Evaluation Report

Eurolyser iFOB (Faecal occult blood) Test Kit (ST0200)

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1. Limit of Detection and Limit of Quantitation

Lower Limit of Detection (LLD) is the minimum quantity of the analyte that can be discriminated from background noise with a stated probability (usually 95%).

Lower Limit of Quantification (LLQ) is defined as the minimum quantity of the analyte that the method can reliable detect. Depending on the defined goal for error, the LLQ could be equal to the LLD or higher, but never lower.
The lower limit of detection and the lower limit of quantification are determined by repeated measurements (n=20) of the ClNa 9 g/L solution, following the manufacturer's package insert T3100 and using the iFOB Control L-I ref: 3900010 from ST0200 Chemicals. This test is run in a BS-300 analyser from Mindray.

Results

Test nº	Result (µg/l)
1	9.6
2	11.8
3	0
4	0
5	12.3
6	0
7	0
8	0
9	0
10	0
11	0
12	15
13	0
14	0
15	0
16	0
17	0
18	0
19	0
20	0
Average	2.435
SD	5.07

The results are calculated according the next formulas:

Lower Limit of Detection (LLD) =
Blank value + 3.29 x Standard Deviation (SD)= **19.3 µg Hb/l**
 ≈ 1.55 µg Hb/g faeces

Lower Limit of Quantification (LLQ) =
Blank value +10 x Standard Deviation (SD) = **53.2 µg Hb/l**
 ≈ 4.28 µg Hb/g faeces

Using the collection tube, and according to the faecal mass sample / stick, the sensitivity of the method referred to **µg Hb / g faeces** is:

> **Cut off 50 µg/l: 4 µg Hb / g faeces**
> **Cut off 100 µg/l: 8 µg Hb / g faeces**
> **Cut off 150 µg/l: 12 µg Hb / g faeces**
> **Cut off 200 µg/l: 20 µg Hb/ g faeces**

2. Precision (repeatability, reproducibility)

Precision Evaluation analysis have been run on a Cobas Mira instrument and Kroma iT analysers, with two different lots and two different operators. 3 samples of an appropriate haemoglobin concentration levels (low. medium and high) covering the full range of ST0200ity were prepared using the extraction buffer. The samples were aliquoted and stored at –20ºC.

The samples were tested during 10 consecutive days, and 10 measurements were run each day, using i-FOB Turbidimetric kit and i-FOB Control L-I ref: 3900010 and L-II ref: 3900015 from ST0200 Chemicals.

The following table expresses the coefficient of variation (CV) results for each haemoglobin level:

COBAS MIRA	n	Mean (µg/l)	Mean (µg Hb/g faeces)	SD	CV (%)
Intra-day (repeatability)	10	129.6	10.4	7.1	**5.51**
		356.6	28.5	7.0	**1.9**
		739.4	59.1	10.9	**1.5**
Inter-day (reproducibility)	10	129.6	10.4	8.3	**7.6**
		356.6	28.5	17.2	**4.8**
		739.4	59.1	18.5	**2.6**

i-FOB Turbidimetric Reagent: Pilot Lot, Site: ST0200 Chemicals Lab, Operator: A

KROMA IT	n	Mean (µg/l)	Mean (µg Hb/g faeces)	SD	CV (%)
Intra-day (repeatability)	10	174	13.9	6.84	**3.98**
		349	27.9	11.14	**3.19**
		775	62	20.2	**2.61**
Inter-day (reproducibility)	10	174	13.9	7.9	**4.54**
		349	27.9	14.1	**3.98**
		775	62	32.1	**4.02**

i-FOB Turbidimetric Reagent: # 31499 / 5000, Site: ST0200 Chemicals Lab, Operator: B

3. Uncertainty of the measurement

It is expressed as the double of the inter-day precision (reproducibility) obtained for the nearest haemoglobin concentration to the lowest limit of reference range.

Uncertainty value for an Hb concentration of 129.6 µg/l (10.4 µg/g faeces) = 7.6 x 2 = **15.2 %**   (Cobas Mira)

Uncertainty value for an Hb concentration of 174 µg/l (13.9 µg/g faeces) = 4.54 x 2 = **9.08 %** (Kroma iT)

4.   Blank Reagent

Blank reagent at 650 nm is calculated with manual method using the spectrophotometer Thermo. Nicollet Evolution 300 with a cuvette of 1 cm light path.
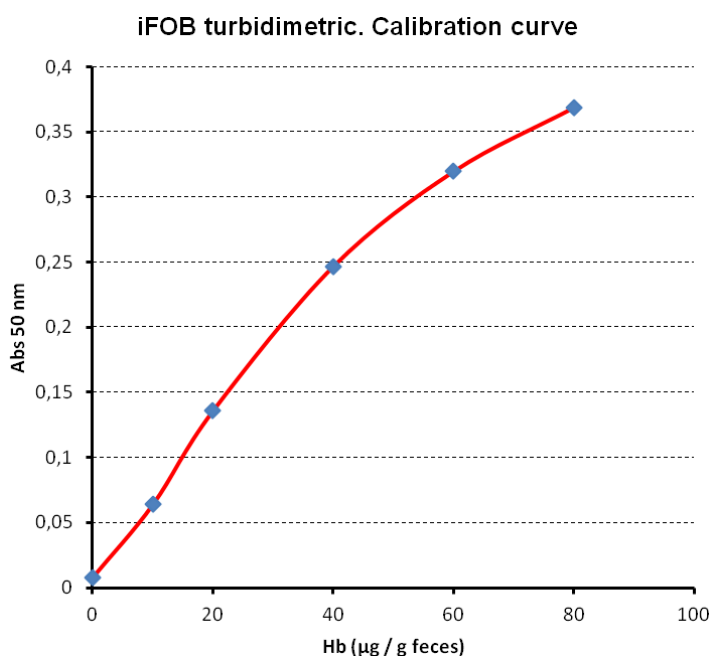
$$A_{650\ nm}: \textbf{< 0.6}$$

5.   Calibration curve

Several samples with different haemoglobin concentrations ranging from 0 to 80 µg/g faeces (1000 µg/l), were prepared by dilution of Internal iFOB calibrator in ClNa 9 g/L. Two replicates of the samples were tested and the absorbance results were plotted against the haemoglobin concentrations in a calibration curve.

MANUAL METHOD:

Hb (µg/l)	Hb (µg/g faeces)	A_{650} nm
0	0	0.008
125	10	0.064
250	20	0.136
500	40	0.247
750	60	0.320
1000	80	0.369

Spectrophotometer: Thermo Nicolet Evolution 300. Manual method

iFOB turbidimetric. Calibration curve

ANALYSER: BS-300 MINDRAY: Units (µg Hb/l)

Linear ChemicalsCurva calibración

Prueba:FOB_____   Método:Logistic 5P_____   Fecha calibrac:10/20/2008_____

Conc.	Respuest
0	111.72
125	340.56
250	566.69
500	932.64
1016	1343.43

Parám.	Valor
K	63627.166
R0	111.725
A	-11.002
B	1.133
C	-0.001

Operario: _____
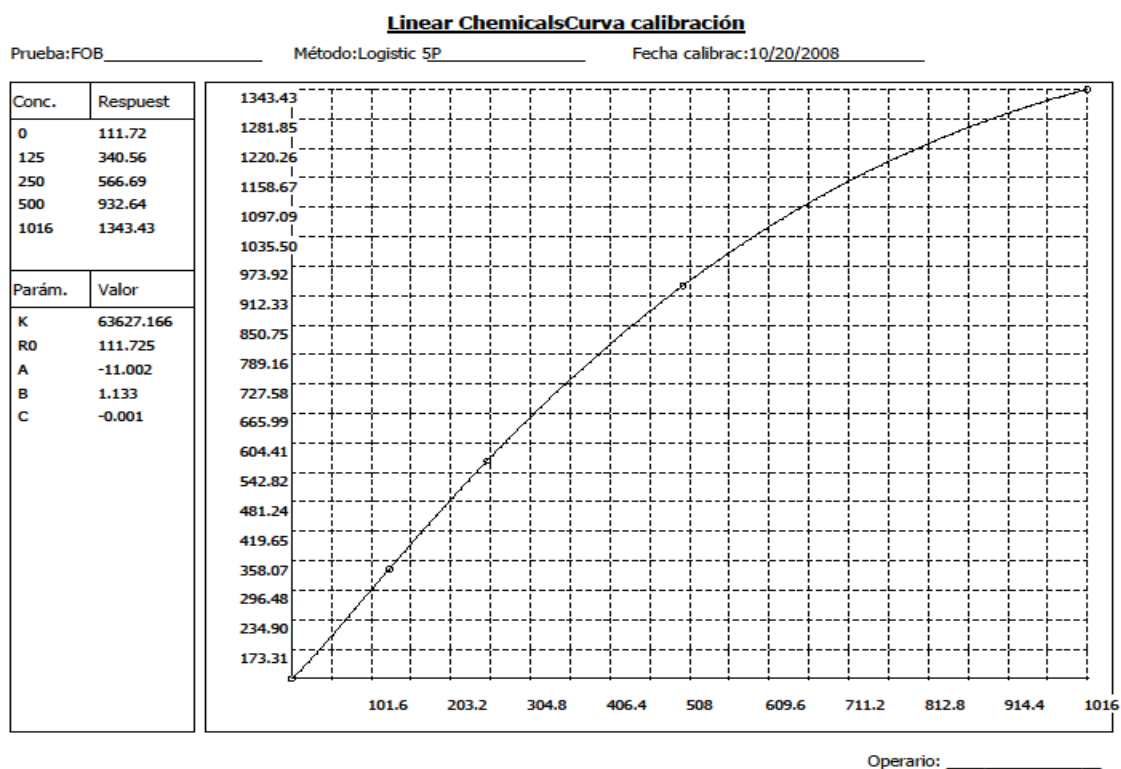
ANALYSER: KROMA IT: Units (µg Hb/l)

i-FOB Turbi

D.O.:	0.0008	0.0508	0.1002	0.1842	0.2701
Conc.:	0.0000	125.0000	250.0000	500.0000	1000.0000
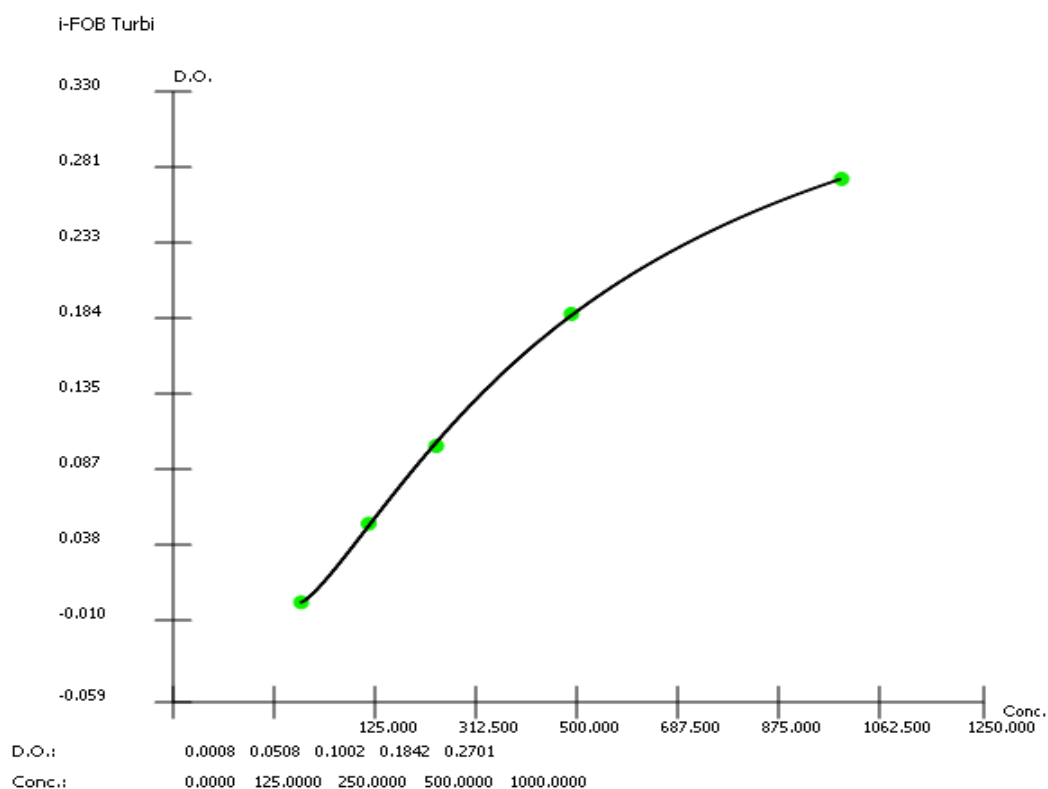
6.   Limit of Linearity

Linearity of the assay is performed running two replicates of several dilutions on extraction liquid of a sample with a haemoglobin concentration of 80 μg/g faeces (1000 μg/l), using the Internal iFOB Calibrator as a calibrator. Measured haemoglobin values are plotted against the expected haemoglobin concentration of the sample dilutions.

