Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency is a commonly occurring erythrocyte enzyme abnormality, estimated to affect hundreds of millions of people worldwide. The condition is frequently associated with neonatal hyperbilirubinemia [1, 2] that may be severe enough to cause kernicterus and death [3]. It is therefore imperative, in high-risk populations, to accurately and rapidly diagnose the condition early in the neonatal period, before the onset of jaundice, and before discharging the infant from the hospital. Many screening tests are available for this purpose; however, the efficacy of two readily available commercially produced kits [qualitative, visual fluorescence screening procedure in blood, procedure no. 202; and visual qualitative determination for G-6-PD deficiency in red blood cells (RBCs), procedure no. 400, both produced by Sigma Diagnostics, St. Louis, MO] has not been evaluated in the neonatal period. As levels of G-6-PD activity may frequently be higher in neonates than in adults [4], and as the screening tests are manufactured primarily according to adult standards, we felt it necessary to test their reliability against newborn standards.

The aim of this study was to compare the accuracy of these two tests in screening for G-6-PD deficiency in the first 48 h of life, to determine if any one was superior to the other.

The principle of all the tests involves the oxidation of glucose-6-phosphate to 6-phosphogluconate, and the concomitant reduction of NADP⁺ to NADPH. These reactions occur in the presence of G-6-PD, and the rate of NADPH formation is proportional to G-6-PD activity. For the quantitative testing, NADPH activity was determined in a narrow-width spectrophotometer (Ocean Scientific Co., Garden Grove, CA) and the G-6-PD activity calculated in relation to RBC count. Fluorescence of NADPH is the basis of the fluorescent screening test, for the detection of which a long-wave ultraviolet light in a viewing box was used, with a General Electric no. F15T8-BL, 15-W lamp, which emits light between 320 and 420 nm. The color reduction test involves reduction of a blue dye, dichlorophenol indophenol, to a colorless state by NADPH.

At the Shaare Zedek Medical Center, newborns at high risk for G-6-PD deficiency, by virtue of their Sephardic Jewish mothers’ families’ origin in Asia Minor [5, 6], are routinely screened for the condition. The following study was performed as part of an assessment of the feasibility of routine screening of newborns with a quantitative commercially available kit (quantitative ultraviolet kinetic determination in blood at 340 nm, procedure no. 345-UV, Sigma Diagnostics), and was approved by the Shaare Zedek Medical Center’s Review Board. In our laboratory, we recently defined the normal value (mean ± SD) in low-risk (Ashkenazi Jews) healthy newborn males for the above-mentioned Sigma quantitative test as 504.8 ± 101.5 (range 361–905) U/10¹² RBC [4]. In the same study, G-6-PD-deficient male neonates were found to have enzyme activity values of 24.5 ± 33.7 (0–110) U/10¹² RBC. Quantitative G-6-PD enzyme activity was determined on term, healthy male newborns born to high-risk mothers, by capillary blood sample, within 48 h of delivery. In those babies in whom sufficient blood was available after the quantitative testing, screening by the above-mentioned commercial kits was performed. Blood was collected into EDTA-containing microtainers and stored in the refrigerator, and all testing was completed within 1 week of collection. Before performing the G-6-PD assay, the RBC count was performed by the Coulter method (Coulter T-890; Coulter Electronics, Luton, UK). For quality control, we assayed a G-6-PD normal control and a deficient control (Sigma Diagnostics, cat. nos. 5888 and G6888, respectively) before running each batch. The test was validated by testing healthy volunteer Ashkenazi Jewish adults at very low risk for G-6-PD deficiency. All these results fell within the manufacturer’s expected adult range (146–376 U/10¹² RBC).

The efficacy of the screening tests was evaluated [7] by determining the number of true positives (both screening and definitive method in the abnormal range), true negatives (both screening and definitive tests in the normal range), false positives (abnormal screening tests, normal definitive test), and false negatives (normal screening test, abnormal definitive test). To compare the accuracy of each of the screening tests in its ability to reflect the true G-6-PD status, the following were calculated: sensitivity, [a/(a+c)] × 100; specificity, [d/(b+d)] × 100, and positive predictive value [a/(a+b)] × 100, where a = true positives, b = false positives, c = false negatives, and d = true negatives.

