Design Verification

IRON liquicolor

CONTENTS

1 Imprecision

The imprecision (within-run and day-to-day) of the IRON liquicolor method was calculated from six determinations per run on ten days. Precisison level low, medium and high control sera were employed as sample material.
Within run imprecision

Hitachi 717

Analyte concentration (µg/dl)	Intra-assay		Inter-assay	
	Standard Deviation	%CV	Standard Deviation	%CV
103	1.69	1.64	2.91	2.82
253	2.25	0.89	5.41	2.14
394	2.77	0.70	5.56	1.41

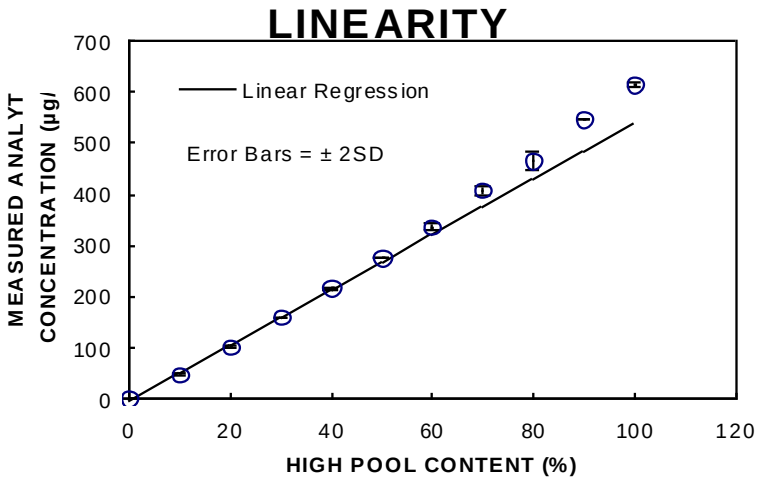
2 Linearity and Detection Limit

Linearity

The linearity of the IRON liquicolor method was controlled by employing a high concentration pool serum and dilutions with phys. saline. The analysed concentrations were calculated vs. the regression line. Deviation from the regression line are expressed in absolute and relative values.

Fresh reagent

High Pool Content (%)	Analytical Data µg/dl	Regressed Data µg/dl	Deviation from Regression Line	
			µg/dl	(%)
0	0.00	-3.46	3.46	-100.0
10	48.30	50.7	-2.41	-4.76
20	101.7	104.9	-3.14	-2.99
30	158.7	159.1	-0.31	-0.20
40	215.6	213.2	2.41	1.13
50	276.4	267.4	8.99	3.36
60	335.8	321.6	14.3	4.43
70	407.6	375.8	31.9	8.49
80	466.1	429.9	36.2	8.42
90	547.8	484.1	63.7	13.1
100	614.4	538.3	76.2	14.1



Conclusion: IRON liquicolor showed a good linearity >500 µg/dl iron measured with Hitachi 717.

Detection Limit

A 20-fold determination of a '0' sample (phys. saline) on a HumaStar 100 analyser revealed an absolute mean of 2.14 µg/dL and a SD of 2.77 µg/dL. From the three-fold standard deviation the detection limit can therefore be calculated on the base of mean +3 SD to 10.45 µg/dL.

