effectively eliminated from the electrophoretic pattern in the plasma samples treated with ethanol at 100 mL/L and incubated in an ice bath for 15 min without a significant change in immunoglobulin concentrations. The 100 mL/L ethanol did not noticeably change the electrophoretic pattern of monoclonal immunoglobulins. This approach allowed analysis of a sample collected from an arteriovenous shunt kept open with heparin.

Conclusions: Ethanol, 100 mL/L, can selectively precipitate fibrinogen without significantly interfering with the immunoglobulins. The precipitation process can be completed in 15 min at 0–4 °C and can avoid the need to obtain another blood sample.

Fibrinogen interferes with serum protein electrophoresis. The presence of fibrinogen in serum specimens is seen in patients who have congenital dysfibrinogenemias or acquired disorders of coagulation, such as anti-phospholipid syndrome, liver disease, vitamin K deficiency, and most commonly, patients who are on anticoagulation therapy or who have an indwelling catheter kept open with low-dose heparin. The protein band for fibrinogen is located at the β/γ junction on serum protein electrophoresis and can be misinterpreted as a monoclonal (M) protein. Some serum specimens from patients in our institutions have exhibited such a discrete band on serum protein electrophoresis, but monoclonal immunoglobulins were not identified by serum immunofixation electrophoresis (IFE).

In such cases, or when fibrinogen is otherwise suspected, it is desirable to identify the paraprotein. Careful examination of the specimen and removal of a tiny fibrin clot may eliminate the paraprotein on repeat electrophoresis (1), but in our experience this technique works poorly. Alternatively, treatment with thrombin (1) or protamine sulfate in combination with thrombin (2) has been used to remove the fibrinogen interference, thereby establishing presumptive identification. However, these agents require proper preparation and storage, and repeat serum protein electrophoresis after treatment is more complicated because of additional proteins added to the serum. IFE with an antibody against fibrinogen is another option for identification, and we have used this technique for research purposes (3). However, anti-fibrinogen antibodies are usually not readily available in hospital laboratories. If the fibrinogen is suspected to be the result of heparin contamination of the serum specimen, a third option is to request another blood sample from the patient, preferably obtained from a peripheral vein. Because there is no standard way to deal with this problem, a simple, inexpensive, and effective method for eliminating fibrinogen from a contaminated serum specimen could be very helpful in clinical laboratories.

It has long been known that fibrinogen is the least soluble of the major plasma proteins and is readily precipitated by salting out with sodium chloride (4) or ammonium sulfate (5), or by precipitation with ethanol (6). We hypothesized that treatment of serum with ethanol would be a simple, efficient method to eliminate...
fibrinogen from suspected specimens that would also be simpler and more effective than the methods customarily used for presumptive identification. To illustrate the utility of this approach, we present a case report from our laboratory.

**Materials and Methods**

Plasma aliquots from a pool of specimens were treated with absolute ethanol at final concentrations of 40, 80, 100, 120, or 160 mL/L or with an equal volume of saline to a final volume of 1 mL. The samples were allowed to stand refrigerated overnight to promote precipitation and then were centrifuged at 1100g for 5 min at 4 °C. We also evaluated 100 mL/L ethanol for precipitation after 15 and 60 min in an ice bath.

After removal of the supernatant from the ethanol-treated samples, the pellets were resuspended in an aliquot of pooled serum with undetectable fibrinogen to a volume of 1 mL and mixed vigorously at 37 °C. The pooled plasma sample and resuspended pellets were tested by routine methods for fibrinogen, using a functional assay that measures the rate of clot formation (BCS; Dade Behring), and the recovery was expressed as the percentage of fibrinogen in the pellet (numerator) divided by the fibrinogen in the untreated pooled plasma (denominator).

In a separate experiment, the pellets were resuspended in 0.2 mL of saline, and serum protein electrophoresis (Beckman Coulter) was performed on the pellets and the corresponding supernatants. To demonstrate the specificity of this treatment, we measured total protein, immunoglobulins, and complement in the supernatant (Image Immunochemistry System; Beckman Coulter) after treatment with 100 mL/L ethanol and compared the results with the specimens treated with 100 mL/L saline to normalize for the volume change.

