Varieties of Human Serum Lipoprotein Pattern: Evaluation by Agarose Gel Electrophoresis

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A previously reported agarose gel electrophoretic technique for the determination of serum lipoprotein patterns has been modified for analysis of a large number of samples for screening and epidemiological purposes. In addition, we demonstrate the varieties of lipoprotein patterns that can clearly be distinguished and visually evaluated for practical applications in the clinical laboratory.

Additional Keyphrases hyperlipoproteinemias · screening

Dyslipidemia has been associated with premature atherosclerosis and other pathological conditions, and the determination of serum lipids is of great importance in health and disease. Most of the lipids are transported in plasma in the form of lipoproteins. Four classes of lipoproteins have been recognized by electrophoresis: chylomicrons, beta-, pre-beta-, and alpha-lipoproteins. Fredrickson, Levy, and Lees (1) classified the hyperlipoproteinemias into five types by paper electrophoresis; their classification is a very useful diagnostic and therapeutic aid.

The renewed interest in serum lipoproteins stimulated the development of improved electrophoretic methods for their determination (2-5). An agarose gel electrophoretic technique that clearly detects the four serum lipoprotein fractions and additional subfractions has previously been reported (6). Our purpose here is (a) to describe improvements in methodology and instrumentation for the simultaneous determination of a larger number of samples, and (b) to illustrate the variety of lipoprotein patterns that can be clearly demonstrated and visually evaluated by this technique.

Materials and Methods

A new electrophoresis cell has been constructed as illustrated in Figure 1. The materials, Plexiglass and platinum electrodes, and the separations are similar to the previous cell (6); the dimensions, however, have been changed so that four slides can be run instead of two. Furthermore, two of these boxes can be used parallel to each other so

![Fig. 1. Photograph of the electrophoresis cell filled with barbital buffer and petroleum ether. Four slides are placed on the agarose blocks.](image-url)
that up to eight slides can be run simultaneously when necessary.

The previously described electrophoretic procedure (6) was used for the separation of the lipoproteins—including the same buffer and agarose reagents, preparation of the slides, application of the sample, electrophoretic migration, and fixing and drying of the slides. However, the Oil Red O stain has been replaced with Sudan Black B. After comparing several lipid stains, the best patterns were obtained with an alcoholic solution of the latter dye. It was prepared as follows:

Stock solution. Sudan Black B, 1 g, in 1 liter of ethanol:water (60:40, by vol). This solution is stable for months at room temperature.

Working solution. Fifty milliliters of freshly filtered stock solution. When the slides are completely dried, they are transferred to a working stain solution and remain for 1 h at room temperature. The slides are then rinsed briefly in ethanol: water (1:1, by vol) until the background stain is removed.

Results

Human serum samples were prepared from freshly drawn and clotted blood. In the example, Figure 2, aliquots from the same serum were used. It illustrates that eight samples can be run simultaneously in two parallel electrophoresis cells. The excellent reproducibility of the lipoprotein patterns may be assessed visually.

Figure 3 illustrates examples of five different patterns obtained by this technique, which are clearly distinguished on the microscope slide and
can easily be evaluated visually. This is a direct photographic reproduction of the actual slides. In the first example are shown the familiar four classes of lipoproteins: chylomicrons, beta-, pre-beta-, and the alpha-lipoprotein, which is resolved into two bands by this technique; this pattern has been defined as a normal control. In the second example two strong pre-beta bands are shown. The third example presents a pattern with a slow migrating band in the pre-beta area. In the fourth and fifth slides, three pre-beta bands can be distinguished, but with a different migration. These examples are not associated with the previously reported five types of hyperlipoproteinemia (6) or of those in liver diseases (7). They represent samples from normal asymptomatic subjects as well as from patients with angina and other pathological conditions. Their significance may become more specific and meaningful when correlated with an abnormal process of defined clinical condition.

Discussion

The electrophoretic cell was modified in response to the need for analyzing a larger number of samples, for screening and epidemiological purposes, by the technique previously described (6). Since two cells can be used concurrently, an operator can process 160 samples daily. The high quality of separation and slide individuality was maintained for visual evaluation, densitometry; and record purposes. The diversity of this system—it can be used to run any smaller number of samples—makes it suitable for small and large laboratories.

In addition to hyperlipoproteinemias, changes of lipoprotein patterns occur in various other pathological conditions. One of the aims of this report was to present the varieties of lipoprotein patterns that are clearly demonstrated by this technique, and to facilitate their correlation with defined pathological conditions. Even more bands are resolved by this and other (3) electrophoretic techniques and can be recognized by the trained eye, but for practical application purposes and meaningful interpretation further improvement of the technique or a combination of two techniques is required.

Chylomicrons are usually absent in the normal fasting serum and in most pathological conditions with the exception of Types I and V hyperlipoproteinemias. Quantitative changes of beta-lipoprotein reflect primarily variations of cholesterol concentration. No significant clinical importance has been attributed to alpha-lipoprotein changes. Determination of the pre-beta-lipoprotein (the bands that migrate between the beta- and alpha-lipoproteins) is of increasing importance because in this area changes occur as a result of genetic variations, abnormal processes, and pathological conditions. It is in this area that our technique has made its greatest contribution by the clear-cut demonstration of several pre-beta bands of potential importance for future developments. We have already presented evidence of such developments (7), myocardial infarction (8, 9), and human genetic variants [Papadopoulos, N. M., Serum lipoprotein patterns of human variants (in preparation)].

References