The ST0200 regression statistics when correlating the mean reported value to the expected value is the following:
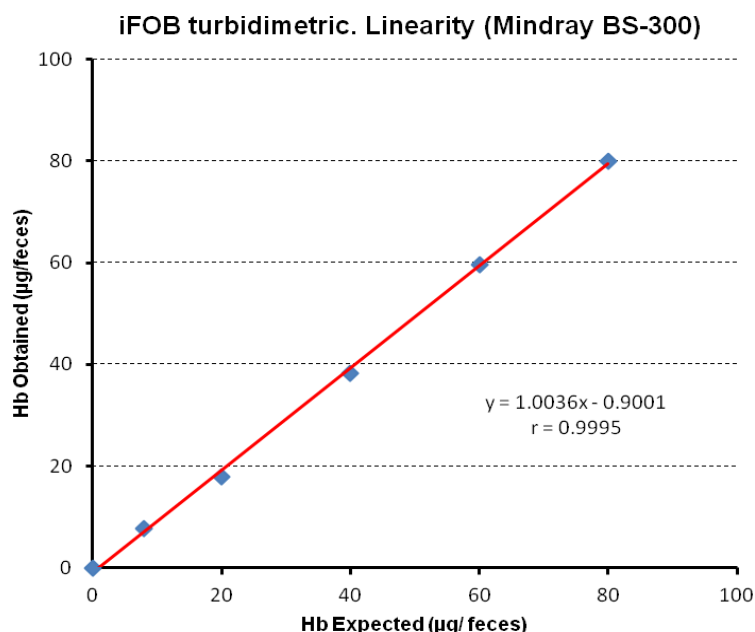
BS-300 MINDRAY

Expected values (μg/l)	Expected values (μg /g faeces)	Reported values (μg/g faeces)
0	0	0
100	8	7.7
250	20	17.9
500	40	38.2
750	60	59.5
1000	80	80

Instrument: BS-300 Mindray

Slope: 1.0
Inetercept: - 0.9
Correlation coefficient (r): > 0.99

iFOB turbidimetric. Linearity (Mindray BS-300)

$y = 1.0036x - 0.9001$
$r = 0.9995$

KROMA IT

Expected values (μg/l)	Expected values (μg /g faeces)	Reported values (μg/g faeces)
0	0	1.4
100	8	11.5
250	20	20.6
500	40	40.1
1000	80	76.7

Slope: 0.93
Inetercept: +2.52
Correlation coefficient (r): > 0.99

iFOB turbidimetry. Linearity (Kroma IT)

$y = 0.9299x + 2.5232$
$r = 0.999$
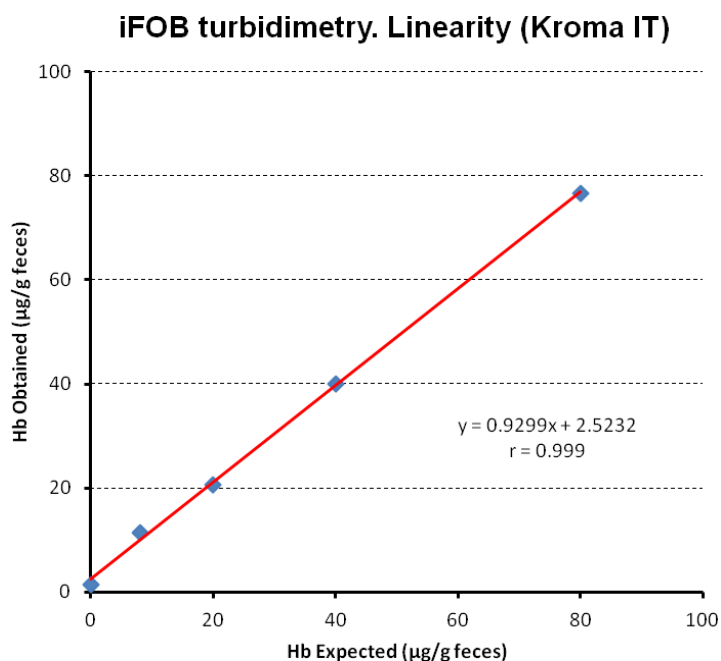
The upper limit of ST0200ity can be established as **1000 μg/l = 80 μg/g faeces**

7.   Measurement range

Measurement range is calculated as the range between the Lower Limit of Detection (LLD) and the Limit of Linearity (LL).

Measurement range: (**19.3 – 1000) µg/l or (1.55 - 80) µg/g faeces**

8.   Analytical sensitivity

5 replicates of two different dilutions of the iFOB Internal Calibrator, 20 and 40 µg/g faeces were tested in the same run in a spectrophotometer with a 1 cm light path cuvette with manual method.

The analytical sensitivity was determined as the quotient between the absorbance and the concentration.

Haemoglobin	$A_{650\ nm}$	Average $(A_{650\ nm})$	Sensitivity (mA / µg/l) (mA / µg/g faeces)
250 µg/l or 20 µg / g faeces	0.134 0.137 0.142 0.136 0.139	0.138	**0.550**
500 µg/l or 40 µg / g faeces	0.238 0.256 0.254 0.238 0.238	0.249	**0.490**

Instrument: Thermo Nicolet Evolution 300. Manual Method.

9.   Prozone effect

Prozone effect is caused when a sample with a high expected haemoglobin concentration gives a low result (absorbance) and a false haemoglobin concentration is measured, below the ST0200ity limit.

Prozone effect was studied by analyzing two replicates of serial dilutions of a high haemoglobin concentration sample in a stool solution, using an Internal iFOB Calibrator as a calibrator and the Thermo Nicollet Evolution 300 Instrument and BS-300 (Mindray). Prozone effect is calculated as an average of the first haemoglobin dilution that produces prozone effect and the next dilution (first dilution not affected by prozone effect).
The highest haemoglobin concentration that has been analyzed is 50000 µg/l or 4000 µg/g faeces.

MANUAL METHOD:

Hb (µg/l)	Hb (µg/g faeces)	Abs 650 nm
0	0	0
125	10	0.064
250	20	0.136
500	40	0.247
1000	80	0.369
1500	120	0.395
3000	240	0.513
6000	480	0.546
12000	960	0.422
25000	2000	0.468
50000	4000	0.431

Instrument: Thermo Nicolet Evolution 300
Method: Manual

iFOBturbidimetric. Prozone Effect (Manual method)
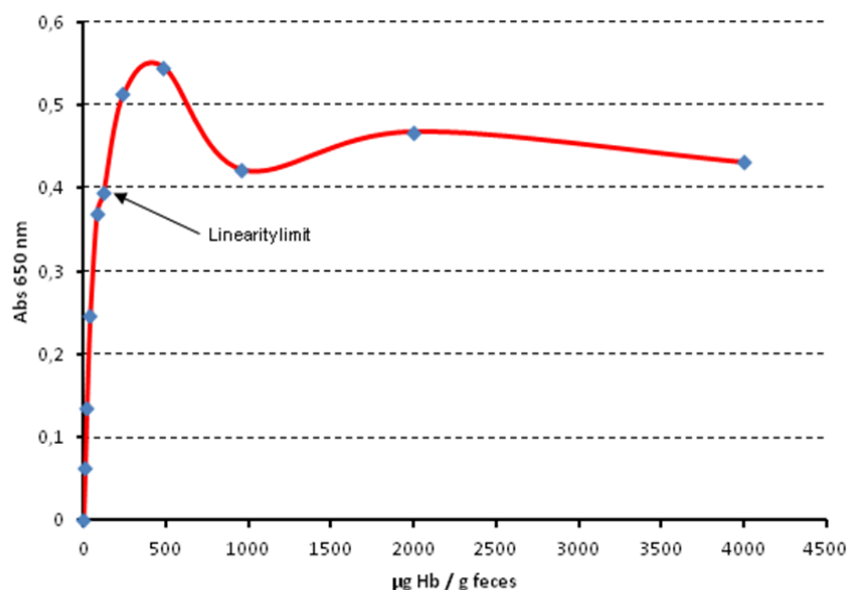
BS-300 MINDRAY:

Hb, Expected (µg/l)	Hb, Obtained (µg/l)
0	0
350	336
700	851
1400	1222
7000	1258
14000	1240
28000	1115
42000	965
56000	890
70000	942

Hb, Expected (µg / g faeces)	Hb, Obtained (µg / g faeces)
0	0
28.1	29.4
56.3	68.4
112	98.3
560	101
1125	100
2240	89.7
3360	77.6
4480	71.6
5600	75.7

iFOB turbidimetric. Prozone Effect (Mindray BS-300)
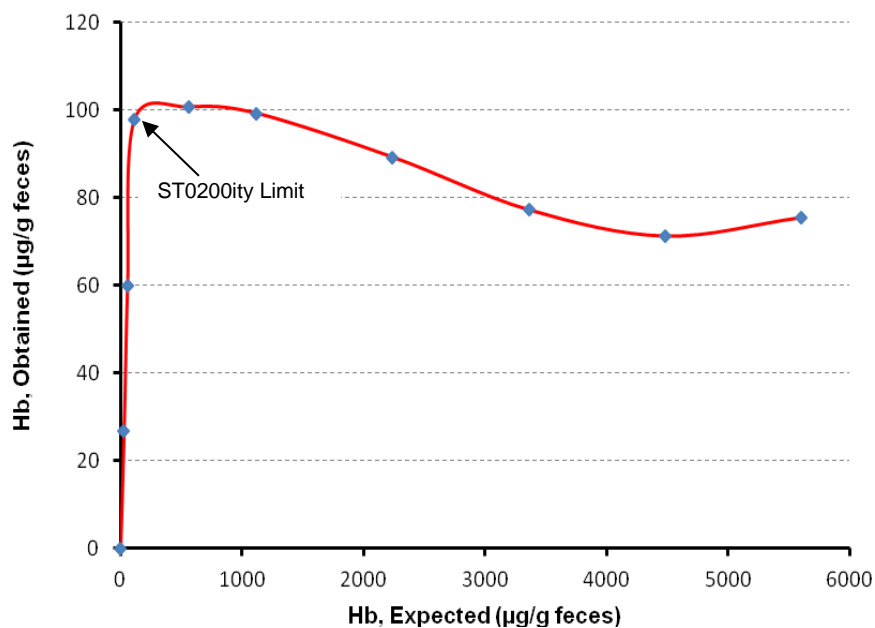
Hb Expected (µg/l)	Hb Obtained (µg/l)
0	24
44	47.3
58	66.3
96	115.5
192	229.7
384	384
3840	1000
38400	1200
384000	1000
3840000	600,6
38400000	632.7

