A New Modification of the ‘Pink Test’ for the Diagnosis of Hereditary Spherocytosis

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The diagnosis of hereditary spherocytosis (HS) is still difficult in some situations because of the variability in the clinical expression of the disease and the inconstant positivity of the laboratory tests. Several tests have been proposed including the autohemolysis test [1], the osmotic fragility test [2] and tests based on the evaluation of the final rate of hemolysis in neutral or acidified glycerol solutions [3–5]. Lately, Vettore et al. [6] proposed the ‘Pink test’ based on the determination of the final hemolysis in a solution containing glycerol at pH 6.66. A simpler method which is especially useful in newborns and infants because it neither requires venipuncture nor anticoagulant has been described by Judkiewicz et al. [7]. This method, however, implies the immediate performance of the test after blood extraction. We suggest a modification of the latter which offers the possibility to collect the blood sample in a special reservoir and test it within the next 3 h.

200 µl of blood taken by fingerprick (or heel puncture in newborns and infants) are introduced in a special tube (Microtainer, Becton Dickinson) containing 0.80 mg of EDTA K and 10 µl of the sample mixed with the hemolysing solution of the ‘Pink test’ [6]. Our study was performed in duplicate, and compared with the standard ‘Pink test’ in 25 healthy donors and 9 patients previously diagnosed as having HS. The results obtained are presented in table 1. The ‘Pink test’ values from healthy controls ranged between 4.1 and 21.7% of hemolysis. Meanwhile, the HS values were always above 41.3% without overlap between both groups. These percentages of hemolysis do not differ significantly from those obtained by Vettore et al. [6] in their work though the threshold value of hemolysis for HS patients is higher than reported by Judkiewicz et al. [7]. Differences between the final hemolysis obtained by both methods for the blood of the same patient were statistically not significant. The correlation coefficient between both sets of results has been higher than 0.90 in control patients and slightly lower in HS patients (0.75) probably because the sample has not been big enough.

In this study we propose a variation of the original ‘Pink test’ described by Vettore et al. [6] which allows, as the one proposed by Judkiewicz [7], the collection of blood by fingerprick without the need of venipuncture but permitting the performance of the test within the first 3 h. This makes the method easier and simpler. In addition, we have confirmed the reproducibility of the ‘Pink test’ for the diagnosis of HS patients.

Table 1. Percent hemolysis of blood from healthy controls and hereditary spherocytosis patients in the original ‘Pink test’ and the proposed modification
### Controls vs. HS patients

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<thead>
<tr>
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<th>Original</th>
<th>Modified</th>
<th>Original</th>
<th>Modified</th>
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<tbody>
<tr>
<td><strong>Mean ± SD</strong></td>
<td>11.1 ± 4.8</td>
<td>11 ± 4.8</td>
<td>57.6 ± 16.7</td>
<td>59.5 ± 19.7</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>4.1–21.7</td>
<td>4.2–22.7</td>
<td>41.3–88</td>
<td>40–97</td>
</tr>
<tr>
<td><strong>r</strong></td>
<td>0.93</td>
<td>0.75</td>
<td></td>
<td></td>
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<tr>
<td><strong>n</strong></td>
<td>25</td>
<td>25</td>
<td>9</td>
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Correlation coefficient; n = number of patients; SD = standard deviation.

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**References**