three methods when analyzed by Student's *t*-test for paired data, even though IgM data obtained with RID were lower. As expected, we found some relevant biases between methods (IgA, 24.1% and 23.8%; IgM, 92% and 86.4%), which probably depend on the calibration material used and the concentration step.

BALF protein evaluation is of particular interest in the study of local immune lung defenses (6) and blood-alveolar barrier permeability (7). Immunoassay methods are frequently used to measure specific proteins because of their specificity and sensitivity. When possible, BALF is sent to a laboratory with sophisticated instrumentation, in which the preferred methods are usually RIA, laser nephelometry, or ELISA. However, very often, only small laboratories are available for routine use, in which simpler and less expensive methods are required. RID is presently appreciated in routine work because of its specificity, conveniences, relatively low cost, and ready commercial availability; no dedicated instrumentation is required. Its disadvantages are long incubation times (2–5 days) and low sensitivity, which necessitates, for BALF samples, concentration steps. Pressure filtration is widely used for this purpose; however, some of the proteins are lost then, as they tend to stick to the filter. Each method of specimen concentration has advantages and disadvantages; as yet, none should be regarded as the standard procedure for BALF (2).

The IgM comparison in this study shows how the underestimate given by RID may also be due to the concentration step. Our aim was to side-step these problems by using modified sensitive immunoturbidimetry kits for urine and serum. This method requires only a spectrophotometer, which is found in all laboratories. The results showed close correlation between the immunoturbidimetric and immunonephelometric methods for all proteins. However, both immunoturbidimetry and immunonephelometry showed poorer correlation with RID (Table 1).

In our study, BALF protein evaluation with RID, though highly specific, was in several cases less sensitive and always more time consuming than immunoturbidimetry. However, as previously stated, the immunoturbidimetric method is less expensive than immunonephelometry, which needs dedicated instrumentation and skilled personnel. For these reasons we think that a modified immunoturbidimetric method can also be considered as a simple and useful tool for measuring protein fractions in BALF.

References

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Determination of Malonaldehyde in Hyperlipemic Sera

To the Editor:

Malonaldehyde (1,1,3,3-tetraethoxypropane; MDA) is the most commonly used marker of lipoperoxidation. It is now accepted that Yagi's fluorometric assay (1), the most widely used method for measuring MDA in biological samples, is not sufficiently specific. We recently developed an analytical improvement of this technique (2) that avoids the long and tedious manipulations of Yagi's assay and gives results that correlate strongly with those from an HPLC method (3). Serum samples were incubated with ethylthiobarbituric acid (DETBA) for 60 min at 96 °C and reaction products were extracted with butanol. The organic layer was submitted to synchronous fluorescence detection (2).

We applied this technique to serum samples from patients with type I hyperlipoproteinemia (4). Despite their high serum lipid concentrations, we found no atheromatous lesions in biopsies or at autopsy, even in old patients with renal diseases (5) or pulmonary hypertension (6). To test the possibility of a link between protection against atheromatous lesions and a possible decreased lipoperoxidation, we measured MDA concentrations in the sera of these patients.

The high hypertriglyceridermia of the patients led to erroneous values for MDA. In fact, lipids were extracted together with the reaction products of MDA and DETBA, and the organic layer became turbid and opalescent. The opalescence interfered with the fluorescence measurement, causing diffraction of the excitation light (Figure 1b). To prevent this, we added perchloric acid and butanol simultaneously to extract and clarify the organic layer. The best results were obtained when 50 μL of perchloric acid (2 mol/L) and 5 mL of butanol were added to the mixture [50 μL of serum in 1 mL of DETBA (10 mmol/L) in phosphate buffer (75 mmol/L), pH 3]. This acid treatment eliminated interference in samples containing up to 12 mmol/L triglycerides, and the spectra obtained were not altered. The yield from butanol extraction was not affected, as assessed with standard or biological samples containing known amounts of added MDA.

We also tested this modification on clear serum samples, which served as

![Fig. 1. Synchronous fluorescence spectra of (a) standard solution of MDA, (b) hyperlipemic serum without acid treatment, and (c) hyperlipemic serum after acid treatment](image-url)
we noticed that patients' samples with Ca values >120 mg/L consistently produced lower Ca values with Generations 18 and 19 Ektachem Ca slides than with another automated method in our laboratory that utilizes the Ca-binding dye cresolphthalein. For 10 samples having Ca values of 130–160 mg/L by the cresolphthalein method, we found the Ektachem values to average 15 mg/L lower (maximum, 23 mg/L lower). Samples with Ca values <110 mg/L had excellent agreement on the two instruments (± 3 mg/L). We therefore performed linearity studies with both methods to examine these discrepancies. Parallel dilutions of plasma samples with Ca values >170 mg/L were prepared with use of the manufacturer's bovine albumin-based diluent, which we further diluted with a half-volume of water to reduce the Ca content of the diluent. The diluent as used had a Ca concentration of 22 mg/L and a total protein content of 660 g/L as measured by the Ektachem. As seen in Figure 1, all samples with Ca values <105–110 mg/L produced values within 3 mg/L of their expected value by both methods. In addition, both methods had a linear response for samples with Ca concentrations <110 mg/L, indicating that the diluent was not producing a "matrix effect" that could complicate interpretation of the study. However, for samples with Ca values >110 mg/L, there was an increasing disparity between the Ektachem and the other method as Ca concentrations increased. For instance, the Ektachem produced values that were 7–14 mg/L lower than expected at Ca concentrations of 120–150 mg/L and 20 mg/L lower at Ca concentrations of 150–160 mg/L. On the basis of these data, we informed the manufacturer and began repeating all Ca determinations performed on the Ektachem, using a 1:2 dilution if the original value was >105 mg/L.

