

## WCX-TOF MS reference values for serum Hepcidin-25

Reference ranges for serum hepcidin (nM) per 5-year age group for men and women in a reference population<sup>1</sup> as measured by weak cation exchange time-of-flight mass spectrometry (WCX-TOF MS)<sup>2,3,4</sup>.

The reference levels for the WCX-TOF MS method are recalculated from those of our ELISA method<sup>1</sup>, based on the regression line:  $(\text{ELISA} - 1.00)/1.52 = \text{WCX-TOF MS}$  that was derived from the results obtained by both methods for the same samples without hepcidin isoforms<sup>3</sup>. Results for heparine plasma do not differ from those for serum.

Age, years	Men (n=1066)					Women (n=882)*, #				
	N	(%)	Median	95% reference range		N	(%)	Median	95% reference range	
				P2.5	P97.5				P2.5	P97.5
18-24	10	(1)	5.3	0.8	11.0	21	(2)	1.1	< 0.5	6.3
25-29	16	(2)	4.9	< 0.5	15.3	28	(3)	1.4	< 0.5	6.6
30-34	18	(2)	4.2	< 0.5	15.8	24	(3)	1.9	< 0.5	13.1
35-39	22	(2)	3.6	< 0.5	12.1	36	(4)	1.5	< 0.5	9.9
40-44	19	(2)	6.1	< 0.5	11.0	65	(7)	2.5	< 0.5	15.2
45-49	76	(7)	3.4	< 0.5	13.2	110	(12)	1.6	< 0.5	8.9
50-54	106	(10)	4.0	< 0.5	13.8	140	(16)	2.9	< 0.5	14.3
55-59	173	(16)	4.4	< 0.5	15.7	129	(15)	4.9	< 0.5	13.6
60-64	179	(17)	4.5	< 0.5	14.3	137	(16)	4.7	< 0.5	17.3
65-69	186	(17)	5.3	< 0.5	13.9	95	(11)	4.9	< 0.5	14.2
70-74	133	(12)	4.9	< 0.5	17.0	62	(7)	5.1	< 0.5	24.2
75-79	99	(9)	3.8	< 0.5	16.1	16	(2)	5.4	0.8	18.4
80-84	22	(2)	3.8	1.6	12.6	10	(1)	7.2	< 0.5	12.0
≥85	7	(1)	6.8	1.6	12.8	9	(1)	3.7	< 0.5	15.4
<b>All</b>	<b>1066</b>	<b>(100)</b>	<b>4.5</b>	<b>&lt; 0.5</b>	<b>14.7</b>	<b>882</b>	<b>(100)</b>	<b>3.6</b>	<b>&lt; 0.5</b>	<b>14.6</b>

\*Pre-menopausal women (age <55; n=424)

Median = **2.0 nM**

P2.5 = 0.1 nM (<0.5 nM)

P97.5 = 12.3 nM

#Post-menopausal women (age ≥55; n=458)

Median = **4.9 nM**

P2.5 = 0.2 nM (<0.5 nM)

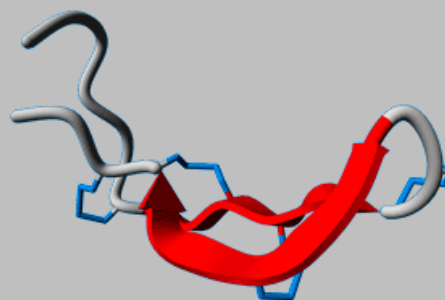
P97.5 = 15.6 nM

<sup>1</sup>Galesloot TE, Vermeulen SH, Geurts-Moespot AJ, Klaver SM, Kroot JJ, van Tienoven D, Wetzels JF, Kiemeny LA, Sweep FC, den Heijer M, Swinkels DW. Serum hepcidin: reference ranges and biochemical correlates in the general population. *Blood* 2011; **117**: e218-25

<sup>2</sup>Swinkels DW, Girelli D, Laarakkers C, Kroot J, Campostrini N, Kemna EH, Tjalsma H. Advances in quantitative hepcidin measurements by time-of-flight mass spectrometry. *PLoS ONE* 2008; **3**: e2706.

<sup>3</sup>Kroot JJ, Laarakkers CM, Geurts-Moespot AJ, Grebenchtchikov N, Pickkers P, van Ede AE, Peters HP, van Dongen-Lases E, Wetzels JF, Sweep FC, Tjalsma H, Swinkels DW. Immunochemical and mass-spectrometry-based serum hepcidin assays for iron metabolism disorders. *Clin Chem* 2010; **56**: 1570-1579.

<sup>4</sup>Kroot JJ, Tjalsma H, Fleming RE, Swinkels DW. Hepcidin in human iron disorders: diagnostic implications. *Clin Chem* 2011 **57**: 1650-1669.



## WCX-TOF MS reference values for [Hepcidin-25/Ferritin] and [TSAT/Hepcidin-25] ratios

Hepcidin values as given on page 1 should, like other hormones, be interpreted in the context of other indices of iron metabolism. For instance, in the absence of inflammation a low Hepcidin-25/ferritin ratio may be consistent with the presence of hereditary hemochromatosis or an iron loading anemia. On the other hand, a low transferrin saturation(TSAT)/Hepcidin-25 ratio may be consistent with Iron Refractory Iron Deficiency Anemia (IRIDA) due to a defect in the TMPRSS6 gene.

### WCX-TOF MS – [Hepcidin-25/ferritin] ratios

#### **Men (n=1064)**

Median = **26.7 pmol/μg**

P2.5 = 2.9 pmol/μg

P97.5 = 87.9 pmol/μg

#### **Pre-menopausal women (age <55; n=424)**

Median = **35.7 pmol/μg**

P2.5 = 3.0 pmol/μg

P97.5 = 167.3 pmol/μg

#### **Post-menopausal women (age ≥55; n=458)**

Median = **40.5 pmol/μg**

P2.5 = 9.1 pmol/μg

P97.5 = 143.1 pmol/μg

### WCX-TOF MS – [TSAT/Hepcidin-25] ratios

#### **Men (n=1059)**

Median = **7.3 %/nM**

P2.5 = 1.7 %/nM

P97.5 = 256.3 %/nM

#### **Pre-menopausal women (age <55; n=422)**

Median = **13.9 %/nM**

P2.5 = 2.0 %/nM

P97.5 = 330.0 %/nM

#### **Post-menopausal women (age ≥55; n=457)**

Median = **5.7 %/nM**

P2.5 = 1.5 %/nM

P97.5 = 73.4 %/nM