Labile plasma iron (LPI) assay

Method
The LPI assay is based on the measurement of the redox-active and readily chelatable fraction of serum NTBI. The assay measures iron-catalyzed radical generation in the presence of a low ascorbate concentration. Radical generation is measured with the fluorogenic redox-sensitive probe dihydrorhodamine (DHR) 123, and iron-catalyzed radical generation is calculated by subtracting the radical generation in the presence of 100 μmol/L of the iron chelator desferrioxamine (Figure 1).

\[ \text{Figure 1: LPI, the redox active and chelatable fraction of NTBI, is measured by exposing native serum to a physiological concentration of ascorbate in the presence of the fluorogenic DHR which will be converted from non-fluorescent (NF) to green fluorescent (F). This is followed in a plate reader and the fraction of the signal change inhibited by deferrioxamine (DFO) or another strong and specific iron chelator is attributed to LPI. Figure adapted from Aferrix Inc.} \]

Volume needed
150 μL serum

Lower limit of detection (mean + 3SD of a blank serum sample, n=28): 0.24 μM

Coefficient of variation
Intra-assay range
- At 0.38 μM: 19.4 %
- At 0.99 μM: 7.1 %
- At 1.77 μM: 9.1 %

Inter-assay range:
- At 0.42 μM: 26.4 %
- At 1.00 μM: 12.1 %

Reference values LPI assay\(^a\)

<table>
<thead>
<tr>
<th>LPI (μM)</th>
<th>N</th>
<th>Median</th>
<th>P2.5</th>
<th>P97.5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>33</td>
<td>&lt;0.24</td>
<td>&lt;0.24</td>
<td>1.00</td>
</tr>
</tbody>
</table>

\(^a\)Obtained from measurement of 33 samples of healthy volunteers (11 male, 22 female; mean age 34.7 years, range 18-61 years), unpublished.

Literature