Stability of Frozen Liquid Control Sera for Assay of Cholesterol in High-Density Lipoprotein

P. N. M. Demacker, A. G. M. Hilmans, D. F. van Sommen-Zondag, and A. P. Jansen

Liquid serum pools with low-, normal-, or above-normal concentration of high-density lipoprotein cholesterol were prepared by selection and dilution of sera having low lipoprotein content or by enrichment with concentrated high-density lipoprotein. Stability of the serum pools depended on storage conditions. On storage between -2 and -12 °C, the high-density lipoprotein cholesterol content decreased. At a constant temperature of -19 to -21 °C the concentrations remained stable for more than 18 months. The imprecision (CV) of the high-density lipoprotein cholesterol assay during this period as established with enzymic cholesterol analysis (Clin. Chem. 28: 1780–1786, 1980) of these serum pools was between 2.7 and 4.8% (n = 51). Serum pools prepared and stored as described are suitable for internal quality-control procedures. In external quality-control trials these sera may be superior to the commercially available lyophilized lipid control sera.

Additional Keyphrases: suitability of quality-control specimens • enzymic methods • variation, source of

In view of increased interest in the determination of high-density lipoprotein (HDL) cholesterol (HDL-cholesterol), clinical laboratories need suitable control sera for calibration and for both internal and external quality control of this assay. Lyophilized lipid control sera are preferred, being easily sent to other laboratories for external quality-control programs. However, most of the commercially available sera are not suitable for this purpose, and reportedly show poor day-to-day precision or large bias when tested with different precipitation procedures (1–3). Furthermore, whether these sera results in accurate values when the corresponding HDL fractions are determined by enzymic cholesterol methods has not been studied.

The use of frozen liquid control sera prepared from large serum pools may be an alternative. Hohenwallner et al. (1) stated that these control sera can be used only for six to eight weeks because the results increase slowly with duration of storage. Warnick et al. (3) nevertheless observed that pool serum was stable when stored at a constant temperature of -15 °C.

We here report our findings with regard to the stability of liquid human pool serum stored frozen for 12 to 18 months.

Materials and Methods

Serum (not necessarily obtained from overnight-fasted persons) was either fresh or stored at -20 °C for as long as one month.

The low-value serum pool was prepared from serum with known below-normal values. Serum containing 8 mmol/L or more of triglycerides was excluded. To decrease the HDL-cholesterol concentration further, to approximately 0.75 mmol/L, we diluted three volumes of this serum pool with one volume of saline.

We made normal-value pool serum from serum obtained from apparently healthy persons. Above-normal pool serum was prepared by adding concentrated HDL to normal serum. To obtain concentrated HDL, we increased the density of 120 mL of fresh serum to 1.225 kg/L by dissolving in it 42.2 g of solid KBr (added in small portions, to prevent denaturation of the lipoproteins by high concentrations of salts). We isolated the d <1.225 kg/L fraction after centrifugation at 168 000 × g for 22 h (14 °C) as described before (4), using a large-capacity rotor (20 × 6 mL). We aspirated the upper 2 mL and dialyzed this fraction for 48 h against a pH 7.4 buffer containing, per liter, 0.02 mol of tri(hydroxymethyl)aminomethane, 0.15 mol of NaCl, and 1.34 mmol of ethylenediaminetetraacetate. The buffer was charged four times during dialysis. To increase the density to 1.063 kg/L we dissolved 83.4 mg of solid KBr per milliliter of dialyzed solution and ultracentrifuged again for 16 h. After removing as much of the upper layer as possible we aspirated the HDL from the bottom of the tubes (7 to 8 mL). Usually we diluted this with 250 mL of serum, which yielded an above-normal HDL-cholesterol value of approximately 1.6 mmol/L.

The three pools obtained in this manner were apportioned into glass ampoules with air-tight caps, stored in the freezers indicated below, and specimens were analyzed almost each week.

Procedures. We determined HDL-cholesterol in serum by the heparin/Mn²⁺ method as described before (5). The HDL fraction was obtained after high-speed centrifugation for 10 min at 10 000 × g. Turbid supernates were cleared by ultrafiltration. We determined cholesterol in duplicate by the enzymic catalase method of Röschlau et al. (6), as slightly modified (5). The resulting HDL-cholesterol values (y) were correlated with time of storage in days (x) by use of Pearson's correlation test.

