may then be used to make the precipitin lines visible. The stain that seemed to give the best contrast between precipitin lines and membrane background was Procion Brilliant Blue M-RS (Inolex Corp., Glenwood, IL). An aqueous stain stock solution, 1.2 g/L was prepared and membranes placed in a working mixture of stain stock/water/acetic acid/methanol (2/2/2/1) for 10 min. Excess stain was then removed by two 10-min rinses in acetic acid/methanol/water (1/3/5). Both Ponceau S and Coomassie Brilliant Blue R were also tried. The first gave too light a contrast; the second did not rinse well from the membrane background.

After the membrane was dried in warm air for 5 min, the heights of the precipitin peaks could be measured. EIA peak heights were reproducible to within ±4.3% across a membrane and within ±5.0% between membranes.

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Quality Control in Assay of High-Density Lipoprotein Cholesterol

To the Editor:

Routine determination of high-density lipoprotein (HDL) cholesterol by means of precipitation assays gives results that compare well with those by ultracentrifugation (*1*). As a logical consequence, quality control of these methods requires attention. Therefore, we tested two precipitation assays by use of pooled human serum and lyophilized control sera:

(*a*) *The precipitation method with phosphotungstate–Mg^{2+}* (2): For this we used the reagents of Boehringer, Mannheim, Germany (lot no. 1179101). The precipitating reagent was five parts of phosphotungstate solution mixed with one part of $MgCl_2$ solution. The final concentration in the mixture was 40 g of phosphotungstate and 0.5 mol of $MgCl_2$ per liter. The test procedure was as follows: we added 50 μL of this precipitating reagent to 500 μL of sample, incubated at room temperature for 10 min, and centrifuged (1500 × *g*, 20 min). We mixed 25 μL of the supernates with 1000 μL of the cholesterol oxidase–PAP cholesterol reagent. Then the tubes were incubated at room temperature for 30 min. We measured absorbances at 546 nm (Eppendorf PCP 6121) and calculated the values with use of the factor 381.8.

(*b*) *Precipitation method with dextran sulfate–Mg^{2+}* (3): For this we used the reagents of Dow Diagnostika (Paul Hauser-Chepharin, 1097 Vienna, Austria), lot no. 8H5V. A 20 g/L solution of dextran sulfate 500 and a 1.1 mol/L solution of $MgSO_4$ were prepared, 500 μL of the samples was mixed with 25 μL of the dextran sulfate solution, and then 50 μL of the $MgSO_4$ solution was added. These samples were incubated at room temperature for 5 min, then centrifuged (1000 × *g*, 10 min). The supernates were used for determination of cholesterol as described above (factor: 399.2).

The control materials were Seronorm Lipid, lot no. 52 (Nyegaard, Oslo), Precilip, lot no. 662 (Boehringer Mannheim) and Target Lipid Control, lot no. 5496 (SmithKline Instruments). These lyophilized control sera as well as human pooled serum were tested in the two precipitation assays. Centrifugation was at 1600 × *g* for 10, 20, 30, and 40 min.

Table 1 shows our results (means of duplicates) and their dependence on the duration of centrifugation.

Table 1. Results by Two Procedures, Showing Effects of Duration of Centrifugation

Centrifugation, min	High-density lipoprotein cholesterol, mg/L: Phosphotungstate–$MgCl_2$	Dextran sulfate–$MgSO_4$
Seronorm Lipid,[a] lot no. 52		
10	675	1840
20	700	1871
30	666	1916
40	560	1900
Precilip, lot no. 662, mean value: 333, mg/L confidence limits: 259–408, mg/L		
10	573	246
20	358	236
30	336	259
40	155	259
Target Lipid Control,[a] lot no. 5496		
10	2112	645
20	1617	342
30	2264	537
40	2835	540
Pooled serum		
10	377	386
20	352	376
30	350	336
40	369	385

[a] No expected values are indicated by supplier.

In Seronorm Lipid there was no precipitate with dextran sulfate–Mg^{2+}. Precilip gave a precipitate in both methods. Moreover, a significant dependence on the duration of centrifugation was demonstrated when the phosphotungstate–Mg^{2+} reagent was used. The expected values were obtained only after 20 to 30 min of centrifugation. In contrast, in the case of Target Lipid Control, precipitation was possible with dextran sulfate–Mg^{2+} reagents but not with phosphotungstate–Mg^{2+}.

In contrast to these lyophilized control materials, we saw no influence of the precipitating reagents or duration of centrifugation for pooled human serum. In our laboratory the precision (CV) in pooled serum within series was 3%, the day-to-day precision 6%.