Hb Expected (µg / g faeces)	Hb Obtained (µg / g faeces)
0	1.9
3.52	3.8
4.64	5.3
7.68	9.2
15.4	18.4
30.7	30.7
307.2	80
3072	96
30720	80
307200	48
3072000	50.6

iFOB turbidimetric. Prozone effect (Kroma IT)
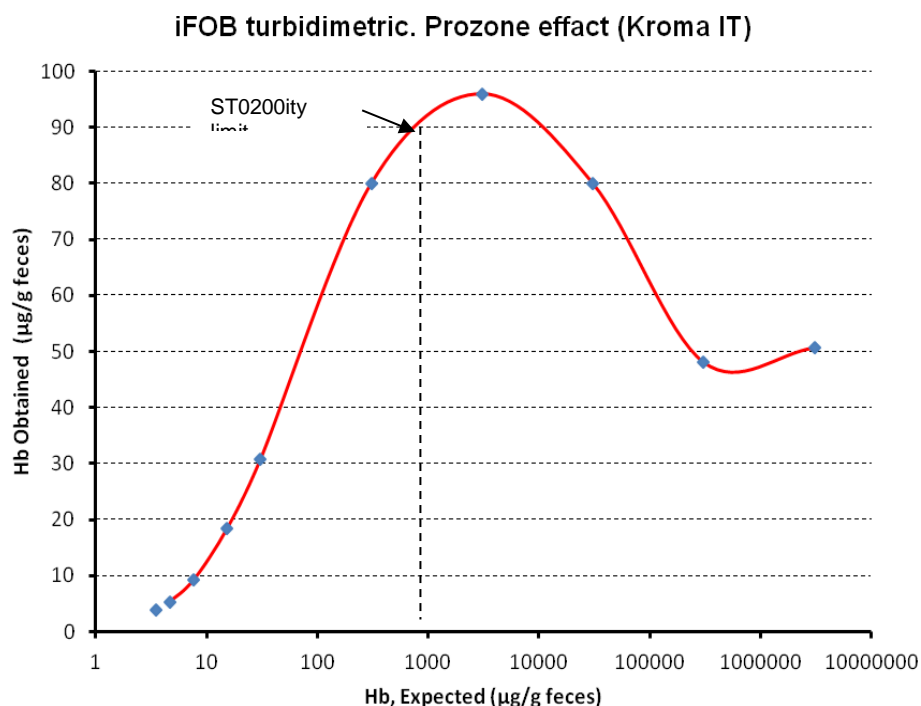
The prozone effect is up to **3072000 µg Hb (3072 mg Hb) /g faeces**

= 38400000 µg Hb (38400 mg Hb) / l

10. Accuracy

10.1. Reference Material

5 replicates of a commercial i-FOB Calibrator traceable to the International Reference Material CRM 522 were assayed in a Cobas Mira analyser, using an Internal iFOB Calibrator as a calibrator.

The haemoglobin average measured was 63.05 µg/g faeces (788 µg/l) and the value of the iFOB Calibrator is 62 µg/g faeces (775 µg/l)

Measurements	Hb (µg/l)	Hb (µg/g faeces)
1	793.4	63.5
2	783	62.6
3	754	60.3
4	800.2	64.0
5	807.2	64.6
Average	788	63.0
Accuracy (%)	**98.32**	

10.2. Comparison of different methods

The i-FOB Turbidimetric method from ST0200 has been compared with FOB Gold (Turbidimetric latex method) from Sentinel and OC Sensor/Diana from Eiken.

SENTIFOB /FOB Gold (SENTINEL) vs i-FOB TURBIDIMETRIC (ST0200)

70 samples from the Hospital Carlo Borromeo from Milan (Italy) that have been evaluated with the FOB-Gold kit on the Modular Instrument from Roche, have been also evaluated with the I-FOB turbidimetric kit used in ST0200.

Samples covering all the analytical range, and some of them with Hb concentrations > 80400 ng/g faeces (1000 µg/l) were analyzed in duplicate on the Hitachi 717 instrument.

Agreement was assessed by Linear Regression (Slope and Intercept) and Correlation Coefficient by Pearson.

COMPARISON ST0200 reagent vs SENTINEL

$y = 1.1618x - 4.3231$
$r = 0.98$
$n = 57$
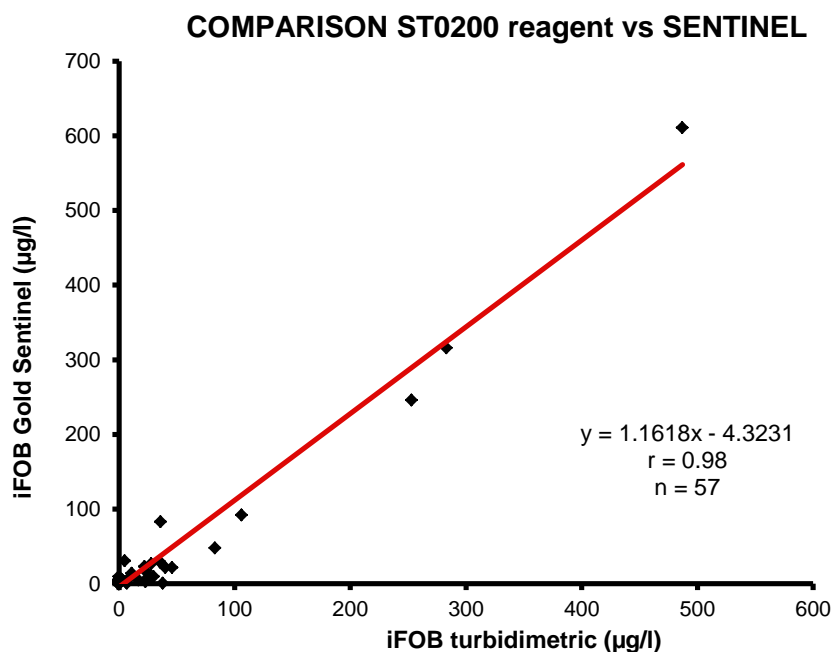
Note: samples > 1000 µg/l and outliers have been excluded

Results: Units (µg/l)

	Analysis on Hitachi 717		Sample ID	Sentinel X	ST0200 Y	Sample ID	Sentinel X	ST0200 Y
ST	[FOB]		1	0	2	36	0	1
ASSAY CODE	[2POINT]:[31]-[50]		2	0	3	37	893	1989
SAMPLE VOLUME	[13] [2]		3	0	1	38	487	611
R1 VOLUME	[200] [50] [NO]		4	22	23	39	40	22
R2 VOLUME	[50] [20] [NO]		5	27	7	40	46	22
WAVE LENGHT	[ ] [660]		6	818	1912	41	30	10
	[NONST0200] [4]		7	283	316	42	9	8
CALIB. METHOD	[6]		8	3	5	43	23	17
STD (1) CONC. -			9	0	3	44	23	3
POS.	[0] - [1]		10	776	23	45	0	0
STD (2) CONC. -			11	38	1	46	23	5
POS.	[63.5] - [2]		12	0	3	47	6	2
STD (3) CONC. -			13	0	0	48	253	246
POS.	[127] - [3]		14	0	0	49	83	48
STD (4) CONC. -			15	0	0	50	0	4
POS.	[254] - [4]		16	0	1	51	0	2
STD (5) CONC. -			17	0	2	52	0	0
POS.	[508] - [5]		18	0	2	53	0	4
STD (6) CONC. -			19	35	30	54	7	1
POS.	[1016] - [6]		20	36	83	55	17	5
SD LIMIT	[999.9]		21	182	646	56	0	1
DUPLICATE LIMIT	[1000]		22	28	27	57	1050	2018
SENSITIVITY LIMIT	[0]		23	0	5	58	1025	1981
ABS. LIMIT	[32000]		24	106	92	59	1009	1863
(INC/DEC)	[INCREASE]		25	11	14	60	1051	2132
PROZONE LIMIT	[-32000] [LOWER]		26	0	0	61	203	228
EXPECTED VALUE	[0] - [10000]		27	0	6	62	0	4
PANIC VALUE	[0] - [10000]		28	0	2	63	0	4
INSTRUMENT			29	5	31	64	837	1828
FACTOR	[1.00]		30	0	0	65	0	11
			31	0	10	66	201	182
			32	0	4	67	895	2006
			33	1012	2161	68	82	57
			34	0	1	69	951	1626
			35	0	0	70	19	134

Comments:

Samples 6, 33, 37, 57, 58, 59, 60, 64, 67, 69 had an Hb concentration over the ST0200ity range of ST0200 kit and Sentinel Kit. For instance, a sample of 2149 µg/l (968 µg/l for Sentinel) has been diluted up to 1:16 giving a result of 647 µg/l x 16 = 10352 µg/l. This would explain the big differences between these two methods regarding the samples with high level of Hb. These discrepancies are due to the different calculation between the two instruments (Modular and Hitachi). Our method gives results over the last calibration point (i.e. 1016 µg/l for the calibrator used), while Modular instrument does not. The Hospital has a flag on the Roche Modular. As they are not interested into the actual value, they reflect results as "positive" and "negative", and high samples are not post-diluted for investigation.

Samples 10, 21 and 70, give some discrepancies, but confronting them to the results of colonoscopy the results are negative.

When analyzing the results from the point of view of qualitative results, considering different "cut off" levels of Hb, the results are the following:

Sample ID	Cut-off					
	50 µg/l (4 µg / g faeces)		100 µg/l (8 µg/g faeces)		150 µg/l (12 µg/ g faeces)	
	Sentinel	ST0200	Sentinel	ST0200	Sentinel	ST0200
1	Negative	Negative	Negative	Negative	Negative	Negative
2	Negative	Negative	Negative	Negative	Negative	Negative
3	Negative	Negative	Negative	Negative	Negative	Negative
4	Negative	Negative	Negative	Negative	Negative	Negative
5	Negative	Negative	Negative	Negative	Negative	Negative
6	Positive	Positive	Positive	Positive	Positive	Positive
7	Positive	Positive	Positive	Positive	Positive	Positive
8	Negative	Negative	Negative	Negative	Negative	Negative
9	Negative	Negative	Negative	Negative	Negative	Negative
10	Positive	Negative	Positive	Negative	Positive	Negative
11	Negative	Negative	Negative	Negative	Negative	Negative
12	Negative	Negative	Negative	Negative	Negative	Negative
13	Negative	Negative	Negative	Negative	Negative	Negative
14	Negative	Negative	Negative	Negative	Negative	Negative
15	Negative	Negative	Negative	Negative	Negative	Negative
16	Negative	Negative	Negative	Negative	Negative	Negative
17	Negative	Negative	Negative	Negative	Negative	Negative
18	Negative	Negative	Negative	Negative	Negative	Negative
19	Negative	Negative	Negative	Negative	Negative	Negative
20	Negative	Positive	Negative	Negative	Negative	Negative
21	Positive	Positive	Positive	Positive	Positive	Positive
22	Negative	Negative	Negative	Negative	Negative	Negative
23	Negative	Negative	Negative	Negative	Negative	Negative
24	Positive	Positive	Positive	Negative	Negative	Negative
25	Negative	Negative	Negative	Negative	Negative	Negative
26	Negative	Negative	Negative	Negative	Negative	Negative
27	Negative	Negative	Negative	Negative	Negative	Negative
28	Negative	Negative	Negative	Negative	Negative	Negative
29	Negative	Negative	Negative	Negative	Negative	Negative
30	Negative	Negative	Negative	Negative	Negative	Negative
31	Negative	Negative	Negative	Negative	Negative	Negative
32	Negative	Negative	Negative	Negative	Negative	Negative
33	Positive	Positive	Positive	Positive	Positive	Positive
34	Negative	Negative	Negative	Negative	Negative	Negative
35	Negative	Negative	Negative	Negative	Negative	Negative
36	Negative	Negative	Negative	Negative	Negative	Negative
37	Positive	Positive	Positive	Positive	Positive	Positive
38	Positive	Positive	Positive	Positive	Positive	Positive
39	Negative	Negative	Negative	Negative	Negative	Negative
40	Negative	Negative	Negative	Negative	Negative	Negative
41	Negative	Negative	Negative	Negative	Negative	Negative
42	Negative	Negative	Negative	Negative	Negative	Negative
43	Negative	Negative	Negative	Negative	Negative	Negative
44	Negative	Negative	Negative	Negative	Negative	Negative
45	Negative	Negative	Negative	Negative	Negative	Negative
46	Negative	Negative	Negative	Negative	Negative	Negative
47	Negative	Negative	Negative	Negative	Negative	Negative
48	Positive	Positive	Positive	Positive	Positive	Positive
49	Positive	Negative	Negative	Negative	Negative	Negative
50	Negative	Negative	Negative	Negative	Negative	Negative
51	Negative	Negative	Negative	Negative	Negative	Negative
52	Negative	Negative	Negative	Negative	Negative	Negative
53	Negative	Negative	Negative	Negative	Negative	Negative