We have become accustomed to minor changes in patients' values (2–3 mg/L) between different generations of Ektachem Ca slides, but these data demonstrate that Generations 18 and 19 Ektachem Ca slides have a clinically significant nonlinear response above 110 mg/L. The manufacturer recently provided customers with a software update and new Supplementary Assigned Values for Ca calibrations, which has improved the linear response to 140 g/L in our laboratory. Because this problem existed for at least several months before the software update, it is important that users be aware that reported Ca values from hypercalcemic patients determined with Generations 18 and 19 Ektachem Ca slides had the potential to be falsely low at concentrations beyond 110 mg/L.

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Editor's note: The manufacturer's representative offered no additional reply for publication.

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Digoxin Immunoassay and Chinese Medicine

To the Editor:

We read with great interest the Letter on Chinese medicine and digoxin assay reported by Panesar (1). Unfortunately, however, our previous reports (2, 3) on this problem were not cited. We had clarified that Chinese medicine containing Ch' an Su had immunoreactive digoxin-like activity.

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Nonlinearity of Calcium Measurement on the Kodak Ektachem at Concentrations >110 g/L (Slide Generations 18 and 19)

To the Editor:

The Ektachem (Eastman Kodak, Rochester, NY) slide for measuring serum calcium is based on a change in absorption when calcium binds the dye Arsenazo III. The manufacturer has claimed an analytical range of 10–160 mg/L for the Ektachem Ca slide. During routine patient comparisons, we noticed that patients' samples with Ca values >120 mg/L consistently produced lower Ca values with Generations 18 and 19 Ektachem Ca slides than with another automated method in our laboratory that utilizes the Ca-binding dye cresolphthalein. For 10 samples having Ca values of 130–160 mg/L by the cresolphthalein method, we found the Ektachem values to average 15 mg/L lower (maximum, 23 mg/L lower). Samples with Ca values <110 mg/L had excellent agreement on the two instruments (± 3 mg/L). We therefore performed linearity studies with both methods to examine these discrepancies. Parallel dilutions of plasma samples with Ca values >170 mg/L were prepared with use of the manufacturer's bovine albumin-based diluent, which we further diluted with a half-volume of water to reduce the Ca content of the diluent. The diluent as used had a Ca concentration of 22 mg/L and a total protein content of 660 g/L as measured by the Ektachem. As seen in Figure 1, all samples with Ca values <105–110 mg/L produced values within 3 mg/L of their expected value by both methods. In addition, both methods had a linear response for samples with Ca concentrations <110 mg/L, indicating that the diluent was not producing a "matrix effect" that could complicate interpretation of the study. However, for samples with Ca values >110 mg/L, there was an increasing disparity between the Ektachem and the other method as Ca concentrations increased. For instance, the Ektachem produced values that were 7–14 mg/L lower than expected at Ca concentrations of 120–150 mg/L and 20 mg/L lower at Ca concentrations of 150–160 mg/L. On the basis of these data, we informed the manufacturer and began repeating all Ca determinations performed on the Ektachem, using a 1:2 dilution if the original value was >105 mg/L.

We have become accustomed to minor changes in patients' values (2–3 mg/L) between different generations of Ektachem Ca slides, but these data demonstrate that Generations 18 and 19 Ektachem Ca slides have a clinically significant nonlinear response above 110 mg/L. The manufacturer recently provided customers with a software update and new Supplementary Assigned Values for Ca calibrations, which has improved the linear response to 140 g/L in our laboratory. Because this problem existed for at least several months before the software update, it is important that users be aware that reported Ca values from hypercalcemic patients determined with Generations 18 and 19 Ektachem Ca slides had the potential to be falsely low at concentrations beyond 110 mg/L.

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Editor's note: The manufacturer's representative offered no additional reply for publication.

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Fig. 1. Plasma samples with Ca concentrations of 172 (A) and 202 (B) mg/L by the Hitachi 747 (Shanghai Manf. Inc., Indianapolis, IN) method diluted with Kodak bovine serum albumin diluent containing 22 mg of Ca and 560 g of total protein per liter. Expected values (x) were determined by multiplying the dilution factor by the value obtained from the Ektachem method for a 1:2 dilution plus the Ca content of the diluent in each sample. Ektachem values for the 1:2 dilutions were 97 (A) and 110 (B) mg/L.

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