ESR results obtained with the three ESR methods. The bias between TEST-1 and the manual Westergren method was −2.7 mm/h (agreement limits, −33 to 8 mm/h). Concerning reproducibility, the mean interassay CV was 7.9%. The ESR values for samples stored for up to 24 h were, on average, 4% lower than the initial values. However, after storage for 48 h, ESR values were, on average, 24% lower than the initial values. Therefore, we consider the maximum permissible storage time to be 24 h.

In conclusion, the Test-1 offers a fast determination of the ESR with acceptable intraassay variability and good correlation with traditional Westergren methods. The bias and agreement limits are comparable to those found by others (6, 7). The use of samples with EDTA as anticoagulant instead of sodium citrate has two possible advantages: the avoidance of a dilution step and consequent analytical errors, and the use of one sample for multiple hematologic analyses (e.g., ESR and complete blood count) (2).

Improved Sensitivity of Capillary Electrophoresis for Detection of Bisalbuminemia

To the Editor:
We wish to report our findings on bisalbuminemia detected by capillary electrophoresis. Bisalbuminemia is an abnormality characterized by a double band of albumin seen on electrophoresis. This anomaly can be either inherited or acquired. The inherited familial bisalbuminemia is a rare abnormality with no pathologic or therapeutic consequences, being of interest only for human genetics or anthropology (1). By contrast, the detection of an acquired and transitory bisalbuminemia may point to an overdose of antibiotics or the presence of ascites or a pancreatic pseudocyst (2, 3).

Since the introduction of the Paragon 2000™ capillary zone electrophoresis (CZE) system (Beckman Instruments) in our laboratory, we have noted an increased frequency of identified cases of bisalbuminemia. The electrophoretic separation was performed according to the manufacturer's instructions, in borate buffer (pH 10) at 9000 V with online detection at 214 nm. We have analyzed 6500 samples by CZE in the last year and detected 8 bisalbuminemas (Fig. 1A). This frequency of bisalbuminemia is high: in a similar number of analyses performed annually by classical agarose gel electrophoresis, we detected only one case of bisalbuminemia in the last 4 years.

We were able to reinvestigate five of these eight patients. Agarose gel electrophoresis performed on the same samples from these five patients did not detect any abnormality of the albumin fraction (Fig. 1B). The morphology of the albumin peak in the eight cases detected with CZE and in the only case that could be detected by both methods was similar. These patients did not take antibiotics or present any pancreatic disease (suggesting an absence of pancreatic pseudocysts). Study of two of these patients 3 month later gave the same result: a bicuspid electrophoretic pattern in the albumin fraction detected by capillary electrophoresis that was not seen with agarose electrophoresis. The albumin composition of this serum component has been confirmed by immunofixation of the sample with anti-albumin antisera (data not shown). Moreover, no discordances were found between the nephelometric quantification of albumin with a Behring BN100 (Behringwerke) instrument (46 g/L) and the albumin concentration determined by capillary electrophoresis (43 g/L). Im-

References

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munofixation with anti-IgG, -IgA, and -IgM κ and λ light chains did not show the presence of monoclonal components. Reduction with mercaptoethanol (1:2 dilution), which dissociates complexes but does not dissociate albumin dimers, excluded the possible formation of complexes with α1-antitrypsin. Interferences by radio-opaque agents or medications, which could lead to the appearance of albuminemia detected by CZE.

The demonstration of a double albumin line on protein electrophoresis depends on the method used for separating blood proteins. Agarose gel electrophoresis has good separation of the β and γ globulins, but CZE shows improved separation of the albumin, α1, and α2 fractions (5). Meiers et al. (6) reported that the method of choice is cellulose-acetate foil electrophoresis because of its high sensitivity, but this method lacks specificity. Our results indicate that with the new capillary electrophoresis, more cases of this rare and interesting electrophoretic abnormality may be detected. However, further research is needed to confirm the clinical importance of the additional cases of bisalbuminemia detected by CZE.

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References

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Silver Staining of Denaturing Gradient Gel Electrophoresis Gels

To the Editor:
The continuing progress in the identification and characterization of genes that cause human genetic diseases has greatly increased the requests for a method that can rapidly identify mutations in these genes. The introduction of PCR has made it possible to rapidly obtain a relatively large amount of single-copy genomic DNA that can be used for subsequent analysis. Direct sequencing of amplified DNA, although a standard procedure in many laboratories, is still time-consuming and laborious. Consequently, different methods have been developed to pre-identify the region of the gene in which the mutation is located, which can subsequently be amplified and sequenced. We have chosen denaturing gradient gel electrophoresis (DGGE) of PCR-amplified material, by which fragments up to 800 bp in length can be screened for the presence of mutations. DGGE, as described originally (1), relies on the partial denaturing of a DNA duplex, which slows its movement in an increasing gradient of denaturant. If a mutation is present in the melted portion, the denaturing properties are changed and the duplex is arrested at a different point, signaling the mutation. This method has been modified so that it detects 90% or more of single base-pair substitutions (2). In standard DGGE methods, polyacrylamide gels are ethidium-bromide stained.

As far as we know from searching the literature and from personal communications, most laboratories using DGGE use ethidium bromide although it is a powerful mutagen and is not as sensitive as silver staining. When DGGE is used for screening large genes (e.g., CFTR, FVIII) and to screen >20 exons, it is convenient to rely on a method that is both sensitive and economical. Silver staining reduces the cost of testing by reducing the volume of the PCR mixture to 10 µL, improves the sensitivity, and provides a permanent record of results.

To illustrate the advantages of the combination of DGGE and silver staining, we performed DGGE of multiplex C for the CFTR gene under the conditions given by Fanen et al. (3). PCR was performed in a final volume of 10 µL, and a 1.5-µL aliquot was used for DGGE. The gel was silver-stained using the following protocol (Fig. 1). After fixation in 150 mL of a solution of absolute ethanol (100 mL/L) and acetic acid (5 mL/L) for 10 min at room tempera-