54	Negative	Negative	Negative	Negative	Negative	Negative
55	Negative	Negative	Negative	Negative	Negative	Negative
56	Negative	Negative	Negative	Negative	Negative	Negative
57	Positive	Positive	Positive	Positive	Positive	Positive
58	Positive	Positive	Positive	Positive	Positive	Positive
59	Positive	Positive	Positive	Positive	Positive	Positive
60	Positive	Positive	Positive	Positive	Positive	Positive
61	Positive	Positive	Positive	Positive	Positive	Positive
62	Negative	Negative	Negative	Negative	Negative	Negative
63	Negative	Negative	Negative	Negative	Negative	Negative
64	Positive	Positive	Positive	Positive	Positive	Positive
65	Negative	Negative	Negative	Negative	Negative	Negative
66	Positive	Positive	Positive	Positive	Positive	Positive
67	Positive	Positive	Positive	Positive	Positive	Positive
68	Positive	Positive	Negative	Negative	Negative	Negative
69	Positive	Positive	Positive	Positive	Positive	Positive
70	Negative	Positive	Negative	Positive	Negative	Negative

Calculations:

According to the population and purposes, the cut-off is different. As a general role, diagnostic sensitivity and specificity may depends on the cutoff selected:

Cut off (µg/l)	Cut off (µg/g faeces)	Sensitivity (%)	Specificity (%)
50	4	79.4	89.7
100	8	76.5	95.3
150	12	70.6	95.9

The calculations of relative sensitivity and specificity have been made according to the different cut-off.

Cut-off : 4 µg/g faeces 50 µg / L	Sentinel (+)	Sentinel (-)	Total
ST0200 (+)	18	2	20
ST0200 (-)	2	48	50
Total	20	50	70

Relative sensitivity: **90%**

Relative specificity: **96%**

Cut-off: 8 µg/g faeces 100 µg/l	Sentinel (+)	Sentinel (-)	Total
ST0200 (+)	16	1	17
ST0200 (-)	2	51	53
Total	18	52	70

Relative sensitivity: **88.9 %**

Relative specificity: **99%**

Cut-off : 12 µg/g faeces 150 µg/l	Sentinel (+)	Sentinel (-)	Total
ST0200 (+)	16	1	17
ST0200 (-)	1	52	53
Total	17	53	70

Relative sensitivity: **94 %**

Relative specificity: **99 %**

OC SENSOR (EIKEN) vs i-FOB TURBIDIMETRIC (reagent used in ST0200)

Faecal samples were collected in Eiken sampling collecting tubes and sent to *"Hospital Clínico de Barcelona"* and tested with Eiken and ST0200 Reagents in Diana analyser (Eiken Reagnts) and Kroma IT analyser (ST0200 Reagents).

iFOB Eiken *vs* iFOB turbidimetric (Linear)

$$y = 1.0374x + 0.5096$$
$$r = 0.993$$
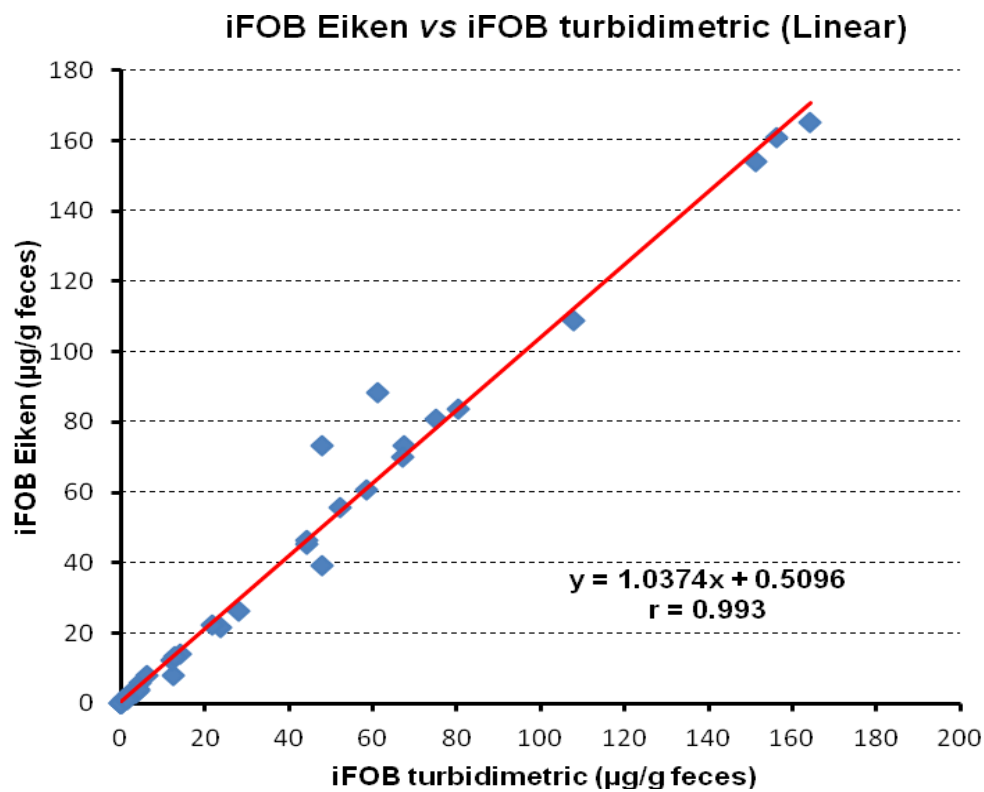
RESULTS	ST0200 (µg/l)	ST0200 (µg/l)			Units: µg / g faeces	
SAMPLE	run 1	Run 2	Mean	EIKEN (µg/l)	ST0200 reagent	EIKEN iFOB
1	0	0	0	0	0.0	0.0
2	0	0	0	0	0.0	0.0
3	10.1	0	5.05	0	0.4	0.0
4	17.7	19.1	18.4	16	1.5	1.3
5	26.8	21.4	24.1	22	1.9	1.8
6	44.4	41	42.7	36	3.4	2.9
7	52.6	41.3	46.95	46	3.8	3.7
8	75.7	40.6	58.15	75	4.7	6.0
9	159.6	154.8	157.2	101	12.6	8.1
10	80	79.2	79.6	100	6.4	8.0
11	147.1	157.7	152.4	153	12.2	12.2
12	172.5	182.4	177.45	178	14.2	14.2
13	283.5	307.1	295.3	272	23.6	21.8
14	581.9	617.8	599.85	492	48.0	39.4
15	119.3	1078.5	598.9	915	47.9	73.2
16	587.3	941.2	764.25	1104	61.1	88.3
17	1554.2	1139.9	1347.05	1360	107.8	108.8
18	2311.2	1792.9	2052.05	2064	164.2	165.1
19	639.4	665.2	652.3	698	52.2	55.8
20	269.4	271.8	270.6	281	21.6	22.5
21	25.1	23.8	24.45	32	2.0	2.6
22	359.2	340.7	349.95	330	28.0	26.4
23	837.9	842.8	842.8	918	67.4	73.4
24	0	0	0	0	0.0	0.0
25	36.7	28.4	32.55	34	2.6	2.7
26	3.6	4.8	4.2	6	0.3	0.5
27	936.1	945	940.55	1010	75.2	80.8
28	56.1	52.8	54.45	47	4.4	3.8
29	1935	1847	1891	1925	151.3	154.0
30	736.6	728.1	732.35	758	58.6	60.6
31	5.7	6.9	6.3	10	0.5	0.8
32	8.4	9.1	8.75	15	0.7	1.2
33	3.2	0	1.6	1	0.1	0.1
34	1012	998.2	1005.1	1046	80.4	83.7
35	0	1.9	0.95	0	0.1	0.0
36	28.3	25.7	27	28	2.2	2.2
37	847.3	831.7	839.5	876	67.2	70.1
38	54.8	50.1	52.45	50	4.2	4.0
39	1925	1980	1952.5	2012	156.2	161.0
40	23.6	18.7	21.15	23	1.7	1.8
41	0	0	0	0	0.0	0.0
42	560.1	549.9	555	568	44.4	45.4
43	0	0	0	2	0.0	0.2
44	13.8	18.7	16.25	18.4	1.3	1.5
45	12.4	17.6	15	13	1.2	1.0
46	559.4	549.8	554.6	579	44.4	46.3
47	157.3	160.6	158.95	167	12.7	13.4

STATISTICAL ANALYSIS

	Cut-off: 8 µg/ g faeces 100 µg/l		
	Eiken (+)	Eiken (-)	Total
I-FOB turbidimetric (+)	22	0	22
I-FOB turbidimetric (-)	1	24	25
Total	23	24	47

Relative sensitivity: 95.6%
Relative specificity: 100%

11. Interfering substances and cross-reactivity

Several substances supposedly interfering are studied: haemoglobin from bovine, goat, pork, rabbit, and ascorbic ac, lipids, and bovine albumin. Serial concentrations from a high concentration solution of each interfering substance were prepared in a collection solution.
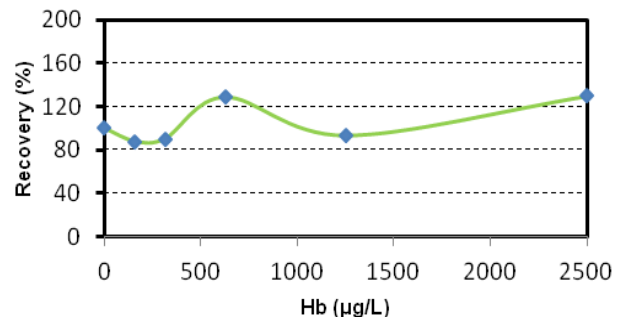
Studied concentrations:

- Bovine haemoglobin: from 156 to 2500 µg/l
- Goat haemoglobin: from 156 to 2500 µg/l
- Pork haemoglobin: from 156 to 2500 µg/l
- Rabbit haemoglobin: from 156 to 2500 µg/l
- Lipids: from 0.5 to 5 g/l
- Ascorbic acid: from 20 to 100 mg/l
- Bovine albumin: 2.2 to 17.5 g/l

HAEMOGLOBIN INTERFERENCES

Goat Hb Interferences
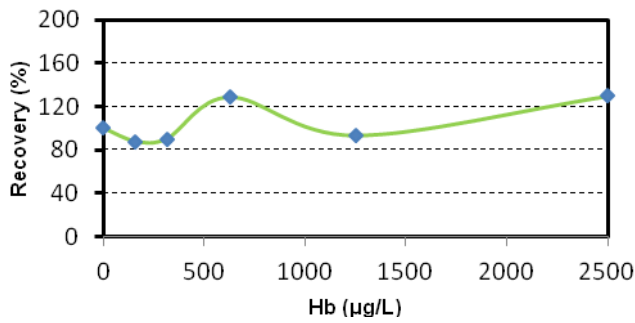
Goat Hb Interferences

