Evaluation of Light-Scattering Index (Nephelometry) for Assessing Serum Triglycerides and Lipoprotein Phenotypes

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Light-scattering measurements (nephelometry) of serum after filtration through appropriate filters is a rapid method for physically determining triglyceride concentrations and lipoprotein phenotypes. A correlation coefficient of greater than 0.9 was obtained when aliquots of the same specimens were tested for triglycerides by light scattering in an Aminco-Bowman Spectrophotofluorometer and by a chemical (Van Handel and Zilversmit) method. Lipoprotein phenotyping by agarose gel electrophoresis and by light-scattering measurement corresponded excellently. In equivocal cases, nephelometric results agreed better with the triglyceride and cholesterol data than did the electrophoretic method.

Additional Keyphrases colorimetry-nephelometry relationships • Fredrickson's phenotypes

Recent studies show that if lipoprotein is estimated by use of LSI¹ properties of serum, after it has been filtered appropriately, the results correlate well with those obtained by ultracentrifugation and electrophoresis (1-3).

These authors and others (4-6) have also shown that there is a good correlation between concentration of triglycerides in serum and LSI measurements.

Baty and Batsakis (7), however, concluded that nephelometry provides too indirect an assay to give consistent results for serum triglycerides.

In the present study, we compared triglyceride in sera as calculated from LSI measurements with data obtained by a chemical method (8). Also, lipoprotein phenotyping by agarose gel electrophoresis and by light-scattering measurements was compared.

Materials and Methods

Methods

Total lipids were determined by a colorimetric method based on a sulfo-phospho-vanillin reaction (9).

Cholesterol was determined with the 12/60 AutoAnalyzer (Technicon Corp., Tarrytown, N.Y. 10591) (10).

Triglycerides were determined manually, by the method of Van Handel and Zilversmit (8).

Lipoprotein electrophoresis was performed on thin agarose gel (Elevitch, F. R., and Austin, D. B., Lipoprotein electrophoresis in thin agarose gel. Presented at the Annual Meeting of the California Medical Association, February 1969). Sample wells of “Agarose Universal Electrophoresis Film” (Analytical Chemists, Inc., Palo Alto, Calif. 94303) are filled with 1.0 µl of freshly drawn serum. The agarose film is electrophoresed for 60 min in a cell at a constant current and 100 V. Filter paper wicks make contact between the inverted agarose gel surface and the outer cell buffer chambers, which are filled with barbital

¹ Nonstandard abbreviations used: LSI, light-scattering index; VLDL, very low-density lipoprotein; TG, triglycerides.

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buffer (pH 8.6, 0.05 mol/liter). After electrophoresis, the lipoproteins are fixed by drying in a convection oven at 80–90°C for about 20 min.

Lipoproteins are stained for 15 min with a working solution of Fat Red 7B (see below) in a closed container, rinsed for 1 min in a glycerol solution (2 ml glycerol plus 98 ml water), air dried, and scanned at 520 nm in a densitometer.

Stock Fat Red 7B. Dissolve 0.9 g of Fat Red 7B (Ciba, Inc., Fair Lawn, N.J. 07410) in 4 liters of methanol.

Working solution (Prepare just before use). Add eight drops of Triton X-100 to 200 ml of stock stain solution in a clean, dry graduated cylinder. Cover the mouth of the graduated cylinder with "Parafilm" and mix by inversion, slowly add 40 ml of de-ionized water and re-mix.

Light scattering (nephelometry) was measured, after filtration through appropriate filters (see below), with the Amino-Bowman Spectrophotofluorometer (American Instrument Co., Silver Spring, Md. 20910; cat. no. 4-8202). Wavelength of both incident and emitted light was set at 625 nm. Since there is no generally accepted standard for turbidity, the instrument was standardized with "Hyland Special Chemistry Control Serum" (Hyland, Costa Mesa, Calif. 72626). Unassayed Hyland serum (value determined by the Van Handel-Zilversmit method) was used as a control.

Calculations. Chylomicon TG, mg/100 ml = 0.75 (LSI_T - LSI_T)

VLDL TG, mg/100 ml = 9 (LSI_T - 5)

Total TG = chylomicon TG + VLDL TG

If chylomicon TG is more than 10% of the total, it is doubtful that the patient has fasted; if he has, an abnormal pattern of type I or V is probably indicated.

Lipoprotein phenotyping by SML (small, medium, and large particles) profile. (See "Micronephelometer Application Bulletin 2A," Particle Data, Inc.)

Serum is prepared as for triglyceride LSI measurements. From LSI measurements, "Particle Scores" for small (S), medium (M), and large (L) particles are determined from a modified nomogram published by the Thorp Micronephelometer Corp. (obtainable from Particle Data Inc.), which relates Particle Scores for lipoprotein particles to ultracentrifugation values for these particles (Figure 1). The nomogram has been modified so that normal cholesterol values, as determined by the Technicon SMA 12/60, include values up to 300 mg/100 ml.

The L LSI value is the LSI for serum filtered through a filter of 0.45-µm pore size minus the LSI for serum filtered through a 0.05-µm filter; particle score is read from a graph relating LSI of L to particle score (Figure 1A).