From these experiments we conclude that quality control of determination of HDL-cholesterol by use of lyophilized lipid control sera is often questionable, because there sometimes is no precipitation at all, probably owing to the influence on lipids of lyophilization and the addition of stabilizers. Only certain control sera are suitable for control use and in these cases only under exact performance of the described methods. The duration of centrifugation is of special importance. Therefore this fact should be mentioned in the package-insert instructions.

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Aldehyde Dehydrogenase Interference in the Haeckel Enzymic Technique for Uric Acid Determination

To the Editor:

At the 8th International Symposium on Clinical Enzymology we presented a report on different enzymic methods for uric acid determination (*1*), one of them being Haeckel's (*2*), which at that time was produced in the form of trial kit by SmithKline Instruments, Sunnyvale, CA 94086 (product no. 89517).

The kits we used at that time, when used in our centrifugal analyzer (CentrifiChem; Union Carbide, Tarrytown, NY 10591), showed some performance problems. Higher values for uric acid concentration were obtained than with the 293-nm method performed on the same instrument according to the procedure of Pesce et al. (*3*).

In a first approach (the one presented in Venice) we corrected this anomaly by use of a serum blank, with water in place of the reagent, obtaining values that correlated perfectly with both the Kageyama method (*4*) and with the 293-nm method. We also found that the reason for this lack of correlation was a spurious serum-blank increase. An aqueous solution of uric acid showed no such blank increment with time. The blank reaction was followed in the absence of uricase (EC 1.7.3.3), and when the reaction product spectrum was scanned vs a freshly prepared blank, it appeared to be the NAD(P)H spectrum. This was even more clear for sera from patients with liver disease, and as the role of aldehyde dehydrogenase (EC 1.2.1.5) in this kind of reaction had been mentioned by Haeckel (*2*), we tried to correct the blank increase by adding

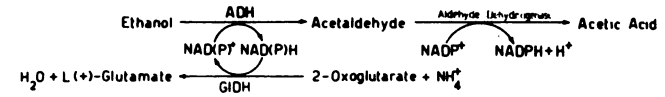

Figure 1.

pyrazole (2.0 g/L final concentration) to the reagent, but saw no substantial improvement. We noticed that the glutamate dehydrogenase (EC 1.4.1.3) activity was proportional to the rate of serum-blank increase, so we hypothesized the reaction scheme (*5*) shown in Figure 1. If this is correct, addition of NH_4^+ or oxoglutarate should diminish NAD(P)H, both endogenous and exogenous, in the sera, delaying for some time its appearance as reaction product.

In the meantime, the aforementioned kit was modified by the producer (buffer changed to bicine, 50 mmol/L, a larger quantity of KCl, up to 90 mmol/L was added to the reagent, and different lots of aldehyde dehydrogenase were used). Very small blank increases were observed with this new formulation.

When we compared data obtained with the old kits (lot no. 615911) as modified by us ($(NH_4)_2SO_4$, 50 mmol/L, added), with those obtained with the new kits (lot no. 633111) and with many other methods for uric acid determination, enzymic and nonenzymic, we obtained the same values with the new kits as the old, and data that were perfectly correlated with the other methods (*5*). Specifically with respect to the Kageyama method, our data are superimposable on data recently obtained by researchers from Boehringer Mannheim (*6*): $y = 3.27 \pm 0.97x$; $r = 0.97$; $n = 107$; $SD_d = 19.6$ μmol/L [y: uric acid by our modified SKI method (50 mmol/L $(NH_4)_2SO_4$ added) measured at 30 °C with a CentrifiChem S 300; x: uric acid by the manual Kageyama method].

In the paper by Bartl et al. (*6*), the anomalous blank increase is attributed to some aldehyde dehydrogenase isoenzyme present in sera of patients with liver disease, but this would not explain how addition of NH_4^+ can correct (in the SKI kit) the blank increase.

Apart from differences in the kits from SKI and Boehringer as far as composition and enzyme source are concerned, the SKI kit showed different behaviors when different lots of aldehyde dehydrogenase were used (Bucolo, G., personal communication). This fact seems to confirm our hypothesis that sera are only partly responsible for the blank increase with the SKI kit.

Perhaps the problems arising from the old SKI reagent formulation and from the Boehringer reagent formulation are basically analogous, but not identical.

We are now perfectly satisfied with our results, which have been kept under control for more than a year, and we are sure that our approach was fundamentally correct.

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