Pork Hb Interferences
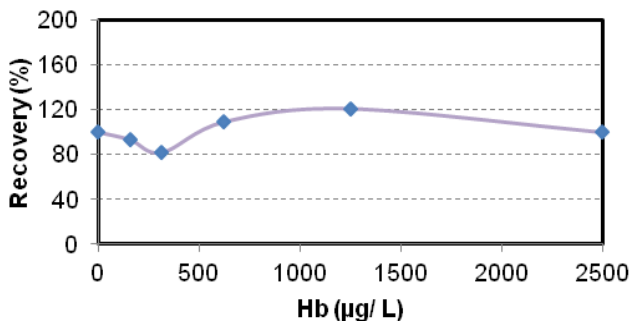
Rabbit Hb Interferences
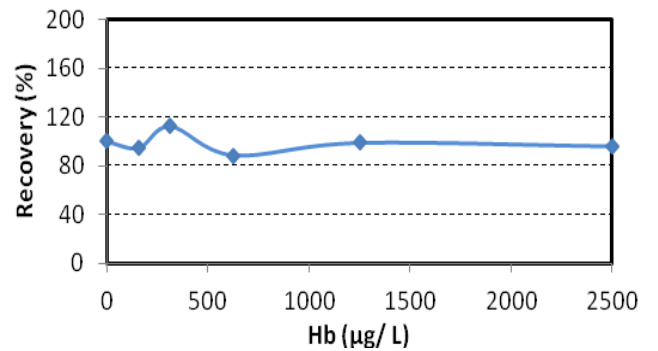
OTHER INTERFERENCES

Ascorbic ac. Interferences
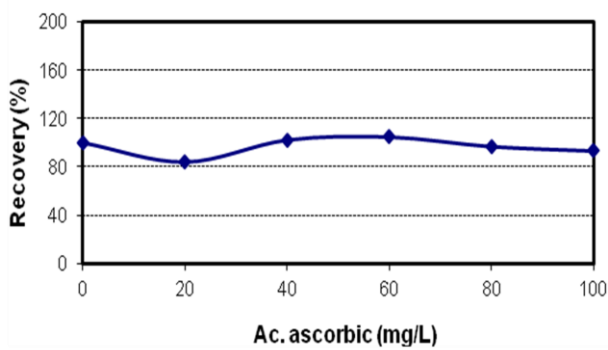
BSA Interferences
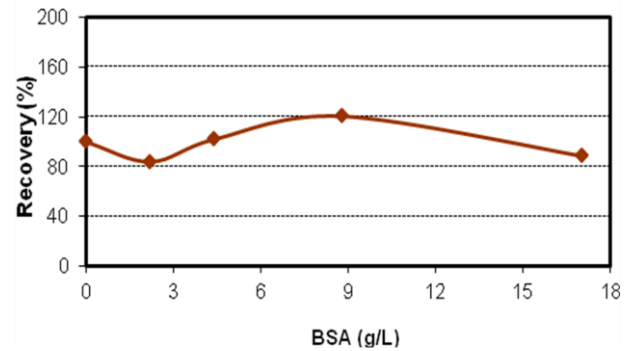
Lipids Interferences
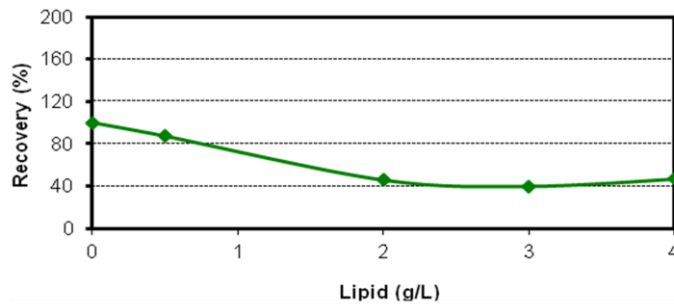
Conclusions

- Bovine haemoglobin: up to 2500 µg/l does not interfere
- Goat haemoglobin: up to 2500 µg/l does not interfere
- Pork haemoglobin: up to 2500 µg/l does not interfere
- Rabbit haemoglobin: up to 2500 µg/l does not interfere
- Lipids (Intralipid): ≥ 2 g/l interfere
- Ascorbic acid: up to 100 mg/l does not interfere
- Bovine albumin: up to 17.5 g/l does not interfere

12. Reaction to haemoglobin variants

Hb-variants Level 2 Control from Eurotrol has been used to test the specicificity of iFOB turbidimetric with some haemoglobin variants. This Control contains 3 Hb variants: Hb A (85-95%); HbS (7 – 13%); Hb C (7 – 13%). A dilution 1×10^{-5} of this control has been done in ClNa 9 g/L to place the Hb concentration into the test range. The results obtained are shown in the table below:

Hb obtained x Dilution Factor (10^5)	Hb expected x Dilution Factor (10^5)	Recovery (%)
28.1 g/l	26.8 g/l	104.85

The monoclonal antibody of this reagent recognizes all the 3 variants of Hb of this control as the result is > 100% of recovery.

13. Accelerate Stability: at 37ºC

I-FOB turbidimetric Reagents

The stability studies were performed at 37ºC. Aliquots of i-FOB Turbidimetric reagents from ST0200 were stressed for 9 days at 37ºC. After this time, all aliquots are tested at the same time against two-fold dilutions in ClNa 9 g/l of Internal iFOB Calibrator, in a Cobas Mira analyser. Two levels of iFOB controls are included in each run.

iFOB turbidimetric stability at 37ºC.
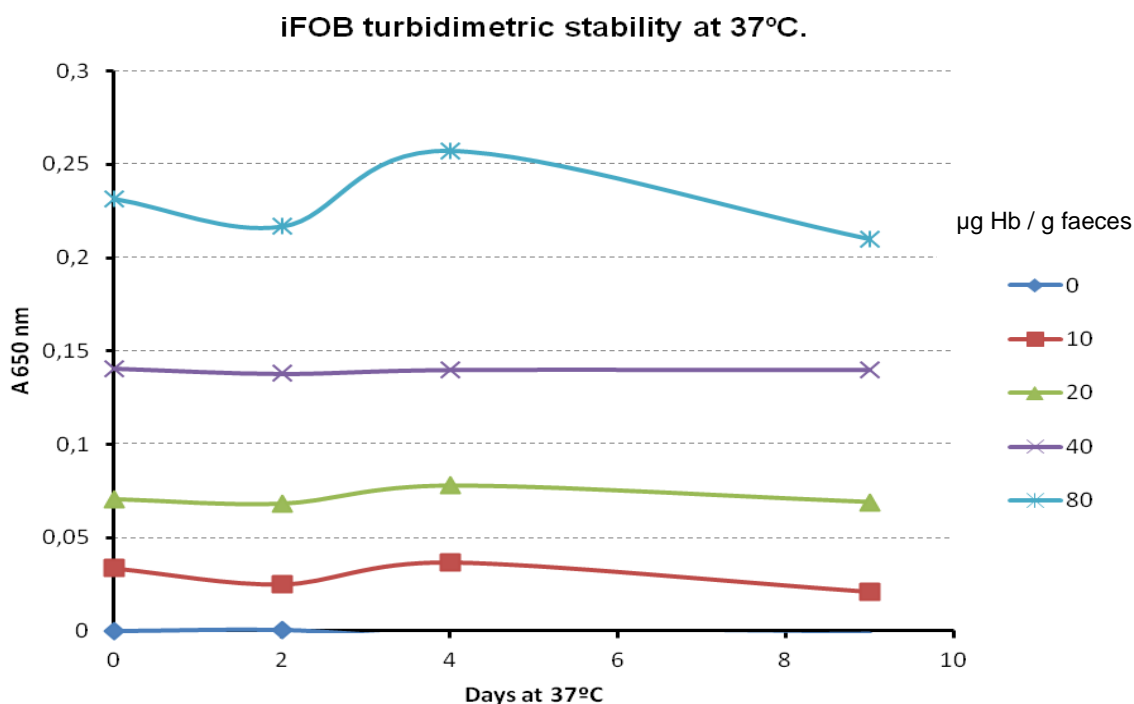
Δ A 650 nm	iFOB Calibrator. µg Hb / g faeces or (µg/l)				
Days at 37ºC	0	10 / (125)	20 / (250)	40 / (500)	80 (1000)
0	0.00015	0.03365	0.07055	0.14070	0.23145
2	0.00075	0.02500	0.06155	0.12460	0.21670
4	-0.00030	0.03690	0.07815	0.15795	0.25735
9	-0.00125	0.02095	0.04540	0.09865	0.20255

iFOB turbidimetric. Stability at 37ºC: Accuracy
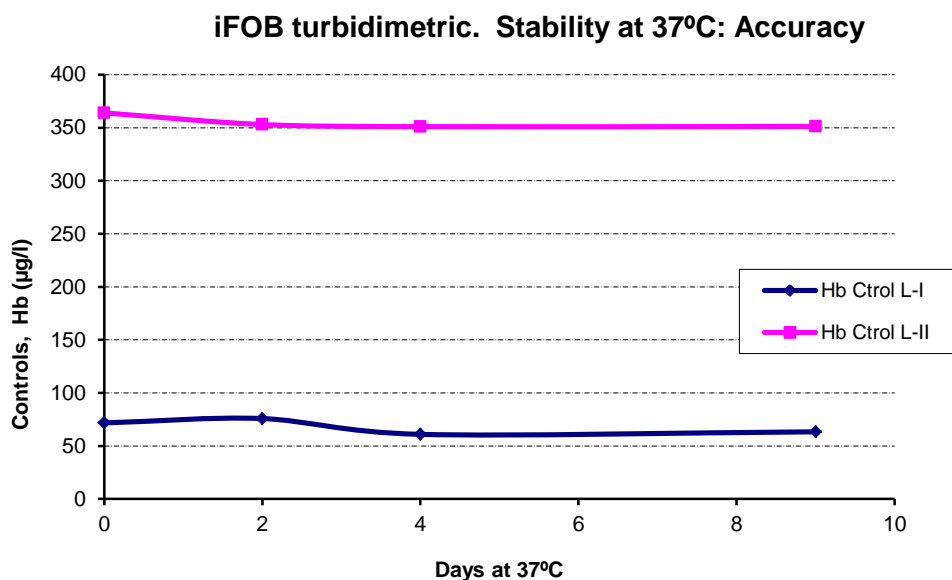
Hb CONTROL	Days at 37ºC			
	0	2	4	9
L-I (µg/l)	71.7	75.8	60.9	63.3
Recovery (%)	100	105.7	84.9	88.2
L-II (µg/l)	364	353	351	351.2
Recovery (%)	100	97	96.4	96.5

From the stability results the reagent used in ST0200 establishes a shelf life of **18 months at 2-8ºC**

MANUFACTURED LOTS

The stability control of each manufactured lot measures the results of absorbance's at time 0 (To) and after 7 days at 37ºC and an accuracy control with two levels of control as well.

i-FOB TURBIDIMETRIC LOT: 31262

Δ A 650 nm	iFOB Calibrator: µg Hb / g faeces or (µg/l)				
Days at 37ºC	0	10 / (125)	20 / (250)	40 / (500)	80 (1000)
0	0.00050	0.03385	0.07950	0.16200	0.24615
7	-0.00118	0.02850	0.07350	0.15405	0.22815

Instrument: Cobas Mira

iFOB turbidimetric # 31262. Stability 37ºC
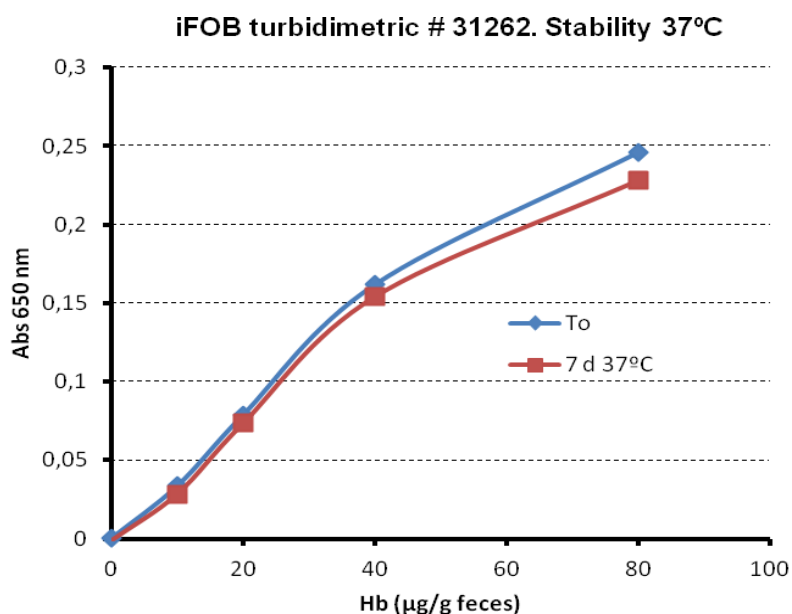
			Hb (µg/l)		
Control	Lot	Target	Range	To	7 d 37ºC
L-I	39065	87	69.7 - 104	88.2	89.7
L-II	39066	394.8	316 - 474	373.6	352.3