Definitive quantitative G-6-PD determination, along with results of the two screening tests, were available for a total of 141 neonates. Of these, 32 had enzyme activity [36.4 ± 31.4 (0–108) U/10¹² RBC] that clearly fell in the range previously defined as G-6-PD deficient [4], while 84 had values [481 ± 109 (363–1017) U/10¹² RBC] above the minimum previously defined cutoff point for being G-6-PD normal. A further 25 neonates had values in the intermediate range [300 ± 55 (166–357) U/10¹² RBC]. In the absence of a definitive molecular genetic study, the designation of those neonates in the intermediate range was necessarily arbitrary. Luzzatto [8] has suggested classifying as deficient any individual having <30% of the normal activity, because above this level, he believes it unlikely that clinical manifestations will be encountered. As enzyme activity of all the neonates in the intermediate range was >30% of the lowest point of the normals, and
as there were no newborns with readings between the lowest of the intermediates (166 U/10^{12} RBC) and the highest of the G-6-PD deficient (108 U/10^{12} RBC), these intermediate neonates were included among the G-6-PD normal group.

In all 32 cases of G-6-PD deficiency, both screening tests always gave a “deficient” reading. Of those within the normal G-6-PD range, in the patient with the lowest quantitative enzyme activity (166 U/10^{12} RBC), both screening tests gave a false-positive result. In addition to this patient, one reading in each of the two screening tests gave a false-positive result in two separate patients. The enzyme activity value of that patient in whom the fluorometric test was inaccurate was 419 U/10^{12} RBC, and that of the patient in whom the colorimetric test was inaccurate was 526 U/10^{12} RBC. Analysis of the ability of each of the screening tests to accurately reflect the G-6-PD status is summarized in Table 1.

An ideal screening test should not give false-negative results, but can be allowed to give a few false-positive results [8]. Thus G-6-PD-deficient neonates should not be misclassified as normal, whereas it is permissible for a few G-6-PD-normal individuals to be designated deficient. The ability of both screening tests to accurately identify both the G-6-PD-deficient and normal neonates was excellent. Both tests correctly identified all those with G-6-PD deficiency, whereas the number of neonates incorrectly diagnosed as G-6-PD deficient was minimal. The tests were equally accurate, and none displayed any advantage over the other regarding its ability to diagnose or refute G-6-PD deficiency. However, an abnormal screening test should be confirmed with a definitive, quantitative test. In this study, we assessed only healthy male newborns, since neither the quantitative test nor the screening tests can accurately assess the female heterozygote state.

Recent reports of kernicterus due to G-6-PD deficiency emanate not only from developing countries [3] but also from the US [9]. While the incidence of G-6-PD deficiency is naturally high in the Middle East and Asia, in the US, communities with a high proportion of Southeast Asian immigrant families can also be expected to have a high frequency of G-6-PD deficiency [10]. Thus, practicing pediatricians and neonatologists worldwide should maintain a high state of awareness of this condition. Access to a reliable and rapidly performable screening test is imperative to identify neonates at risk and institute appropriate therapy.

The International Committee for Standardization in Haematology has recommended the fluorescent spot test as a screening test for G-6-PD deficiency [11]. However, some recently published studies of G-6-PD deficiency in neonates [3, 12] have utilized the commercial color reduction kit. The latter test is cheaper, easier to perform, less time consuming, and requires less sophisticated equipment (ultraviolet lamp) than the fluorescent spot test. Thus it may be more appropriate for mass use in developing countries than the more complicated fluorescent spot test. It can be set up and run by unskilled workers in a doctor’s office or neighborhood clinic. On the other hand, the quantitative method is not suitable for mass screening, because of its high cost, special equipment required, and the high level of technician training necessary. In light of the equal accuracy we determined for both screening methods in neonates, these additional factors should be taken into account before introducing any individual test.

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References


Table 1. Calculated parameters assessing accuracy of the two screening tests in the identification of G-6-PD status.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Color reduction test</th>
<th>Fluorescent spot test</th>
</tr>
</thead>
<tbody>
<tr>
<td>True positive</td>
<td>32/32</td>
<td>32/32</td>
</tr>
<tr>
<td>True negative</td>
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<td>107/109</td>
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<td>2/109</td>
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<tr>
<td>False negative</td>
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<tr>
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<td>98%</td>
</tr>
<tr>
<td>Positive predictive value</td>
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