In addition, M-protein-containing specimens (IgG, IgAκ, and biclonal IgMκ and IgGA) were treated similarly with either 100 mL/L ethanol or saline and held at 4 °C overnight. After centrifugation, the immunoglobulin concentration was measured in the supernatants, and protein electrophoresis was performed. The experiments were performed in triplicate. Finally, the sample from the patient was compared with 100 mL/L ethanol and saline treatment.

The method used to compare the quantitative data in this study was the paired-sample t-test. The difference between results was significant at P < 0.05 (two-tailed test). The experiment was repeated three times.

**Case Report**

A 22-year-old African-American female with a history of HIV/AIDS and end-stage renal disease presented to the University of Louisville Hospital Emergency Department with episodes of seizure and mental status changes for 1 day, and nausea and vomiting for 2 weeks. She had been diagnosed with HIV infection 8 years before presentation. She was also diagnosed with HIV nephropathy 3 years previously and required dialysis. A serum specimen was sent to the laboratory for protein electrophoresis. A band with restricted mobility in the β/γ junction was observed, and the corresponding IFE was negative for a M-protein. It was discovered that the specimen was drawn from an established arteriovenous shunt kept open by a low dose of heparin; therefore, the presence of fibrinogen in the serum specimen was highly suspected.

**Results**

The addition of ethanol to plasma specimens at 80 and 100 mL/L selectively removed fibrinogen. Quantitative measurement of fibrinogen indicated that ethanol concentrations of 160, 120, 100, and 80 mL/L, but not 40 mL/L, reduced fibrinogen below the detection limit of the assay. Correspondingly, protein electrophoresis of supernatants (lanes S in Fig. 1A) after ethanol treatment and pellet fractions (resuspended in 0.2 mL of saline; lanes P in Fig. 1A) showed that 160, 120, 100, and 80 mL/L ethanol completely precipitated fibrinogen from plasma, such that the fibrinogen band was seen mainly in the pellet fraction. However, when the pellets were resuspended in serum and scaled back to 1 mL, most of the fibrinogen was recovered in the resuspended pellets containing 80 or 100 mL/L ethanol (Fig. 1B). Only 60% of the fibrinogen was recovered from the pellets precipitated with 40 mL/L ethanol, and the protein band for fibrinogen was still present in the supernatant. The percentage recovery in the pellets gradually deceased with 120 and 160 mL/L ethanol (Fig. 1B), although on electrophoresis the fibrinogen paraprotein band was eliminated from the supernatants treated with 120 and 160 mL/L ethanol (Fig. 1A). Our goal was to optimize the elimination of fibrinogen without significantly altering other serum proteins. Because the largest recovery of fibrinogen occurred with 80 and 100 mL/L ethanol and 100 mL/L ethanol is more convenient to achieve than 80 mL/L, the clinical laboratory, the following comparison study was performed with 100 mL/L ethanol only.

Addition of 100 mL/L ethanol to the specimen and incubation for 15 min in an ice bath or overnight at 4 °C significantly decreased the fibrinogen concentration (P < 0.001) without altering the concentrations of immunoglobulins (IgG, IgA, and IgM) and complements (C3 and C4; P > 0.05) in the supernatant compared with the controls (Table 1). Most importantly, when serum specimens containing monoclonal proteins were treated with ethanol (100 mL/L) or saline, there was no observable difference in the electrophoretic pattern between the supernatants treated with ethanol and the controls (Fig. 2A). In addition, ethanol-treated samples showed good quantitative recovery of immunoglobulins, approaching 100% in most cases (Fig. 2B). We chose 15 min as the incubation time in the ice bath because there was essentially total precipitation of fibrinogen with no improvement over 1 h (data not shown).
Immunoglobulins and fibrinogen were determined in the patient’s sample after addition of 100 mL/L ethanol or saline (Fig. 3B) with overnight incubation at 4 °C. The presence of fibrinogen in the patient’s heparin-contaminated serum specimen was largely removed by the addition of 100 mL/L ethanol without significant changes in the immunoglobulins. A band similar to that seen in the untreated serum was observed in the saline control, but it was absent in the supernatant after treatment with 100 mL/L ethanol (Fig. 3A).