Tolerance: Inside range

i-FOB TURBIDIMETRIC LOT: 31338

Δ Abs x 10.000 650 nm	iFOB Calibrator: µg Hb / g faeces or (µg/l)				
Days at 37ºC	0	10 / (125)	20 / (250)	40 / (500)	80 (1000)
0	10.54	130.3	282.44	572.85	893.03
7	5.13	151.91	364.18	721.85	1070.10

Instrument: BS-300 Mindray

iFOB turbidimetric # 31338. Stability 37ºC
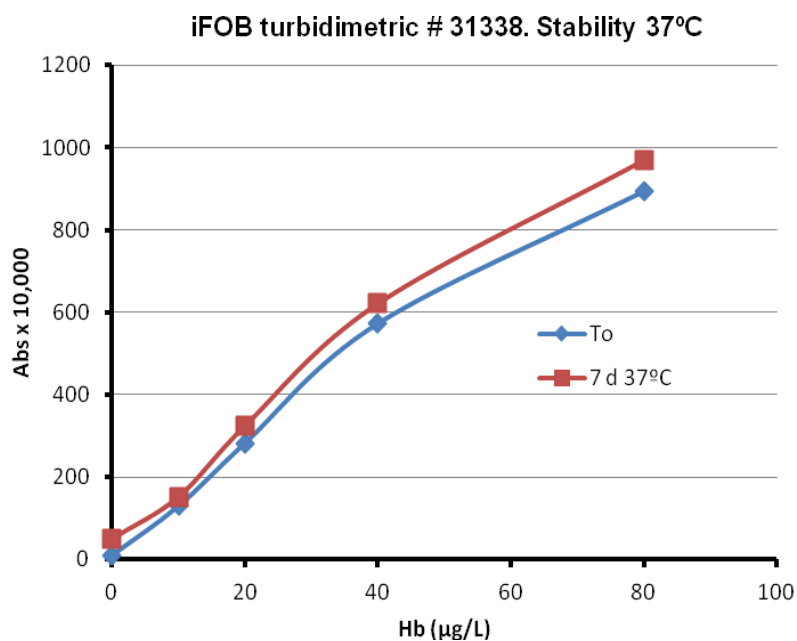
		Hb (µg/l)			
Control	Lot	Target	Range	To	7 d 37ºC
L-I	39065	87	69.7-104	87.5	91.5
L-II	39066	394.8	316 - 474	360	356.5

Tolerance: Inside range

i-FOB Turbidimetric Calibrator

PILOT LOT

The stability studies were performed at 37ºC. Several aliquots of iFOB Calibrator from ST0200 were stressed at different periods of time for a maximum of 14 days at 37ºC. After this time, all aliquots are tested using I-FOB turbidimetric Reagents from ST0200 and an Internal iFOB Calibrator, in a Cobas Mira analyser. Two levels of iFOB controls are included in each run.

	Days at 37ºC			
	0	8	9	14
Calibrator (µg/l)	998.6	934.9	1018.4	885.8
Recovery (%)	100	93.6	101.9	88.7

Tolerance: +/- 10%

From the stability results ST0200 establishes a shelf life of **24 months at 2-8ºC**

iFOB turbidimetric. Stability at 37ºC
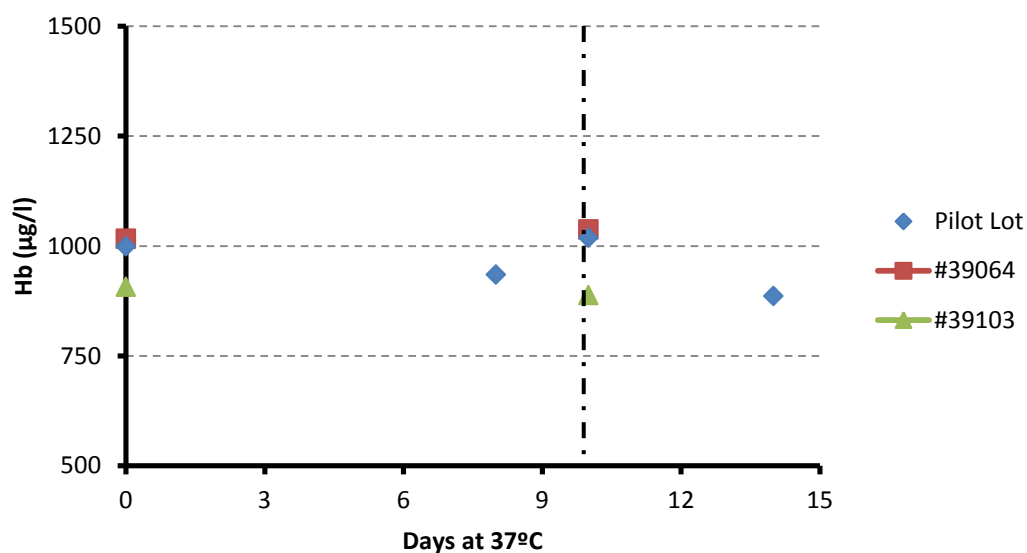
MANUFACTURED LOTS

Stability control of calibrator measures the Hb concentration at time 0 (To) and after 10 days at 37ºC

Hb (µg/l)

i-FOB Calibrator. Lot	To	10 d 37ºC	Recovery (%)
39064	1016	1037	97.9
39103	907	888	97.9

Acceptance tolerance: +/- 10%

14.   Real Time Stability: at 2-8ºC

14.1. i-FOB turbidimetric **Reagent: Lot 31262**

The real time study is performed at 2-8ºC. Sensitivity and ST0200ity range is measured after different periods from the manufacturing date. The assays are run in a BS-300 from Mindray, using the Internal iFOB calibrator of 1000 µg/l. Two levels of iFOB Controls are included in each run.

Calibration curve:

CAL (µg/l)	To	6 months	12 months	18 months	24 months
0	30	20	40	58	32
125	340	339	329	351	312
250	566	659	618	649	604
500	932	988	929	939	898
1000	1343	1300	1267	1278	1197

Analyser: BS-300 Mindray

Accuracy: Two iFOB Controls (L-I and L-II)

Months 2-8ºC	L-I	L-II
0	88	372
12	80	352
18	84.4	358
24	99.4	395

Analyser: BS-300 Mindray

REAL TIME STABILITY (2-8ºC)

REAL TIME STABILITY: ACCURACY

LL: Low limit
HL: High Limit
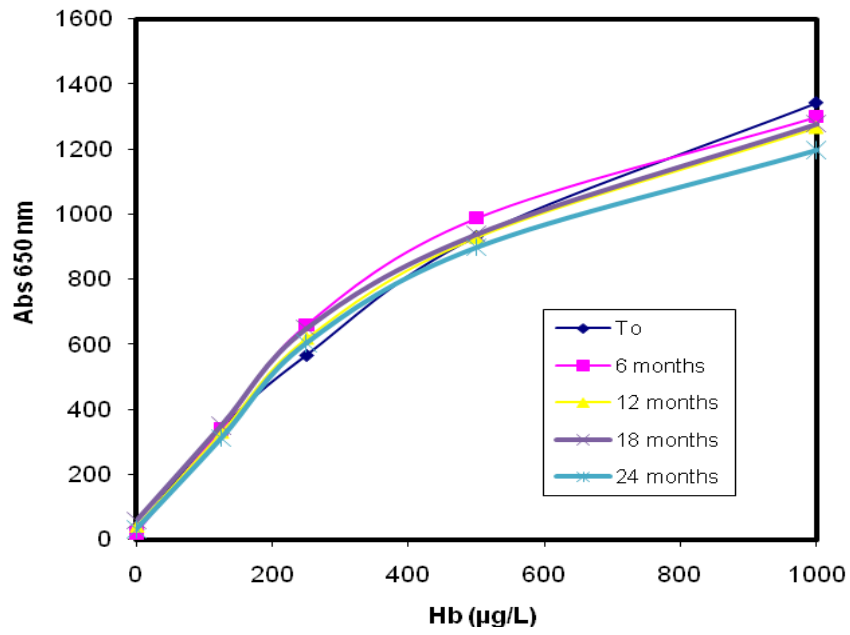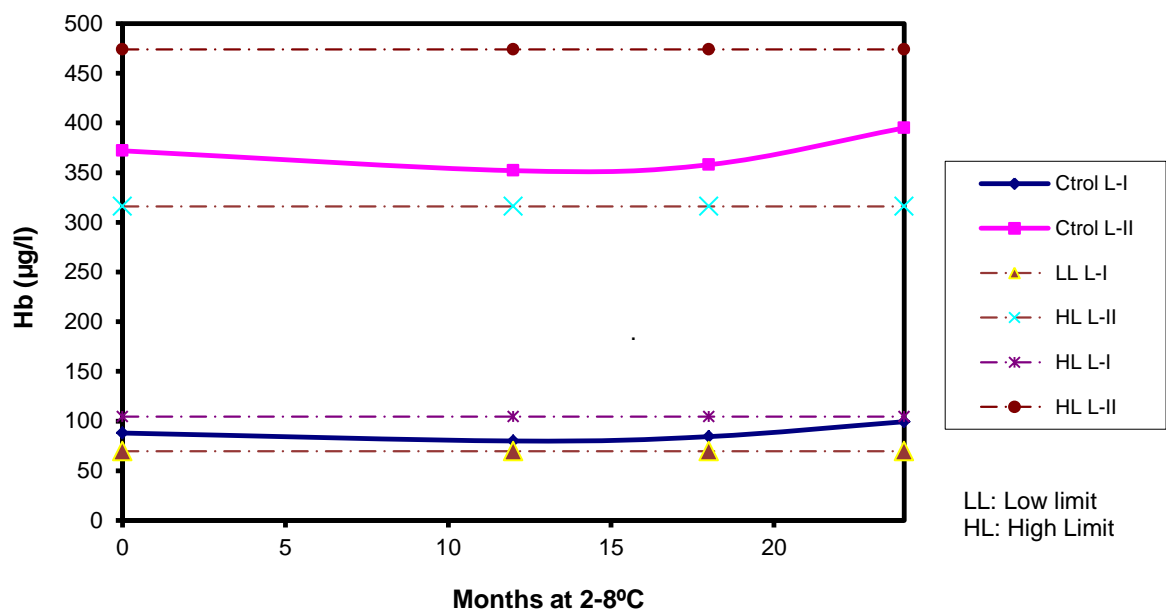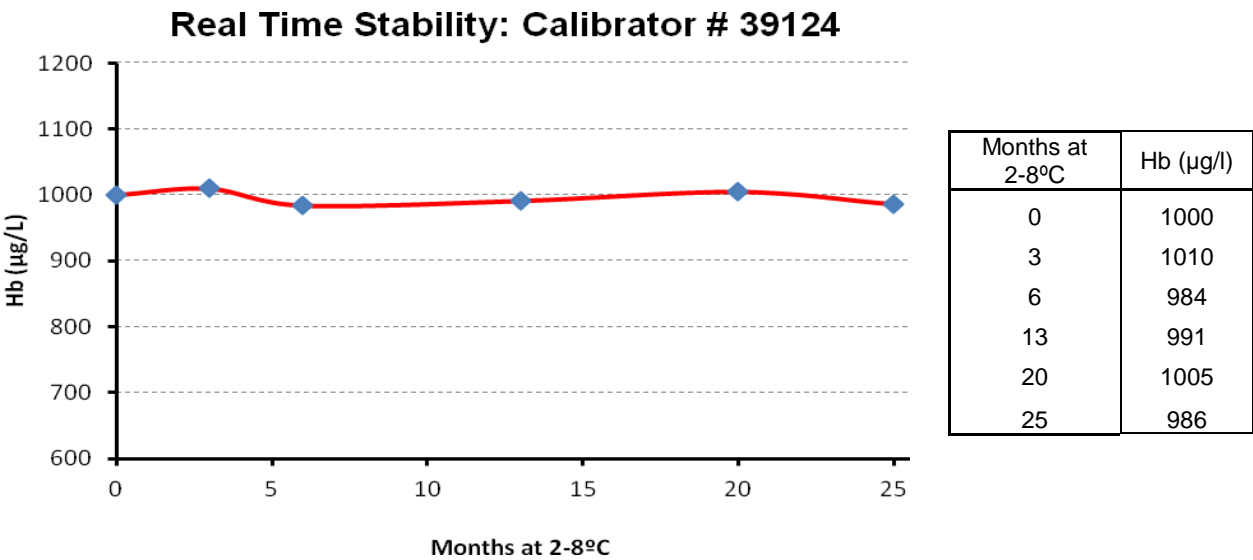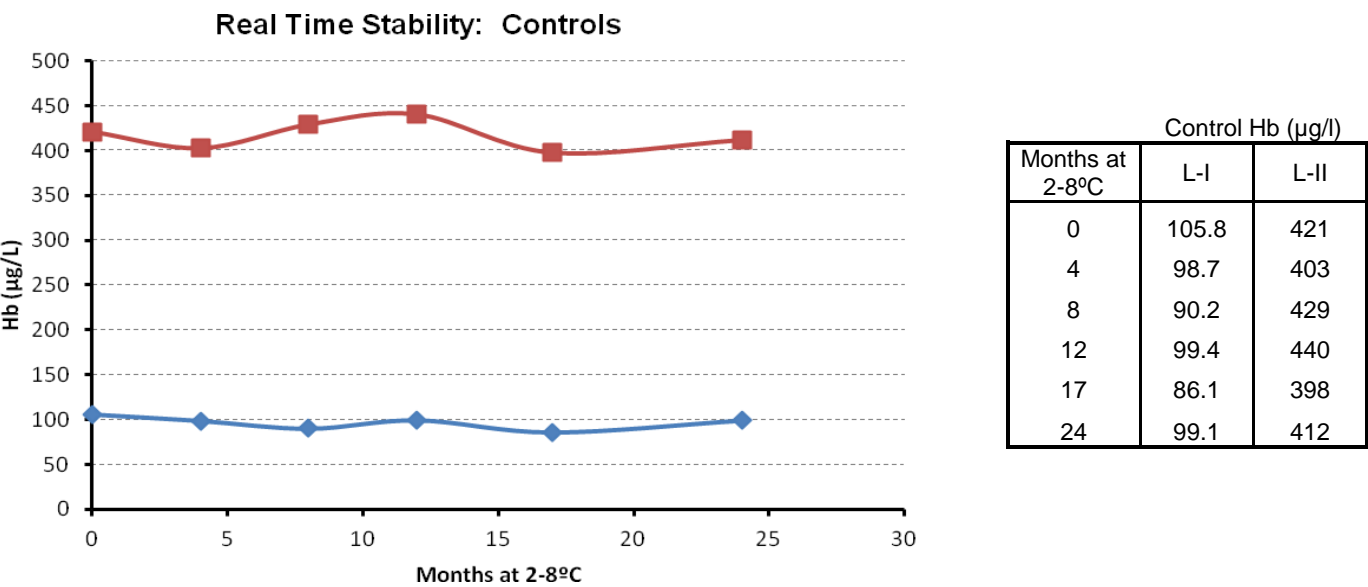
14.2. i-FOB Turbidimetric Calibrator: # 39124

