Table 1. Different Procedures and Results Compared

<table>
<thead>
<tr>
<th>Standards or sera, μL</th>
<th>Qualitative one-step method</th>
<th>Qualitative two-step method</th>
<th>Modified one-step method</th>
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<td>45/25</td>
<td>primary: 90/25</td>
<td>second: 60/25</td>
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</tr>
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<td>300/15</td>
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<tr>
<td>1000</td>
<td>1000</td>
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</tr>
</tbody>
</table>

Results: absorbance of standards

5 int. units/L: 0.093
10: 0.020
50: 0.085
100: 0.095
200: 0.107

*With the qualitative procedure, only two standards are assayed: 10 and 50 int. units/L.

Only one serum gave an uncertain result (10.9 int. units/L) with the one-step method and a negative result (<1 int. unit/L) with the two-step method.

To eliminate the "hook effect" phenomenon, which can occur when sera contain high β-HCG concentrations, it is necessary to test the samples at two different dilutions. At present, we are studying this problem to specify what minimum concentrations of β-HCG induce this phenomenon.

Reference


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Artifactual Peroxidase Activity in Animal Tissues

To the Editor:

Several recent papers (e.g., 1–6) have dealt with determination of peroxidase (EC 1.11.1.7) activity in erythrocytes and some mammalian tissues by use of the guaiacol test (7). However, a rather peculiar behavior of this activity was reported, including its substantial stimulation after gamma-irradiation of erythrocytes with doses on the order of tens of kGy (3, 6). On the other hand, I was unable to detect so-defined peroxidase activity in fresh bovine hemolysates, although it appeared after several days of storage and increased progressively. This suggested that any peroxidase activity estimated in mammalian tissue extracts by use of the guaiacol test is artifactual.

Indeed, when a hemolysate of human erythrocytes was subjected to fractionation on DEAE-cellulose, peroxidase activity so estimated correlated with the hemoglobin content of fractions obtained. No activity was detected in hemoglobin-free supernates obtained from the hemolysates by the Tauchihaishi procedure (8). Therefore, hemoglobin seems responsible for the peroxidation of guaiacol by hemolysates and possibly by other tissue extracts that are contaminated with blood.

Peroxidative activity of (met)hemoglobin is well known. For example, methemoglobin and hematin can catalyze oxidation of uric acid by hydrogen peroxide (9). As in the case of uric acid peroxidation (9), peroxidation of guaiacol catalyzed by human hemolysates or purified hemoglobin was pH-dependent, the pH optimum being about 5. Apparent Kₘ values determined from Lineweaver–Burk plots were: 0.47 mmol/L for guaiacol, 0.67 mmol/L for H₂O₂ (at 21 ± 1 °C) with both hemolysates and hemoglobin. The guaiacol-peroxidizing activity of human hemoglobin increased upon irradiation of 50 g/L hemoglobin solutions with doses of 5 to 30 kGy of gamma radiation, although no clear-cut correlation between this activity and methemoglobin content of the preparations could be seen.

Apparently, the peroxidative activity of hemoglobin reflects some alterations of its structure upon molecular aging and some other types of damage. This may be an interesting problem per se and perhaps might be of biological importance under some conditions of oxidative stress. However, I conclude that determination of "peroxidative activity" with guaiacol in animal tissues gives information on the state of deterioration of the preparations examined rather than on anything else and should not be recommended. As the authors of the guaiacol test warned: "The main sources of error are caused by the use of this method with crude cell extracts" (7).

References


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