3 Recovery in Control Sera

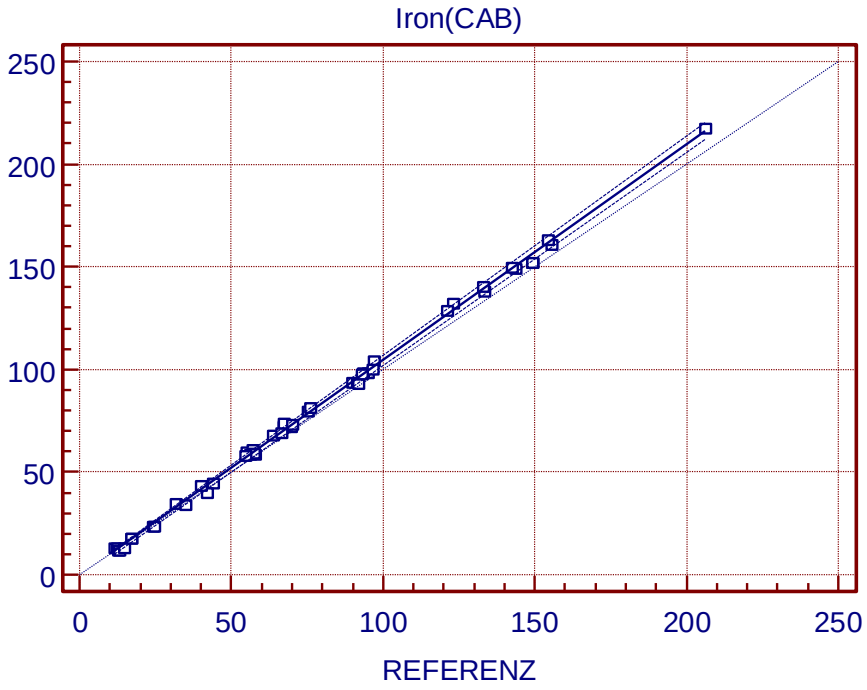
Commercially available control sera have been employed. The control sera have been reconstituted/prepared according to the manufacturer's instructions. Five-fold determinations of each control serum have been performed with the IRON liquicolor method. The means of the fivefold determinations have been calculated and compared with the target values.

CONTROL SERUM RECOVERY				HUMAN fresh # H034		HUMAN #H019 25 months		HUMAN # H020 26 months		HUMAN # H027 27 months	
Control serum	LOT	Target µg/dl	Range µg/dl	Result µg/dl	Deviation (%)	Result µg/dl	Deviation (%)	Result µg/dl	Deviation (%)	Result µg/dl	Deviation (%)
HUMATROL N	# 020	144	124 – 164	157	9.03	147	2.08	148	2.78	145	0.69
SERODOS	# 6868	115	91 – 139	108	-6.09	109	-5.22	106	-7.83	116	0.87
SERO.Plus	# 6795	214	169 - 259	230	7.48	224	4.67	225	5.14	209	-2.34
Precinorm	# 164919	123	102 – 144	117	-4.88	116	-5.69	119	-3.25	120	-2.44
Precipath	# 163661	168	138 - 198	185	10.12	186	10.71	176	4.76	177	5.36
Summary		1050		1158		1163		1120		1107	

4 Comparison of Methods

The IRON liquicolor has been compared against a commercially available iron method. 51 patient samples and controls have been employed in the comparison.
The results have been evaluated by a main component analysis. The linear regression obtained could be described as follows:
IRON liquicolor (Y) = 0,939 * Reference IRON (X) – 3,383
Coefficient of correlation: r = 0,995
Both methods showed a good agreement and no significant deviation could be observed with any specific sample.
An evaluation of the method comparison acc. to the model of Passing&Bablok yielded the following results:

Variable X : REFERENZ
Variable Y : TEST
Sample size = 52
REFERENZ
Lowest value = 11.5050
Highest value = 206.1000
Arithmetic mean = 76.9530
Median = 67.4000
Std. dev. = 46.4912
Std.error mean = 6.4472
TEST
Lowest value = 11.6500
Highest value = 217.3000
Arithmetic mean = 80.1635
Median = 72.0750
Std. dev. = 48.7423
Std. error mean = 6.7593
REGRESSION EQUATION
Y = -0.6268 + 1.0510 X
Intercept A: -0.6268; 95% CI: -1.8550 to 0.1548
Slope B: 1.0510; 95% CI: 1.0373 to 1.0667
Cusum test for linearity: No significant deviation from linearity (P>0.10)



5 Traceability

The internal master standard, against which the kit working standards are calibrated, is traceable to the to the reference method atomic absorbance spectrometry (AAS).