**Discussion**

It has been known that fibrinogen can be specifically precipitated from human plasma by addition of ethanol to a final concentration of ~80 mL/L at a temperature around ~2 °C, which allows maximum precipitation and minimum denaturing of fibrinogen protein (6). The first step of our study was to find the optimum conditions for removing fibrinogen for our clinical purposes. We selected 4 °C as our precipitation temperature because it is essentially the same as in the study by Cohn et al. (6). We decided to test our hypothesis with overnight precipitation first to achieve maximum equilibrium and specificity of this method. By comparing final ethanol concentrations of 40, 80, 100, 120, and 160 mL/L, we demonstrated that ethanol concentrations of 80 and 100 mL/L can effectively and selectively precipitate fibrinogen from plasma with 100% recovery of fibrinogen in the pellet (Fig. 1). The decreased recovery with 120 and 160 mL/L ethanol could be attributable to increased denaturing of fibrinogen, which was no longer measurable in the functional assay (BCS method), or decreased precipitation of fibrinogen because a higher concentration of ethanol is more favorable for precipitating other plasma proteins (6). Incubation of the specimen with 100 mL/L ethanol for 15 min in an ice bath or overnight at 4 °C did not significantly change the concentrations of IgG, IgA, IgM, C3, and C4 (Table 1), indicating that 100 mL/L ethanol is specific for precipitating fibrinogen, although small amounts of albumin, α-globulin, and β-globulin may precipitate with the fibrinogen (Fig. 1A). Because 100 mL/L ethanol is easier to achieve than 80 mL/L, we recommend 100 mL/L ethanol as a convenient way to eliminate interference from fibrinogen-contaminated serum specimens for protein electrophoresis in the clinical laboratory.

The second step of our study was to minimize the incubation time in an ice bath that essentially effected total precipitation of fibrinogen. As shown in Table 1, fibrinogen precipitation can be accomplished with incubation for only 15 min in an ice bath. In hospitals or laboratories where few of these fibrinogen paraproteins

---

**Table 1. Comparison of fibrinogen, immunoglobulin, and complement concentrations in the supernatants of samples treated with 100 mL/L ethanol and saline.**

<table>
<thead>
<tr>
<th>100 mL/L</th>
<th>Saline control</th>
<th>4 °C overnight</th>
<th>Ice bath for 15 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen, g/L</td>
<td>4.06 (0.042)</td>
<td>&lt;0.7°</td>
<td>&lt;0.7°</td>
</tr>
<tr>
<td>IgG, g/L</td>
<td>7.730 (0.420)</td>
<td>7.690 (0.258)</td>
<td>8.163 (0.205)</td>
</tr>
<tr>
<td>IgA, g/L</td>
<td>1.627 (0.047)</td>
<td>1.615 (0.015)</td>
<td>1.720 (0.044)</td>
</tr>
<tr>
<td>IgM, g/L</td>
<td>0.603 (0.010)</td>
<td>0.559 (0.021)</td>
<td>0.643 (0.012)</td>
</tr>
<tr>
<td>C3, g/L</td>
<td>0.951 (0.015)</td>
<td>1.084 (0.036)</td>
<td>1.033 (0.015)</td>
</tr>
<tr>
<td>C4, g/L</td>
<td>0.184 (0.015)</td>
<td>0.166 (0.006)</td>
<td>0.229 (0.002)</td>
</tr>
</tbody>
</table>

*Concentrations are given as the mean (SD).

°P <0.001 compared with saline control.
may be encountered, ethanol treatment is more cost-effective for reagents and labor than preparing thrombin-protamine solutions or performing a repeat IFE with anti-fibrinogen antibody.

Most importantly, treatment with 100 mL/L ethanol did not noticeably precipitate immunoglobulins (Table 1), especially monoclonal immunoglobulins, because recovery of all three classes in the supernatant was near 100% (Fig. 2). Similarly, the treatment did not precipitate polyclonal immunoglobulins because their recoveries were also near 100%. The three M-protein-containing samples in Fig. 2 are representative of three different classes of M-protein. Moreover, we tested several samples with variable amounts of monoclonal immunoglobulins (three with IgG, two with IgM, and two with IgA) and found that 100 mL/L ethanol did not change the electrophoretic pattern compared with untreated specimens.

