Design Verification

TIBC

CONTENTS

1 Introduction

The HUMAN TIBC kit has been designed for the determination of serum/plasma total iron binding capacity. The iron transport protein transferrin is treated with a fixed amount of iron(III) solution, sufficient to enable complete saturation. Unbound iron(III) is removed by adsorption onto aluminium oxide. After precipitation or centrifugation the transferrin bound iron is determined by a suitable iron test kit (HUMAN Iron liquicolor (CAB), HUMAN Iron TPTZ liquicolor HIT).

2 Imprecision

The imprecision (within-run and day-to-day) of the TIBC method was calculated from five respectively six determinations on five consecutive days. Commercially available control sera with low, medium and high TIBC content were employed as sample material. For the iron determination in the supernatant the HUMAN Iron TPTZ liquicolor HIT test kit (cat.-no.: 122917) has been employed. The test has been calibrated with the Autocal HIT calibrator (cat.-no.: 130017, lot: H010, 298 µg/dl iron).

Low TIBC

Day / No.	1	2	3	4	5	
1	211.5	204	225.9	216.3	233.7	
2	214.5	206.7	230.7	221.7	232.5	
3	213.9	205.8	227.7	220.5	233.1	
4	216.6	202.2	226.8	220.8	228.9	
5	211.2	209.4	223.8	216	230.7	
6	214.8	204.6	225	220.5	236.1	
	Within-run					**Day-to-day**
Mean. µg/dl	213.75	205.45	226.65	219.3	232.5	219.53
SD. µg/dl	2.068	2.477	2.406	2.481	2.488	10.614
CV. %	0.97	1.21	1.06	1.13	1.07	4.83

Medium TIBC

Day / No.	1	2	3	4	5	
1	249.3	2442	255.3	258.3	271.5	
2	251.7	243.3	262.5	257.1	273.9	
3	254.1	241.5	263.1	252.9	270	
4	249.3	241.2	264.6	255.3	270	
5	254.4	245.7	261.9	251.4	267.9	
6	254.1		266.4	255.9	271.2	
	Within-run					**Day-to-day**
Mean. µg/dl	252.15	243.18	262.3	255.15	270.75	257.17
SD. µg/dl	2.413	1.881	3.793	2.586	1.997	10.424
CV. %	0.96	0.77	1.45	1.01	0.74	4.05

High TIBC

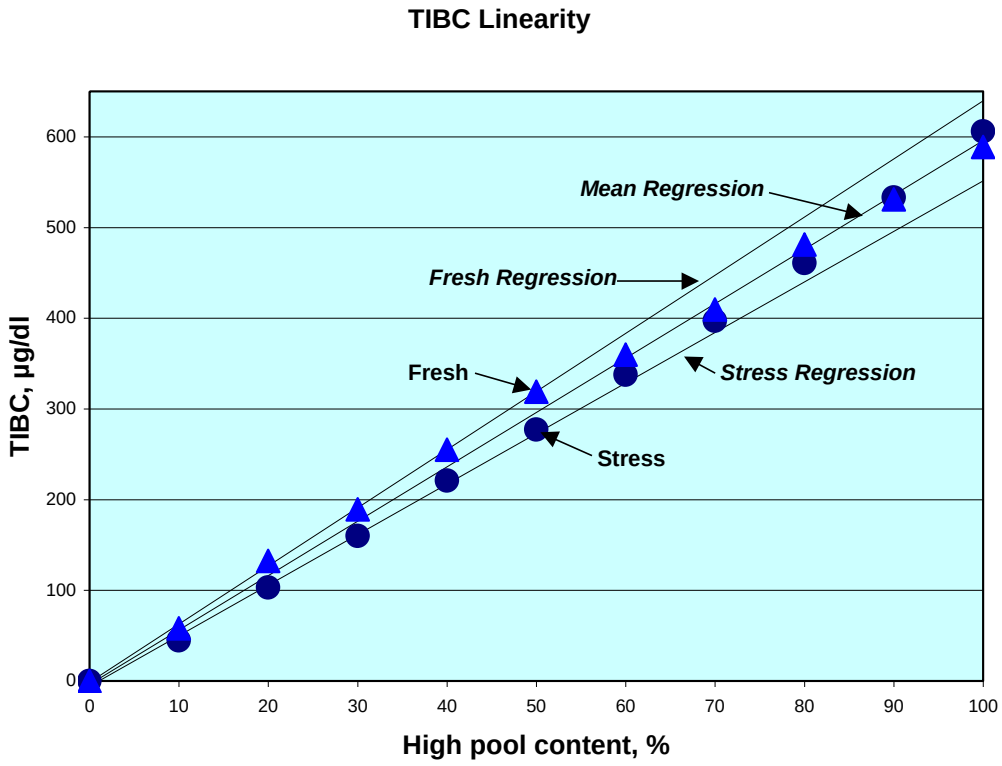
Day / No.	1	2	3	4	5	
1	374.1	351.3	360.1	357.3	351.9	
2	360.1	345.1	355.2	358.5	353.7	
3	366.9	349.2	357.6	358.5	355.5	
4	369.3	352.2	358.2	359.7	350.7	
5	374.1	354.3	362.1	357.3	354	
6		359.7	357.1	356.4	354.6	
	Within-run					**Day-to-day**
Mean. µg/dl	368.9	351.97	358.38	357.95	353.4	357.75
SD. µg/dl	5.824	4.910	2.416	1.176	1.780	6.642
CV. %	1.58	1.40	0.67	0.33	0.50	1.86

3 Linearity and Detection Limit

Linearity

The linearity of the TIBC method was controlled by employing selected high TIBC sera which have been gradually diluted with physiological saline. Recovered TIBC values have been compared against the regressed values. Regression equation was obtained from the first five dilutions (bold). Linearity behaviour of freshly produced reagents and stressed reagents was compared.