6 Interference

Interferences by triglycerides, hemoglobin, bilirubin, copper and ascorbic acid have been studied by adding known amounts of the potentially interfering substance to a known sample. Recoveries have been analyzed according to the method of Glick et al. (Clin.Chem. 1986, 32 470-5).

Triglyceride interference			Hemoglobin interference			Copper interference			Bilirubin interference			Ascorbic acid interference		
Trig.. mg/dl	Result µg/dl	Dev. %	Hemo. mg/dl	Result µg/dl	Dev. %	Cu µg/dl	Result µg/dl	Dev. %	Bili. mg/dl	Result µg/dl	Dev. %	Ascorb mg/dl	Result µg/dl	Dev. %
0	183.05	100.0	0	210.1	100.0	0	220.2	100.0	0	215.8	100.0	0	223.35	100.0
250	184.45	100.8	50	229.8	109.4	100	220.4	100.1	4	216.3	100.2	2	217.25	97.3
500	183.35	100.2	100	242.7	115.5	200	220.9	100.3	8	216.1	100.1	4	216.10	96.7
750	183.35	100.2	150	256.1	121.9	300	221.5	100.6	12	218.7	101.3	6	214.10	95.9
1000	184.60	100.8	200	265.6	126.4	400	223.5	101.5	16	222.3	103.0	8	213.20	95.5
1250	182.10	99.5	250	274.5	130.6	500	222.8	101.2	20	222.7	103.2	10	211.10	94.5
1500	183.45	100.2	300	286.4	136.3	600	225.0	102.2	24	222.4	103.1	12	209.00	93.6
1750	182.30	99.6	350	296.5	141.1	700	225.6	102.5	28	224.7	104.1	14	209.00	93.6
2000	183.85	100.4	400	308.8	147.0	800	227.6	103.4	32	225.8	104.6	16	206.65	92.5
2250	184.00	100.5	450	319.2	151.9	900	229.5	104.2	36	227.3	105.3	18	206.20	92.3
2500	183.35	100.2	500	330.0	157.1	1000	227.0	103.1	40	228.7	106.0	20	205.10	91.8
Glick		**1**			**4**			**2**			**2**			**2**

Conclusion: Triglycerides, copper, bilirubin and ascorbic acid did not show any remarkable interference up to 2500 mg/dl, 1000 µg/dl, 40 mg/dl and 20 mg/dl, respectively. This is expressed by a Glick value of 1 by triglycerides and Glick values of 2 by copper, bilirubin and ascorbic acid. Hemoglobin showed a substantial interference from 100 mg/dl. The package information contributes to this information.

7 Stability

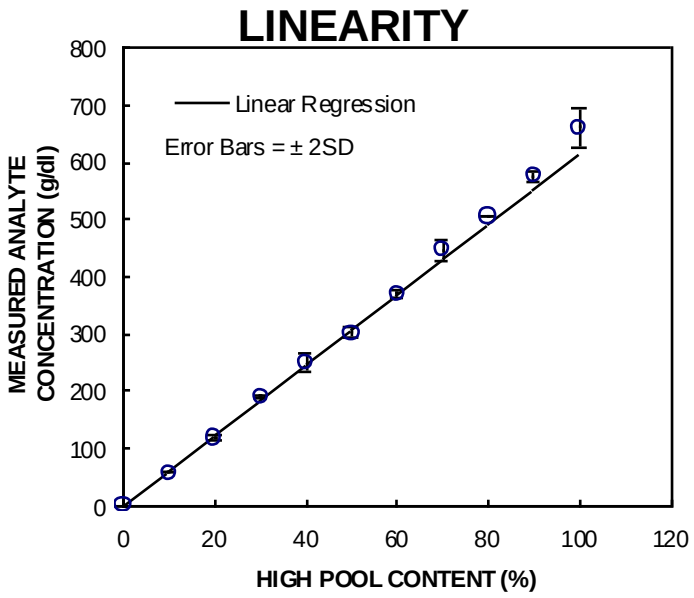
Real time studies have been conducted for three independent production lots of this product on a Hitachi 717.

