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

Method

NTBI is measured by a nitrilotriacetic acid (NTA) ultrafiltration assay [1]. 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).

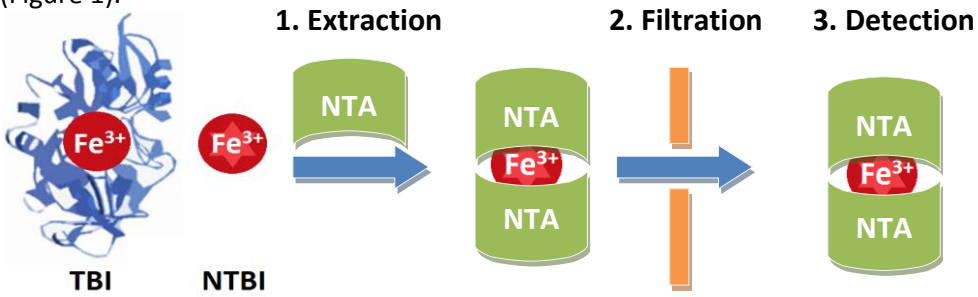

Figure 1: *Principle of the NTBI assay. NTBI is measured by extraction (1) from plasma with nitrilotriacetic acid (NTA), size filtration (2) and iron detection by colorimetric methods (3). Figure adapted from Cabantchik[2].*

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[a]

NTBI (µM)		95% CI	
N	Median	P2.5	P97.5
33	<0.47	<0.47	1.98

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from measurement of 33 samples of healthy volunteers (11 male, 22 female; mean age 34.7 years, range 18-61 years), unpublished.*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

1. Zhang D, Okada S, Kawabata T, Yasuda T. An improved simple colorimetric method for quantitation of non-transferrin-bound iron in serum. Biochem Mol Biol Int. 1995;35(3):635–641.

2. Cabantchik ZI. Labile iron in cells and body fluids: physiology, pathology, and pharmacology. Pharmacol. 2014; 13(5):45.