	TIBC				**Deviation (measured vs. regressed)**					
High pool. %	**Measured. µg/dl**		**Regressed. µg/dl**		**µg/dl**			**%**		
	fresh	stress	fresh	stress	fresh	stress	mean	fresh	stress	mean
0	0	0	-1.33	-5.67	1.33	5.67	3.52	n/a	n/a	n/a
10	57.9	44.7	62.77	50.04	-4.87	-5.34	-5.10	-7.76	-10.67	-9.04
20	132.3	103.2	126.87	105.75	5.43	-2.55	1.43	4.28	-2.41	1.23
30	189.3	159.9	190.98	161.46	-1.68	-1.56	-1.65	-0.88	-0.97	-0.94
40	255	220.95	255.08	217.17	-0.08	3.78	1.80	-0.03	1.74	0.76
50	319.05	277.05	319.18	272.88	-0.13	4.17	1.95	-0.04	1.53	0.66
60	359.85	337.65	383.28	328.59	-23.43	9.06	-7.27	-6.11	2.76	-2.04
70	409.5	397.2	447.38	384.3	-37.88	12.9	-12.60	-8.47	3.36	-3.03
80	481.35	460.65	511.48	440.01	-30.13	20.64	-4.88	-5.89	4.69	-1.02
90	530.85	533.1	575.58	495.72	-44.73	37.38	-3.82	-7.77	7.54	-0.71
100	588.6	606	639.69	551.43	-51.09	54.57	1.57	-7.99	9.90	0.26



Conclusion: Based on a 10% deviation limit the TIBC method is linear up to at least 500 µg/dl. Fresh reagents tend to slightly underestimate high TIBC concentrations, while stressed reagents tend to slight overestimation. A mean regression of fresh and stressed reagents show excellent linearity up to 600 µg/dl.

Detection Limit

A 20-fold determination of a '0' sample (phys. saline) on a Hitachi 717 analyser revealed an absolute mean of 1.30µg/dl and a SD of 1.50µg/dl. From the three-fold standard deviation the detection limit can therefore be calculated on the base of mean +3 SD to 5.80µg/dl.

4 Traceability

The TIBC test requires a separate test for iron determination. For traceability refer to the corresponding design verification reports SU-FE and SU-FE717.

5 Recovery in Control Sera

Commercially available control sera have been employed. The control sera have been reconstituted/prepared according to the manufacturer´s instructions. Fivefold determinations of each control serum have been performed with the iron liquicolor test method. The means of the fivefold determinations have been calculated and compared with the target values.

			Fresh		Stress	
Control serum	**Lot**	**Target range). µg/dl**	**Recovery. µg/dl**	**Deviation. %**	**Recovery. µg/dl**	**Deviation. %**
SERODOS®	6868	289 (228 – 350)	282.5	-2.24	264.6	-8.44
Precinorm	155092	265 (202 – 328)	293.9	10.92	266.6	0.6
Precipath	199459	297 (225 – 369)	314.8	5.99	295.3	-0.57
Lyphocheck 1	14071	413 (326 – 500)	375.2	-9.16	373.8	-9.49
Lyphocheck 2	14072	252 (199 – 305)	211.3	-16.17	213.1	-15.45

Conclusion: Control sera are recovered within the specified ranges. Mean recoveries with fresh TIBC reagents (–2,13%) are slightly higher than those with stressed reagents
(-6,67%).

6 Comparison of Methods

The TIBC has been compared against a corresponding method on the market for long years. 54 patient and control sera have been employed in the comparison. The iron determinations were performed on a Hitachi 717 analyzer, using the HUMAN Iron TPTZ HIT kit. Results have been evaluated by a non-regression model according to Bablok & Passing. The correlation coefficient has been obtained from a linear regression. The equation obtained could be described as follows:

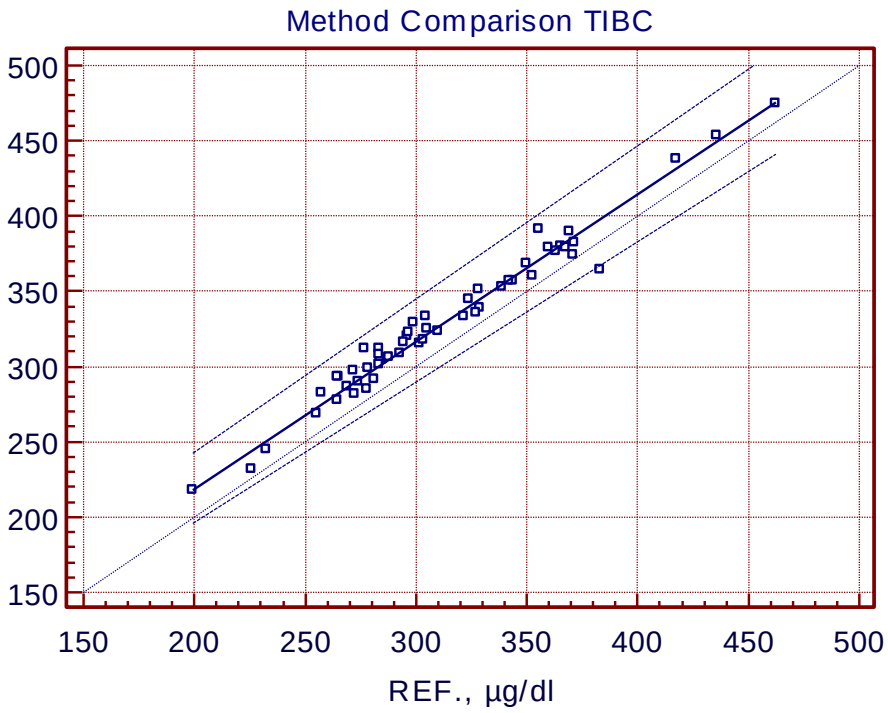
r =	**0.985**
Y =	**0.977 * X + 23.615**
X_{mean} =	**312.153**
Y_{mean} =	**329.265**

Both methods showed a good agreement and no significant deviation from linearity could be observed.

Ref.. µg/dl	**Test. µg/dl**
284.85	304.95
417.3	437.85
296.25	319.95
294.6	315.6
343.8	357
342	356.85
271.65	297
328.05	351.45
349.65	368.4
355.2	391.5
365.4	379.95
363.15	376.35
278.55	299.1
272.55	281.4
329.1	339
264.75	277.35
382.95	363.9
310.05	323.7

Ref.. µg/dl	**Test. µg/dl**
370.8	374.4
298.95	328.8
232.5	245.25
257.55	282.3
265.2	292.95
462.45	474.45
276.75	311.9
304.35	333.15
338.85	353.1
352.5	360.45
327.3	336
321.75	333
324.15	345
301.8	314.85
360	378.75
303.15	317.4
435.6	453
264.45	292.8

Ref.. µg/dl	**Test. µg/dl**
278.1	285.05
371.55	382.35
283.95	305.7
288.15	306.6
296.7	322.5
367.8	378.9
255.15	268.35
281.4	291.4
292.8	309
199.2	217.5
273.9	289.65
268.95	286.8
226.05	231.75
283.35	312.15
305.25	325.2
283.5	308.1
369.15	389.4
283.35	301.05



7 Stability

The stability of this test has been checked by temperature stress and real time stability studies. The recovery of control sera has been tested and compared against the date of QC release. Additional stability data are summarized in this report under section 3 (Linearity) and 4 (Control sera recovery).

Results Real Time and Stress

Package lot: 086; date of manufacture: Dec.2000; expiry date: 01.Jul.2002

Control serum	Target (range), µg/dl	QC release, µg/dl	Nov. 2001, µg/dl	Nov. 2001 after 10 days @ 56°C, µg/dl
Humatrol N, #017	139 (110 – 168)	128		
Humatrol P, #016	255 (201 – 309)	272		
Humatrol N, #019	236 (188 – 283)		235.1	251.3
Humatrol P, #017	299 (239 – 358)		310.2	313.1
Lyphocheck 1, #44651	199 (159 – 238)		206.3	209.6
Lyphocheck 2, #44652	265 (212 – 318)		272.8	271.6

Stress Results

Package lot	061	062	063	064
Date of manufacture	1998-04	1998-05	1998-06	1998-08
Expiry	1999-10	1999-11	1999-12	2000-02

Control serum	Target (range), µg/dl	QC release, µg/dl				Aug. 1998 after 10 days @ 56°C, µg/dl			
		061	062	063	064	061	062	063	064
Humatrol N, #017	139 (110 – 168)	125.5	136.2	142.2	123.7	130.5	131	135	131.7
Humatrol P, #015	213 (168 – 258)	209.2	201.7	223.9	233.1	215.9	229.8	227.1	234.3
Serodos, #6866	283 (224 – 342)	328.2	317.7	339.4		324.7	329.7	341.1	
Serodos Plus, #6792	279 (220 – 338)	272.7	280	270.2	255.5	269.2	270.9	277.9	262.2
Validate N, #6B401	269 (213 – 325)	276.1	253	270.7	253.5	293.3	253.5	274.4	263.4
Fe-Solution	400 – 600	430.4	439.2	427.9	432.5	432.4	438.2	414.9	422.9

Conclusion: Both results from real-time and additional temperature stress data confirm the stability claim of 18 months from the date of production. Latest real time investigations confirm a stability of 20 months from date of production (data on file at Human GmbH).