The real time study is performed at 2-8ºC. Haemoglobin concentration is measured after different periods from the manufacturing date. The assays are run in a BS-300 from Mindray, using the Internal iFOB calibrator of 1000 µg/l. Two levels of iFOB Controls are included in each run.

Months at 2-8ºC	Hb (µg/l)
0	1000
3	1010
6	984
13	991
20	1005
25	986

14.3. i-FOB Turbidimetric Controls: # 39104 (L-I) and # 39105 (L-II)

The real time study is performed at 2-8ºC. Haemoglobin concentration is measured after different periods from the manufacturing date. The assays are run in a BS-300 from Mindray, using the Internal iFOB calibrator of 1000 µg/l. Two levels of iFOB Controls are included in each run.
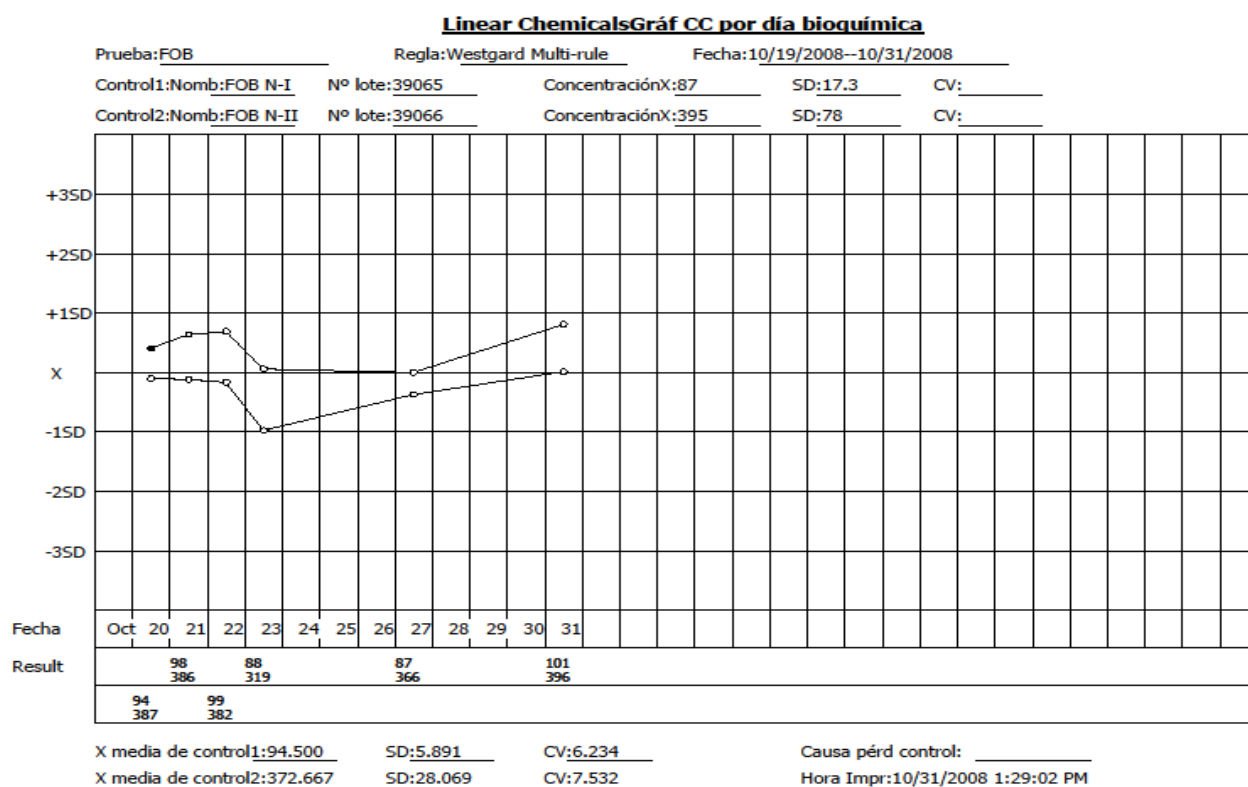

Months at 2-8ºC	Control Hb (µg/l)	
	L-I	L-II
0	105.8	421
4	98.7	403
8	90.2	429
12	99.4	440
17	86.1	398
24	99.1	412

15. Calibration and Reagent "On Board" Stability

On Board Stability was carried out on BS-300 analyser from Mindray and Hitachi 717. Two i-FOB Turbidimetric Controls Level I and Level II from ST0200 were tested during in a period of time, with a calibration run, only the first day. After this period of time, the calibration is stable.

Results:

BS-300 Mindray	Hb (µg/l)						
iFOB Control	Day 1 (10/19/2008)	Day 2	Day 3	Day 4	Day 8	Day 12 (10/31/2008)	Range
Level I	94	98	99	88	87	101	69.3 - 104.3
Level II	387	386	382	319	366	396	317- 473

Linear ChemicalsGráf CC por día bioquímica

Prueba:FOB_____   Regla:Westgard Multi-rule   Fecha:10/19/2008--10/31/2008_____

Control1:Nomb:FOB N-I   N° lote:39065___   ConcentraciónX:87___   SD:17.3___   CV:_____

Control2:Nomb:FOB N-II   N° lote:39066___   ConcentraciónX:395___   SD:78___   CV:_____

X media de control1:94.500___   SD:5.891___   CV:6.234___   Causa pérd control: _____

X media de control2:372.667___   SD:28.069___   CV:7.532___   Hora Impr:10/31/2008 1:29:02 PM

Hitachi 717: Calibration Stability

Std	Hb (µg/l)	Abs x 10000							
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
S1	0	37	33	45	36	41	43	41	31
S2	63.5	227	223	239	235	242	237	234	258
S3	127	448	461	472	425	441	440	458	506
S4	254	883	887	921	890	890	918	904	954
S5	508	1770	1740	1833	1756	1701	1656	1782	1902
S6	1016	3104	3128	3200	3191	3097	3083	3159	3258

Calibration Stability
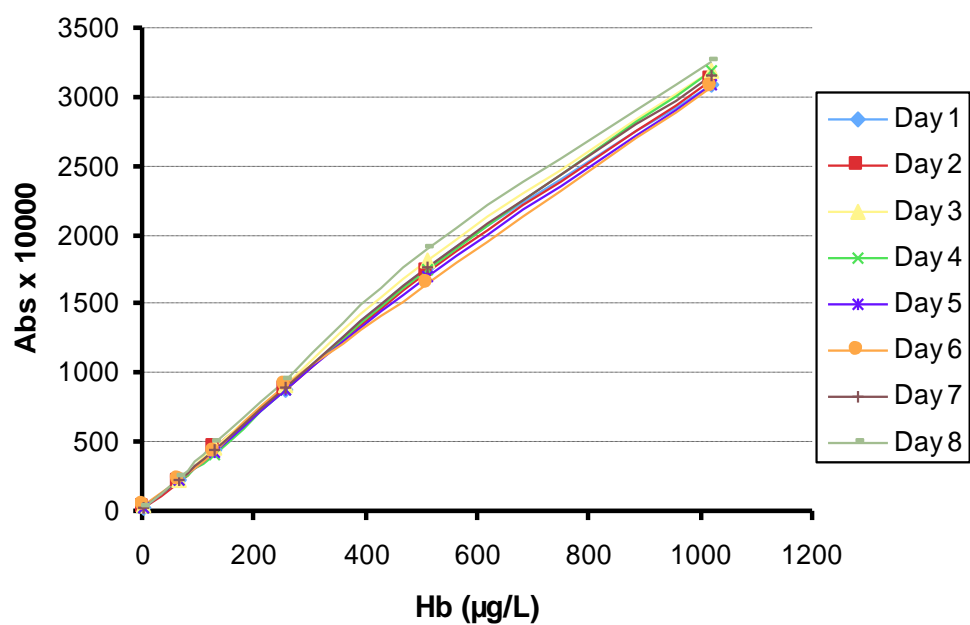
Accuracy stability:

Ctrol	Hb (µg/l)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
L-I	69.7 – 104.5	103	87	102	93	90	93	95	94
L-II	316 - 474	429	453	450	432	412	414	422	404

"On Board" Stability

From the stability results ST0200 stablishes a "On Board" calibration stability > **1 week.**

16-A.   Sample stability in the Sample Collection Tube (RT and 2-8ºC)

Faecal samples were collected from healthy voluntary individuals and resulted negative for blood.
These samples were then pooled and divided into 9 fractions. Each fraction was mixed with different amounts of fresh human haemoglobin. 9 plastic tubes were filled with 5 mL of ST0200 extraction buffer and each tube spiked with *62 µg of each fecal-haemoglobin fraction. Each tube was divided in two more tubes:

- First tube is stored at room temperature (22-25ºC).
- Second tube is stored at 2-8ºC.

