and LDH isoenzyme reagent (both from Helena Laboratories, Beaumont, TX), for LD staining, which is less susceptible than the previously described method to interference by hemoglobin. The final concentrations of the single-reagent mixture for LD staining are as follows: D,L-lactate, 120 mmol/L; NAD⁺, 5 mmol/L; diaphorase (EC 1.8.1.4), 6000 U/L; nitroblue tetrazolium (NBT), 0.5 mmol/L; MTT, 2 mmol/L; and Tris HCl, pH 7.4, 50 mmol/L.

Wash three times with isotonic saline erythrocytes isolated from EDTA-treated venous blood, then disrupt them by adding an equal volume of distilled water. Electrophoretically fractionate the erythrocyte hemolysates, using Titan III Lipo, and make the activity bands visible by staining with Helena LDH isoenzyme reagent. Then quantify the bands with a Clini scan reflectance densitometer (Helena Laboratories) at 570 nm. Figure 1 shows zymograms obtained for normal controls and typical heterozygous individuals with H- or M-subunit deficiencies. From the total LD activity and that of each quantified LD isozyme, we calculated the H/M-subunit ratio in erythrocyte LD. The range of H/M ratios (mean ± 2 SD) obtained for 178 cases from the normal control group was 3.20 to 4.30. Thus, the new criterion is an H/M ratio <3.19 for H-subunit deficiency and >4.31 for M-subunit deficiency.

From a clinical viewpoint, there are no consistent and common symptoms in LD H-subunit deficiency (3). On the other hand, exertional myoglobinuria, abnormalities at delivery, and skin eruptions are commonly seen symptoms in individuals homozygous for M-subunit deficiency (4). Carriers with either subunit deficiency have no clinical symptoms. However, subjects with these subunit deficiencies can easily be misdiagnosed because of the decreased release of LD from affected organs in various disease states. Consequently, it is important to detect patients with LD-subunit deficiency as a potentially misinterpretable hereditary disorder. The above criterion would be useful for detecting such patients.

References

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α1-Antitrypsin Typing Test Evaluated

To the Editor:

In 1984 we published an original method for α1-antitrypsin typing (Pi typing) by use of dried blood specimens absorbed on paper and mailed to the laboratory (1), a method especially suited for low-cost mass screenings in high-frequency or high-risk populations.

We now report results of the "field testing" of the method, after a large survey in the northern Italian province of Bozen, in which α1-antitrypsin deficiency is frequent.

This screening included 16 000 newborns. Blood specimens were absorbed on paper (Schleicher & Schuell, no. 2992, special paper for the Guthrie's test) and mailed to the laboratory. A 0.5-cm-diameter piece of paper was cut out and the blood was rehydrated with a 1 g/L solution of D,L-dithiothreitol (Sigma Chemical Co., cat. no. D-0632). After 1 h at 37 °C, the moist paper samples were placed directly on a polyacrylamide gel for isoelectric focusing as described (1). After fixation, the gels were exhaustively washed in water and stained (2). Parents of those newborns with pathological phenotypes were notified through the local coordination center in Bozen that organized the collection, and 506 serum specimens were frozen and sent to the laboratory for Pi typing, performed as described (2).

Comparing the Pi-typing results obtained for dried blood samples with those obtained for serum allowed us to check the number of diagnostic errors during the screening and thus to verify the reliability of our method. The percentage error during the three years of the study was 7.11%: 36 errors in 506 newborns.

Two different types of errors must be distinguished (3, 4): (a) "type A" errors, or errors with "pejorative disconfirmation" (attribution of a pathological phenotype to an uncertain phenotype, so as to justify obtaining the serum for confirmation) and (b) "type B" errors, or errors with "ameliorating disconfirmation" (attribution of a normal phenotype to a pathological one). During the three years of the screening there were 6.9% (35 of 506) pejorative disconfirmations, but only one ameliorating disconfirmation (0.198%).

The percentage of "disconfirmations" can be assumed to be the "unreliability index" of the method that we used for the mass screening (3, 4). During the three years of screening, this index has changed (Figure 1).

Because so many serum samples were examined during the three years (1986: 100 cases; 1987: 159 cases; 1988: 247 cases) we rule out the likelihood that the continuous decline of the unreliability index is related to chance. We therefore ascribe it to improvements in the method and in the experience of the laboratory staff. In fact, the accuracy of the method is...
increasing exponentially (Figure 1), and a projection of the error index suggests that the method's reliability, now at 97%, should continue to improve.

Evidently the method works quite well, especially if used by experienced laboratory personnel.

References

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