### Procedures

**Triglycerides by LSI** ("Micronephelometer Application Bulletin 1A"; Particle Data Labs. Inc., Box 265 Elmhurst, Ill. 60128). Blood collected after a 12–14 h fast is allowed to clot and centrifuged at 2500 × g for 20 min. One ml of serum is diluted with 9 ml of saline solution (9 g/liter). A 5-ml Yale B-D Lok-Control syringe with a 25-mm filter attachment ("Millipore Swinnex-25," Millipore Corp., Bedford, Mass. 01730), is used (light pressure) to pass the diluted serum through a 0.45-µm membrane filter (Sartorius MF 50). At least 1.5 ml of this prefILTERED serum is placed in a cuvet for LSI measurement P. The remaining serum is passed through a Sartorius MF 12 filter (0.05-µm pore diameter), and at least 1 ml of the filtrate is placed in a cuvet for LSI measurement f. Platelets, fibrin, and other light-scattering debris of diameter greater than 0.45 µm are eliminated by the first filtration; thus, light-scattering in the first filtrate is caused only by triglyceride-containing chylomicra and VLDL (β-lipoproteins, the principal cholesterol carriers, do not scatter light). The second filtration removes the chylomicra, so that light scattering in the second filtrate is attributable to the triglyceride-containing VLDL. Normal fasting serum contains insignificant amounts of chyomicron triglycerides.

![Fig. 1. Nomogram relating LSI measurements to L, M, and S Particle Scores](http://example.com/fig1.png)
The M LSI value is the LSI for serum filtered through a MF 12 membrane (0.05 μm); particle score is read from dual scale relating it to the M LSI value (Figure 1B). M particles relate quantitatively to the LSI values obtained from 0.05-μm filtered serum, because the L particles (chylomicra) have been removed, and the S particles (cholesterol) cause no appreciable light scattering.

S particle score is read from the nomogram relating it to the cholesterol value and M LSI value by aligning a clear plastic straight edge between cholesterol value and the M LSI value and noting the intersect reading of the S particle score (Figure 1B). S and M particles contain a characteristic percentage of cholesterol, L particles have little. Thus, if cholesterol and M are known, S can be evaluated from the nomogram.

S, M, and L values are related to the Fredrickson types of hyperlipoproteinemias ("Micronephelometer Application Bulletin 2A," Particle Data Inc.) as shown in Figure 2.

**Results and Discussion**

The study group consisted of over 300 patients. Triglycerides were determined in the serum of 73 of these by both manual and LSI methods (nephelometry). A correlation coefficient >0.9 was found between the values obtained by the two methods (Figure 3).

Sera from 62 patients that had an abnormal total lipid, cholesterol, or triglyceride value were phenotyped by agarose gel electrophoresis and LSI. Of these, 14 were typed as type II, 30 as type IV, 11 as type V, and seven as type III. (Since type I is usually found in childhood, no such cases have been encountered at the VA Hospital.)

![Fig. 2. Relationship of S, M, and L Particle Scores to Fredrickson types of hyperlipoproteinemias](image)

Of the 14 type II's, results for the two methods agreed in 12. In the other two, the LSI pattern indicated a type II, but the electrophoretic pattern appeared to be a type IV. However, the cholesterol and triglyceride values in the two cases were consistent with a type II hyperlipoproteinemia.

Of the 30 type IV's, results of the two methods corresponded in 21 cases. In the other 9, there was no electrophoretic separation between the β- and pre-β fractions (as fairly commonly occurs with type IV's electrophoresed on agarose gel); however, the LSI pattern was clearly type IV and corresponded to the cholesterol and triglyceride values. Patients with an LSI pattern typical of type II or type IV, but with normal total lipid values were designated as "type II (or IV) tendency." Five of the 300 patients studied showed a LSI pattern in which all three parameters (S, M, and L) were increased; these were classified as type III or type V, depending on whether significant amounts of chylomicron triglyceride were found compared to the total triglyceride. If the percentage of chylomicron triglyceride was high, the patient was typed as a V; otherwise he was typed as type III. The correctness of this typing was verified by ultracentrifugation and by electrophoresis.

We suggest that the results of Hood et al. (11) and Hollender et al. (12), as quoted by Baty and Batsakis (7), did not show LSI to be the method of choice for triglyceride determinations because they did not use filtration before light-scattering was measured. Stone and Thorp (7) and Rouillac et al. (4) also found a gross distortion in the correlation between LSI and triglycerides in unfiltered serum samples.

Table 1 shows the differences in light-scattering units obtained in our laboratory on diluted ali-
Table I. Effect of Filtration on Light Scattering by 11 Sera

<table>
<thead>
<tr>
<th>Unfiltered serum</th>
<th>Serum filtered through 0.45 μm filter</th>
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<tr>
<td></td>
<td>Light-scattering units</td>
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<tr>
<td></td>
<td>16.0</td>
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<td>17.2</td>
<td>16.0</td>
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quotients of unfiltered serum and serum filtered through 0.45-μm filters. Such differences would result in large discrepancies in triglyceride values. Apparently filtration to remove large light-scattering particles is needed if triglyceride values calculated from light-scattering measurements are to be correlated with the chemical method of determining triglycerides.

We conclude that LSI measurements of serum after filtration are a valuable aid in physically determining triglyceride concentrations and in lipoprotein phenotyping. The method is considerably faster than agarose gel lipoprotein electrophoresis, and the results are more consistent with the triglyceride and cholesterol data than are those obtained by the electrophoretic methods, in our hands.

Filtration before light-scattering is measured is necessary for accuracy.

By the use of filters of various pore sizes and correlating the results with clinical data, it should be possible to delineate subgroups of the Fredrickson classification.

References