Design Verification

TIBC

Contents

1 Introduction

The HUMAN TIBC kit (REF 10670) 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), REF 10229, 10230, or HUMAN IRON TPTZ liquicolor, REF 122917, 12291, 12290).

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 (REF 122917) has been employed. The test has been calibrated with the Autocal HIT (REF 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

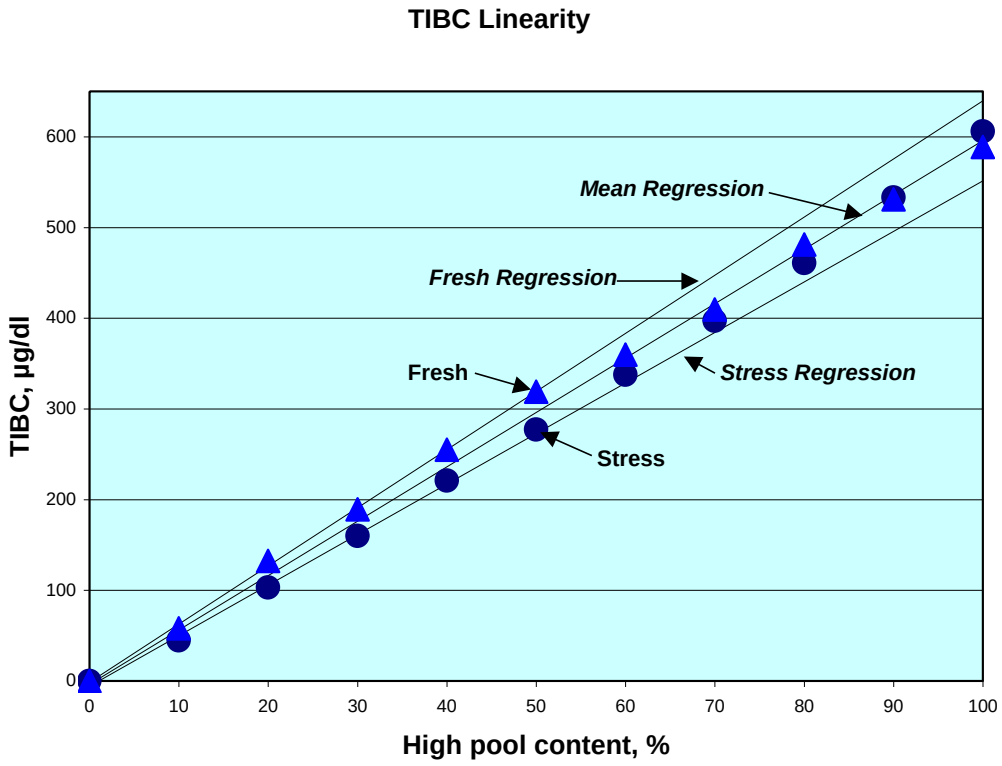
Day / No.	1	2	3	4	5	
1	374.1	351.3	360.1	357.3	351.9	
2	360.1	345.1	355.2	358.5	353.7	
3	366.9	349.2	357.6	358.5	355.5	
4	369.3	352.2	358.2	359.7	350.7	
5	374.1	354.3	362.1	357.3	354	
6		359.7	357.1	356.4	354.6	
	Within-run					Day-to-day
Mean. µg/dl	368.9	351.97	358.38	357.95	353.4	357.75
SD. µg/dl	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 (physiological saline) on a Hitachi 717 analyser revealed an absolute mean of 1.30 µg/dl and a SD of 1.50 µg/dl. From the three-fold standard deviation the detection limit can therefore be calculated on the base of mean +3 SD to 5.80 µg/dl.

4 Traceability

The TIBC test requires a separate test for iron determination. For traceability, refer to the corresponding design verification reports for IRON liquicolor (CAB), REF 10229, 10230, and for IRON TPTZ liquicolor, REF 122917, 12291, 12290.