Linearity

A high concentration pool serum was employed and dilutions with phys. saline were made. The analysed concentrations were calculated vs. regression line. Deviation from the regression line are expressed in absolute and relitive values.

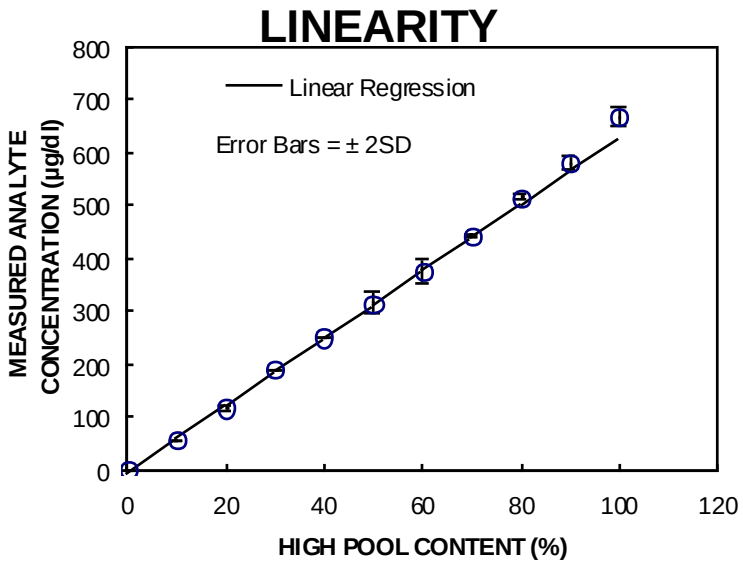
Age: 25 months, Lot H021

High Pool Content (%)	Analytical Data µg/dl	Regressed Data µg/dl	Deviation from Regression Line	
			µg/dl	(%)
0	0.00	-1.39	1.39	-100.0
10	56.7	60.2	-3.50	-5.81
20	118.0	121.8	-3.79	-3.11
30	191.0	183.4	7.62	4.16
40	248.1	245.0	3.18	1.30
50	301.6	306.5	-4.90	-1.60
60	369.9	368.1	1.76	0.48
70	446.0	429.7	16.2	3.79
80	504.8	491.3	13.5	2.74
90	576.4	552.9	23.5	4.25
100	660.5	614.5	46.0	7.49



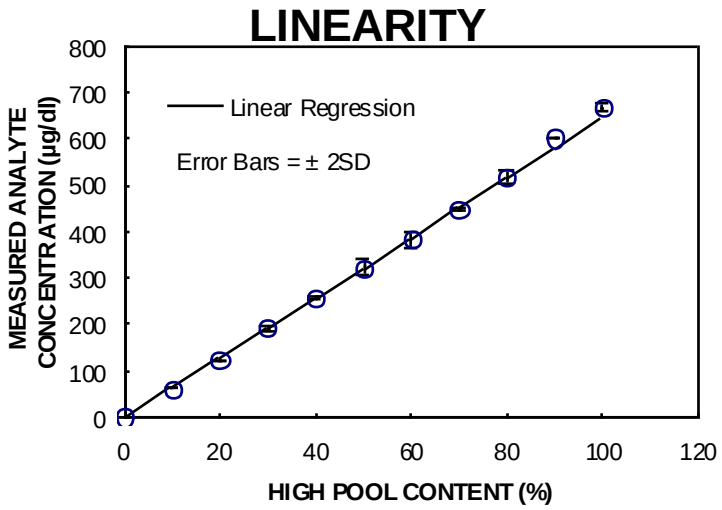
Age: 26 months, Lot H020

High Pool Content (%)	Analytical Data µg/dl	Regressed Data µg/dl	Deviation from Regression Line	
			µg/dl	(%)
0	0.00	-4.91	4.91	-100.0
10	55.7	58.7	-3.03	-5.16
20	115.7	122.4	-6.66	-5.45
30	189.7	186.0	3.75	2.02
40	249.5	249.6	-0.09	-0.03
50	314.5	313.3	1.28	0.41
60	376.7	376.9	-0.16	-0.04
70	442.4	440.5	1.91	0.43
80	515.5	504.2	11.4	2.26
90	580.6	567.8	12.8	2.26
100	667.1	631.4	35.7	5.65