Results

We stored the control sera in a freezer that was in normal use on the laboratory. Table 1 gives the mean ± SD for HDL-cholesterol in the three control sera. The coefficients of variation (CV) over the year were 12.9, 7.4, and 7.1% for the low-, normal-, and above-normal pools of serum, respectively (n = 37). HDL-cholesterol values decreased slowly and progressively by 0.24–0.36 mmol/L in that year. After the low-value pool serum had been stored for three months in the freezer, ultrafiltration was frequently necessary. For all three serum pools the correlation between duration of storage and HDL-cholesterol concentration was highly significant (p <0.001) (Table 1). Furthermore, the decrease in HDL-cholesterol during storage was of the same order for all three control sera and apparently independent of the original HDL-cholesterol value. The freezer used for storage was opened frequently during the day, and the temperature range showed a strong day–night rhythm. In the morning the temperature was -12 °C, but rose to -2 °C at the end of a working day in summer (room temperature 26 °C).

For storage of a new series of sera we looked for a freezer with a constant temperature. The central freezing room of our
laboratory appeared to be unsuitable for our purposes. Although the thermostast indicated a constant temperature of 
-15 °C, the actual temperature inside the freezer was nowhere less than -10 °C. Rather, as a result of insufficient air circulation within that room, the temperature ranged from -10 °C at the floor to +2 °C at the ceiling.

The new sera were now stored in a small freezer that was opened only infrequently. The temperature inside it was constantly between -19 and -21 °C. The results obtained with these new control sera stored 18 months in the freezer were satisfactory (Table 1; pools 4 and 5). The CVs for the low- and above-normal value serum pool were now 4.9 and 2.7%, respectively (n = 51), and there was no correlation between duration of storage and HDL-chol concentration.

Discussion

Evidently the conditions for storage of sera for the HDL-chol assay should be controlled and should also be defined more accurately. When serum was stored at a constant temperature of about -20 °C we noticed stable values for 18 months, but concentrations progressively decreased with time when serum was stored in a freezer that had large fluctuations in temperature. These results support the findings of Warnick et al. (3).

At first we tried concentrating the HDL by isolating the HDL (d <1.225 kg/L) from the d >1.063 kg/L fraction. This procedure made one dialysis step redundant. However, the serum proteins were then eliminated only at the second ultracentrifugation step and a final 15-fold concentration of the HDL-fraction was impossible because of the increased viscosity.

Obviously, the properties of the prepared serum pools are identical to those of normal human serum; therefore, they can be used in chemical as well as enzymic cholesterol analysis and are also suitable for external quality-control trials. However, the special problems encountered with regard to the distribution of these frozen liquid sera make large-scale use impossible. It should be pointed out that these sera cannot be accurately analyzed by the ultracentrifugation method, because freezing changes lipoprotein densities. Furthermore, the enriched serum may contain high concentrations of LP(a) lipoprotein, which results in falsely high HDL-chol values.

Use of lyophilized commercial lipid control serum for external quality-control programs is not recommended, because each lot will require thorough studies to evaluate their use as control sera in the HDL-chol assay. Between-day precision—if possible that of different lots from the same manufacturer—must be characterized, and one must ascertain whether these sera can successfully be fractionated by all currently used precipitation procedures and whether the use of different chemical or enzymic cholesterol reagents results in accurate values. Studies so far indicate that most sera are not suitable (1–3).

We based our evaluations on pool sera with normal concentrations of triglycerides. To test whether apo B-associated cholesterol is removed completely by the precipitation procedure, sera with above-normal concentrations of triglycerides should also be analyzed. That such sera are also stable at -20 °C is not certain. Recently, Bachorik et al. (7) obtained falsely high HDL-chol values by use of the heparin/Mn²⁺ precipitation procedure after storage of sera between -18 to -23 °C, because especially in lipemic sera the low-density lipoproteins were difficult to precipitate completely. Even when sera were stored at -80 °C for one year an increase in HDL-chol was noticed by use of the heparin/Mn²⁺ method (8). We have found that the efficiency of the heparin/Mn²⁺ method for removing apo B-containing lipoproteins from lipemic serum is low (5); fresh sera with triglycerides ≥2.8 mmol/L could not be fractionated successfully. In light of the results of Bachorik et al., perhaps only sera with normal concentrations of triglycerides can be fractionated successfully by the heparin/ Mn²⁺ method after having been frozen unless ultrafiltration is used to clear the turbid supernates. Other precipitation procedures, more effective in removing apo B-containing lipoproteins, should be used for the determination of HDL-chol in thawed frozen sera.