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

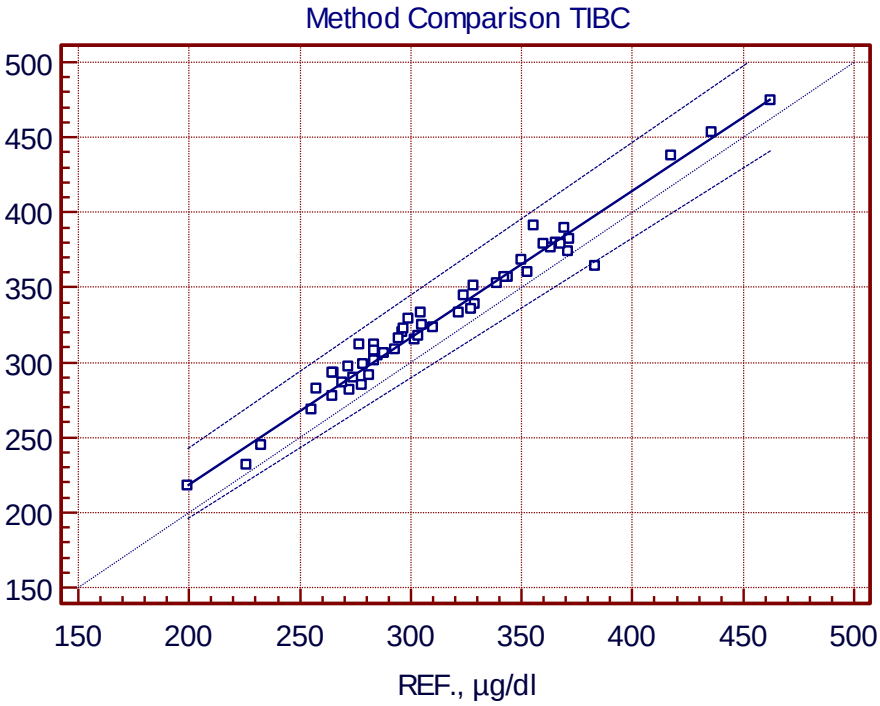
The TIBC has been compared against a corresponding method on the market for long years. 54 patient and control sera have been employed in the comparison. The iron determinations were performed on a Hitachi 717 analyzer, using the HUMAN IRON TPTZ liquicolor kit. Results have been evaluated by a non-regression 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.

Reference (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

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
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 Real Time Stability

Real time studies have been conducted for three independent production lots of this product. The reagents were stored at 15 - 25 °C. The real-time stability was tested up to 125 % of the shelf life at several intervals.

A number of HUMAN control sera have been employed. The control sera have been reconstituted/prepared according to the manufacturer'sinstructions. The mean values (n=2) s obtained with fresh (= reference) and 3 different reagent LOTs have been calculated and compared.

Criteria

Check	Acceptance criteria
Recovery	within range
Deviation result mean from fresh mean	≤ 10 %

Control recovery				TIBC, REF 10670, LOT 12006 after 25 months			
				Reference	Test		
Control serum	LOT	Target	Range	Fresh	Result	Dev.to Ref.	Recovery in range
		µg/dl	µg/dl	µg/dl	µg/dl	(%)	YES/NO
HumaTrol N	0002	246	197 - 295	227	228	0.4	YES
HumaTrol P	0003	570	456 - 684	453	484	7.0	YES
SERODOS	0002	279	223 - 334	250	259	3.5	YES
SERODOS plus	0002	414	331 - 496	345	375	8.7	YES
Mean						**4.9**	

Control recovery				TIBC, REF 10670, LOT 12005 after 26 months			
				Reference	Test		
Control serum	LOT	Target	Range	Fresh	Result	Dev.to Ref.	Recovery in range
		µg/dl	µg/dl	µg/dl	µg/dl	(%)	YES/NO
HumaTrol N	0002	246	197 - 295	227	251	10.4	YES
HumaTrol P P	0003	570	456 - 684	453	493	8.9	YES
SERODOS	0002	279	223 - 334	250	268	7.0	YES
SERODOS plus	0002	414	331 - 496	345	384	11.1	YES
Mean						**9.3**	

Control recovery				TIBC, REF 10670, LOT 12004 after 27 months			
				Reference	Test		
Control serum	LOT	Target	Range	Fresh	Result	Dev.to Ref.	Recovery in range
		µg/dl	µg/dl	µg/dl	µg/dl	(%)	YES/NO
HumaTrol N	0002	246	197 - 295	227	232	2.3	YES
HumaTrol P P	0003	570	456 - 684	453	479	5.8	YES
SERODOS	0002	279	223 - 334	250	261	4.6	YES
SERODOS plus	0002	414	331 - 496	345	371	7.5	YES
Mean						**5.0**	

Conclusion:

All results support the claimed stability of 20 months from production for the test kit.