In the reported case, we illustrate one way to use this approach for identifying fibrinogen interference. Using this technique, we were able to show that the paraprotein in the patient’s serum was fibrinogen, thus confirming our suspicion, because it was removed with the addition of ethanol (100 mL/L; Fig. 3). Ethanol (100 mL/L) effectively and selectively precipitates fibrinogen from a fibrinogen background.

<table>
<thead>
<tr>
<th></th>
<th>IgG Lambda</th>
<th>IgG Kappa</th>
<th>IgM Kappa/IgG Lambda</th>
</tr>
</thead>
<tbody>
<tr>
<td>saline</td>
<td>1.10±1.73</td>
<td>2.64±0.025</td>
<td>13.37±0.208</td>
</tr>
<tr>
<td>ethanol</td>
<td>1.17±2.00</td>
<td>2.82±0.099</td>
<td>13.77±0.569</td>
</tr>
<tr>
<td>saline</td>
<td>0.83±0.002</td>
<td>29.27±0.503</td>
<td>0.59±0.004</td>
</tr>
<tr>
<td>ethanol</td>
<td>0.94±0.007</td>
<td>29.40±0.557</td>
<td>0.54±0.013</td>
</tr>
<tr>
<td>saline</td>
<td>0.74±0.003</td>
<td>2.61±0.005</td>
<td>0.15±0.006</td>
</tr>
<tr>
<td>ethanol</td>
<td>0.12±0.003</td>
<td>1.16±0.007</td>
<td>0.78±0.015</td>
</tr>
</tbody>
</table>

Unit: g/L

**Fig. 2.** Effects of 100 mL/L ethanol on M-proteins. (A), serum protein electrophoretic pattern of M-protein-containing specimens treated with 100 mL/L saline or ethanol. Migration of protein is toward the positive electrode (top). + indicate point of migration of M-proteins. (B), quantitative measurement of immunoglobulins (mean ± SD) in M-protein-containing specimens treated with 100 mL/L saline or ethanol.

**Fig. 3.** Paraprotein profile (A) and quantitative results for fibrinogen and immunoglobulins (B) in the patient's specimen after addition of 100 mL/L ethanol or saline. (A), both the densitometer tracings and the electrophoretic gel patterns are shown. The arrow indicates the point of migration of fibrinogen on the tracings and gel pattern. The fibrinogen band was seen in the saline control sample but absent in the supernatant after treatment with 100 mL/L ethanol.
ogen-contaminated serum sample or plasma. We thus found that ethanol precipitation was a more convenient and inexpensive method for identifying fibrinogen paraproteins because ethanol is readily available and requires no special storage conditions, reconstitution, or solution preparation.

Ordinarily, a paraprotein in the β/γ region of the electrophoretic gel is examined by immunologic techniques to determine whether it is a monoclonal immunoglobulin. If immunologic analysis is negative, it is desirable to define the nature of the unknown protein because immunoglobulins occasionally react poorly or not at all with the antisera used for detection, in which case a legitimate M-protein could be overlooked. In this report, we have shown that a fibrinogen paraprotein can be presumptively identified by simple treatment with 100 mL/L ethanol and repeated protein electrophoresis. The electrophoretic pattern of the ethanol-treated sample is not suitable for quantitative measurement of a M-protein because of the dilution with ethanol. Thus, this method is effective at both presumptively identifying and removing fibrinogen, which allows the laboratorian to quickly rule out monoclonal gammopathy and, ultimately, reduce the cost of further evaluation.

In our laboratories, this approach has been very helpful in classifying several cases of fibrinogen paraproteins. It is especially useful in hospitals with moderate to low numbers of samples requiring electrophoresis such that no more than one or two electrophoretic plates are run each day. The suspect sample can be precipitated overnight in the refrigerator and confirmed as fibrinogen the next day with the next plate run. We find that the overnight approach better fits our testing flow than the 15-min precipitation because it is less labor-intensive. Laboratories with large numbers of samples requiring electrophoresis may wish to use the 15-min precipitation so that they can complete the procedure sooner.

References