Non-transferrin-bound iron (NTBI) assay in serum

**Method**

NTBI is measured by a nitrilotriacetic acid (NTA) ultrafiltration assay. In brief, heparinized plasma will be incubated with NTA to mobilize iron from NTBI to the Fe-NTA complex. Plasma proteins will removed by ultrafiltration and the Fe-NTA in the ultrafiltrate will be measured by a colorimetric assay (Figure 1).

![Diagram of NTBI assay](image)

**Figure 1:** Principle of the NTBI assay. NTBI is measured by extraction (1) from plasma with nitrilotriacetic acid (NTA), size filtration (2) and iron detection by colorimetric methods (3). Figure adapted from Cabantchik.

**Volume needed**

0.5 ml serum

**Lower limit of detection** (mean + 3SD of a blank serum sample, n=40): 0.47 µM

**Coefficient of variation**

- Intra-assay range:
  - At 1.13 µM: 18.8 %
  - At 1.99 µM: 12.0 %
  - At 3.04 µM: 15.4 %
  - At 6.13 µM: 4.3 %

- Inter-assay range:
  - At 1.18 µM: 26.5 %
  - At 2.06 µM: 16.9 %
  - At 2.65 µM: 13.5 %
  - At 3.65 µM: 13.6 %
  - At 6.24 µM: 7.5 %

**Reference Values NTBI**

<table>
<thead>
<tr>
<th>NTBI (µM)</th>
<th>N</th>
<th>Median</th>
<th>95% CI</th>
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<tbody>
<tr>
<td></td>
<td>33</td>
<td>&lt;0.47</td>
<td>&lt;0.47</td>
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</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from measurement of 33 samples of healthy volunteers (11 male, 22 female; mean age 34.7 years, range 18-61 years), unpublished.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**