Age: 27 months, Lot H019

High Pool Content (%)	Analytical Data µg/dl	Regressed Data µg/dl	Deviation from Regression Line	
			µg/dl	(%)
0	0.00	-1.11	1.11	-100.0
10	63.2	63.1	0.07	0.11
20	123.1	127.4	-4.21	-3.31
30	192.0	191.6	0.45	0.23
40	258.0	255.8	2.16	0.85
50	322.5	320.1	2.43	0.76
60	384.0	384.3	-0.26	-0.07
70	446.8	448.5	-1.75	-0.39
80	516.4	512.8	3.67	0.72
90	601.2	577.0	24.2	4.19
100	666.8	641.2	25.6	3.99



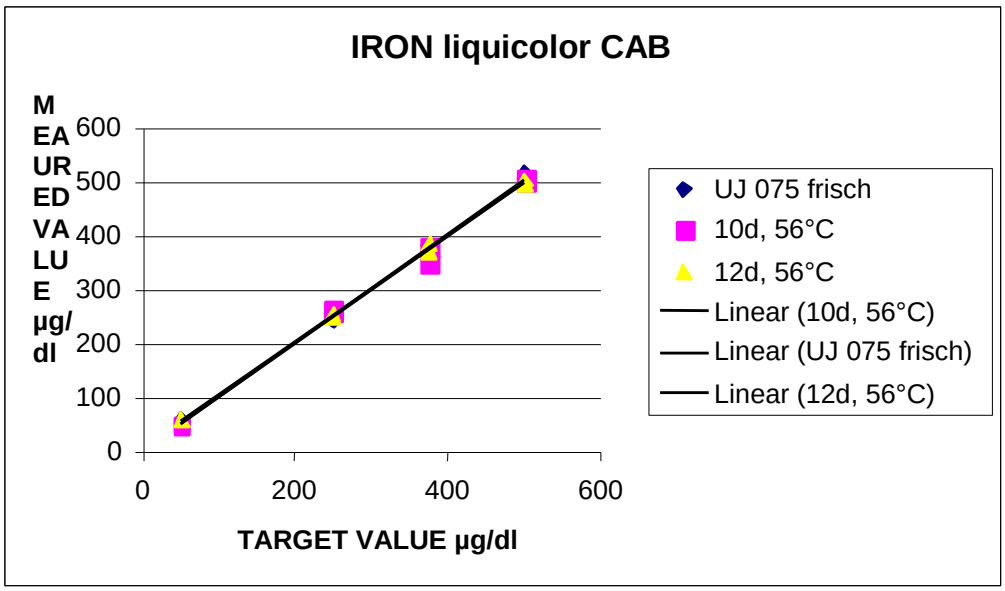
Recovery in Control Sera

See point 3.

Temperature Stress Results

In addition to the real-time storage, temperature stress tests have been applied on the IRON liquicolor test. Stressed reagents have been compared with fresh ones, as sample dilutions of a high stock solution have been employed. The manual pipetting regime has been applied.

% Dilution of high stock solution	Target value µg/dl	Fresh reagent. µg/dl	Stressed for 10 d @ 56°C. µg/dl	Stressed for 12 d @ 56°C. µg/dl
10	50	53.5	50.2	59.4
10	50	59.4	51.9	58.4
50	250	256.1	260.9	256.4
50	250	242.8	264.8	251.4
75	375	368.7	348.8	385.3
75	375	382.7	379.5	370.1
100	500	516.2	498.5	497.2
100	500	502.7	504.7	503.9



Conclusion: All results support the claimed stability of 24 months from production for the test kit.