A Modification of the 'Pink Test' May Improve the Diagnosis of Hereditary Spherocytosis

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Several tests for the laboratory diagnosis of hereditary spherocytosis (HS) have been proposed as screening tests for the detection of spherocytes in the peripheral blood, but at present none of them is a single and unequivocal test for HS. In 1984 Vettore et al. [1] proposed a quantitative determination of hemolysis of small blood samples in a solution containing glycerol at pH 6.66, called 'pink test'. This test showed a high diagnostic sensitivity (100%) whereas the specificity was 97 %. Judkiewicz et al. [2] proposed a modification requiring only 10 μ l of blood taken by finger-prick without anticoagulant, thus eliminating the necessity of venipuncture. In their report the thresh-

old value of hemolysis for suspecting HS was still lower (18 versus 28.5%) and there was no overlap between HS and control subjects (100% of specificity and sensitivity).

We have carried out an investigation aimed to evaluate the reproducibility of the 'pink test' and the effect of storage time of the sample on its results. We examined 50 healthy subjects and 15 patients affected with HS, whose diagnosis was established on the basis of red cell morphology, osmotic fragility, autohemolysis, clinical signs, blood values and family studies. We performed the 'pink test' with the hemolyzing medium according to Vettore et al. [1], at pH 6.66 and Table 1. Percent hemolysis of blood from healthy controls and HS patients in the original version of the 'pink test' and in the proposed modification after 24 h of incubation (24°C)

86.7 100

54

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with the osmolarity carefully adjusted with an osmometer to 290 mosm/l.

We confirm the value of this test; in our cases the choice of a threshold value of hemolysis of 28.5% as proposed by Vettore et al. [1] gave us a specificity of 95% (with sensitivity of 86%). When we chose a value of 18%, the specificity was lower (18% of control subjects had an hemolysis ranging between 20 and 30%).

Hoffmann et al. [3] experienced the effect of incubation (24 h at room temperature) on acidified gly-cero lysis time and showed that 24 h after blood collection the test achieves a sensitivity of 100% without any false-positive result in normal subjects. According to this experience we repeated the 'pink test' 24 h after blood collection, with storage of samples at room temperature. While the specificity was the same (96%), the sensitivity increased to 100% (table 1). Our experience with the 'pink test' after incubation suggests that in this way a sensitivity approaching 100% in HS is achieved without a loss of specificity.

References

- Vettore, L.; Zanella, A.; Molaro, G.L.; De Matteis, M.C.; Pa-vesi, M.; Mariani, M.: A new test for the laboratory diagnosis of spherocytosis. *Acta haemat.* 72: 258–263(1984).
- Judkiewicz, L.; Szczepanek, A.; Bugala, I.; Bartosz, G.: Modified end-point glycerol hemolysis assay as a screening test for hereditary spherocytosis that requires no venipuncture. *Am. J. Hemat.* 26: 89–91 (1987).
- Hoffmann, J.J.M.L.; Swaak-Lammers, N.; Bucx, M.J.L.; Breed, W.P.M.: Evaluation of the acidified glycerol lysis test. *Br. J. Haemat.* 65: 252(1987).