References

Evaluation of a New Serum Separator

Daniel L. Seckinger, D. Antonio Vazquez, Phyllis K. Rosenthal, and Z. Herbert Heller

We tested the Centri-Sep filter (DADE) for its effectiveness in separating serum from clotted blood samples used for chemical analysis. Although statistical analysis by paired t-test showed differences in results for some analytes with the use of this device as compared with use of no separator or of a serum-decanting device, we concluded from the small bias of the paired means that the new separator device did not interfere with the clinical usefulness of reported values for the analytes studied. The separator is not an effective barrier for long-term storage of serum on its clot; however, we could obtain about 10% more serum with the separator than with decanting devices.

Additional Keyphrase: sample preparation

The Centri-Sep fluid filter (American Dade, Div. of American Hospital Supply Corp., Miami, FL 33152) is a cylindrical plug, 13 or 16 mm in diameter, of cellulose acetate fibers bonded together with glycerol triacetate. The average pore size is 18 μm² and the average fiber size before expansion is 21 μm. After the blood specimen has clotted, but before centrifugation, the filter is inserted into the blood tube. During centrifugation (1200 X g for 10 min) the filter moves down into the tube. When it first touches the serum, the filter absorbs fluid and expands against the tube walls in a "wiping" action as it moves; it then settles onto the compacted clot. If the specimen has not clotted, such as with patients on anticoagulant therapy or with coagulation defects, the cells will pass through the pores and the filter will settle on the bottom because the relative density of the filter material exceeds that of the cells.

We did four evaluation studies to assess the efficacy of the "Centri-Sep" for serum/clot separation. In the first, we compared both sizes of filter with no separation device. In the second, we compared the fluid filter separator with the Filter Sampler (Glasrock Plastics, Fairburn, GA 30213) separator. After determining that the Centri-Sep did not interfere with any chemical analysis, we evaluated the possibility of using it as a barrier in stored blood. We also compared the serum yield of the Centri-Sep and the Filter Sampler. Free hemoglobin was determined in paired samples with and without the Centri-Sep to determine if there was any trauma to the erythrocytes because of the filter.

Materials and Methods

Sample collection and processing. The samples were typical of our hospital patient population, including such conditions as hyperlipidemia and hyperbilirubinemia.

For all the various comparative studies, duplicate samples were drawn simultaneously. We removed the stopper from one tube of each pair, inserted a fluid filter in the top of the tube, then recapped the tube with the plastic cap to prevent aerosol formation. After centrifuging the paired samples together at 1200 X g for 10 min, we decanted the serum from the blood fluid filter tube into a clean test tube and stored it for chemical analysis. Serum from the other evacuated tube was either decanted directly or separated from its clot with use of the Filter Sampler.

Chemical analysis. Thirty different analyses for chemical analytes, including some common therapeutic drugs and radioimmunoassay determinations, were performed. For continuous-flow analysis we used an SMA 12/60 and single-channel AutoAnalyzer systems (both from Technicon Instrument Corp., Tarrytown, NY 10591). Electrolytes were analyzed with a C-800 ion-specific electrode continuous-flow analyzer (Technicon). Insulin, thyroxin, triiodothyronine uptake, and thyrotropin were determined by radioimmunoassay. Creatine kinase, alanine aminotransferase, triglycerides, and lactate were determined with a VP Bichromatic Analyzer (Abbott Laboratories, North Chicago, IL 60064). Magnesium was determined by atomic absorption. Amylase and lipase were determined by a nephelometric method (Coleman 91; Perkin-Elmer, Oakbrook, IL 60521). Acid phosphatase, cholinesterase, and total iron were determined by manual procedures. Free hemoglobin was determined by the oxidation of benzidine.

In all of the comparison studies, we alternated the order of analysis, i.e., fluid filter/reference separator, reference separa-