Tubes were tested daily for haemoglobin using I-FOB turbidimetric kit from ST0200. Results are shown below.

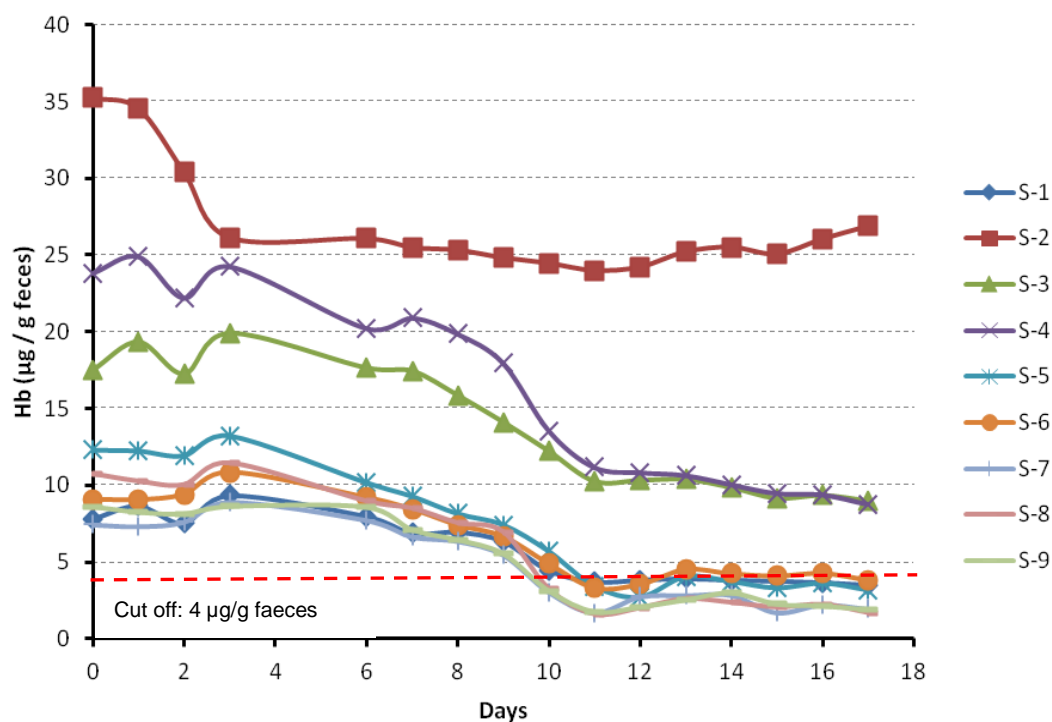(*): This figure corresponds to the amount of faeces dosed for each Sample Collection Tube (20 µg/1.6 ml)

RESULTS	STABILITY AT RT (22-25ºC)								
	SAMPLES (µg/l)								
DAYS	S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8	S-9
0	97.8	441	218.6	297.8	153.7	113.6	92.9	134.2	107.7
1	108.3	432.2	241.6	311.3	153	113.3	91.4	128.2	103.2
2	94.2	380.2	215.6	277.6	149.2	117.7	95	125.3	101.8
3	116.8	326.6	248.6	303.2	165.3	135.6	111.3	143	107.9
6	99.8	325.9	220.5	252.8	127.3	115.1	96.5	112	107.3
7	86.0	318.6	218.1	261.3	116.1	105.6	83.1	106.2	88.9
8	87.1	316.4	198.3	248.3	101.9	92.8	79.4	94.3	80.7
9	79.6	310.2	176.5	224.6	92.8	83.4	68.3	86.9	69.4
10	55.8	305.7	153	169.6	71.3	61.4	38	40.6	39.1
11	46.7	299.8	128.5	139.7	42.8	41.8	21.2	20	22.1
12	48	302.6	129.2	135.5	34	44.6	34.2	25	26
13	49.3	315.1	130	133.2	50.8	56.7	35.2	32.4	31.8
14	48.1	318.9	123.5	125.7	46.7	53.2	35.5	29.5	37.5
15	47.2	313.3	114.7	118.6	41.4	51.4	21.3	25.6	28.4
16	46.1	325.6	117	117.5	45.3	53.5	27.7	27.9	26.9
17	44	336	112.5	109.7	39.8	48.1	24.2	21.2	23.8
Decrease	53.8	127.8	104	179.3	112.3	62.3	71.6	108.6	79.4
Average	99.9								

Decrease / day	3.2	7.5	6.1	10.5	6.6	3.7	4.2	6.4	4.7
(%)	3.24	1.70	2.80	3.54	4.30	3.22	4.53	4.76	4.33
Average (%)	3.60								

RESULTS	STABILITY AT RT (22-25ºC)								
	SAMPLES (µg Hb/ g faeces) 1 µg Hb/ll = 0.08 µg Hb / g faeces								
DAYS	S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8	S-9
0	7.82	35.28	17.49	23.82	12.30	9.09	7.43	10.74	8.62
1	8.66	34.58	19.33	24.90	12.24	9.06	7.31	10.26	8.26
2	7.54	30.42	17.25	22.21	11.94	9.42	7.60	10.02	8.14
3	9.34	26.13	19.89	24.26	13.22	10.85	8.90	11.44	8.63
6	7.98	26.07	17.64	20.22	10.18	9.21	7.72	8.96	8.58
7	6.88	25.49	17.45	20.90	9.29	8.45	6.65	8.50	7.11
8	6.97	25.31	15.86	19.86	8.15	7.42	6.35	7.54	6.46
9	6.37	24.82	14.12	17.97	7.42	6.67	5.46	6.95	5.55
10	4.46	24.46	12.24	13.56	5.70	4.91	3.04	3.25	3.13
11	3.74	23.98	10.28	11.18	3.42	3.34	1.70	1.60	1.77
12	3.84	24.21	10.33	10.84	2.72	3.57	2.74	2.00	2.08
13	3.94	25.20	10.40	10.66	4.06	4.53	2.82	2.59	2.54
14	3.84	25.51	9.88	10.06	3.73	4.26	2.84	2.36	3.00
15	3.78	25.06	9.17	9.48	3.31	4.11	1.70	2.05	2.27
16	3.68	26.04	9.36	9.40	3.62	4.28	2.21	2.23	2.15
17	3.52	26.88	9.00	8.78	3.18	3.84	1.94	1.69	1.90
Decrease	4.30	10.22	8.32	14.34	8.98	4.98	5.73	8.69	6.35
Average	7.99								
Decrease / day	0.3	0.6	0.5	0.8	0.5	0.3	0.3	0.5	0.4
(%)	3.24	1.70	2.80	3.54	4.30	3.22	4.53	4.76	4.33
Average (%)	3.60								

Hb stability in Sample Collection Tube 22-25ºC

CUMULATIVE DECREASE AT 22- 25ºC (%)									
(µg/l)									
SAMPLE	S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8	S-9
Initial Conc (µg/l)	87.8	441	228.6	297.8	183.4	133.6	92.9	155.6	127.7
Initial Conc (µg Hb/ g faeces)	7.82	35.28	17.49	23.82	12.30	9.09	7.43	10.74	8.62
DAY									
1	-10.7	2.0	-10.5	-4.5	0.5	0.3	1.6	4.5	4.2
2	3.7	13.8	1.4	6.8	2.9	-3.6	-2.3	6.6	5.5
3	-19.4	25.9	-13.7	-1.8	-7.5	-19.4	-19.8	-6.6	-0.2
6	-2.0	26.1	-0.9	15.1	17.2	-1.3	-3.9	16.5	0.4
7	12.1	27.8	0.2	12.3	24.5	7.0	10.5	20.9	17.5
8	10.9	28.3	9.3	16.6	33.7	18.3	14.5	29.7	25.1
9	18.6	29.7	19.3	24.6	39.6	26.6	26.5	35.2	35.6
10	42.9	30.7	30.0	43.1	53.6	46.0	59.1	69.7	63.7
11	52.2	32.0	41.2	53.1	72.2	63.2	77.2	85.1	79.5
12	50.9	31.4	40.9	54.5	77.9	60.7	63.2	81.4	75.9
13	49.6	28.6	40.6	55.3	67.0	50.1	62.1	75.9	70.5
14	50.9	27.7	43.5	57.8	69.6	53.2	61.8	78.1	65.2
15	51.7	29.0	47.6	60.2	73.1	54.8	77.1	80.9	73.7
16	52.9	26.2	46.5	60.5	70.5	52.9	70.2	79.2	75.1
17	55.0	23.8	48.5	63.2	74.1	57.7	74.0	84.2	77.9
RECOVERY (%)	45.0	76.2	51.5	36.8	25.9	42.3	26.0	15.8	22.1

RESULTS	STABILITY AT 2-8ºC								
	SAMPLES (µg/l)								
DAYS	S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8	S-9
0	97.8	441	218.6	297.8	153.7	113.6	92.9	134.2	107.7
1	93.9	433.5	236.3	315.4	181.7	133.2	110.2	150.3	130.3
2	95.5	443	237.5	313.5	171.3	124.3	104.3	146.3	110.9
3	114.9	476.5	264.2	354.7	184.1	154.9	101.9	168.3	146.5
6	108.9	465.2	257.6	339.3	187.9	136.2	111.9	160.4	128.6
7	84.4	470.8	260.1	338.1	189.7	132.3	118.6	170.5	118.2
8	82.1	467.3	257.6	328.4	176.4	130.4	112.4	164.1	112.4
9	78.6	448.9	239.1	312.7	151.6	106.7	99.8	136.7	110.1
10	63	426	207.4	277.6	128.1	88.3	67.3	103.8	76.4
11	46.7	382.6	197.4	269.6	125.6	81.3	61.4	93.25	69.4
12	46.5	382.2	197.8	258.4	120.8	86.6	65.6	98.8	74.3
13	46.5	383	197.4	263.4	126	92.7	69.3	101.9	78.1
14	47.8	381.8	196.2	261.5	123.7	91	69.2	102.8	77.2
15	46.3	381.9	197.3	262.4	124.7	90.9	68.4	100.3	73.4
16	48.8	384.5	198.6	267.2	125.4	94.6	70.7	106.8	82.4
17	49.8	385.8	199.6	266.1	125.5	93.3	69.1	104.7	82.1
21	59.2	432.9	228.1	301.1	144.7	109.4	86.5	122	92.5
24	61.2	432.6	218.4	305.6	145.8	114.5	85.7	119	91.4
Decrease	36.6	8.4	0.2	-7.8	7.9	-0.9	7.2	15.2	16.3
Average	9.23								
Decrease/day	1.5	0.3	0.0	-0.3	0.3	0.0	0.3	0.6	0.7
(%)	1.56	0.08	0.00	-0.11	0.21	-0.03	0.32	0.47	0.63
Average (%)	0.35								

RESULTS	STABILITY AT 2-8ºC								
	SAMPLES (µg Hb/ g faeces) 1 µg Hb/l = 0.08 µg Hb / g faeces								
DAYS	S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8	S-9
0	7.82	35.28	17.49	23.82	12.30	9.09	7.43	10.74	8.62
1	7.51	34.68	18.90	25.23	14.54	10.66	8.82	12.02	10.42
2	7.64	35.44	19.00	25.08	13.70	9.94	8.34	11.70	8.87
3	9.19	38.12	21.14	28.38	14.73	12.39	8.15	13.46	11.72
6	8.71	37.22	20.61	27.14	15.03	10.90	8.95	12.83	10.29
7	6.75	37.67	20.81	27.05	15.18	10.58	9.49	13.64	9.46
8	6.57	37.38	20.61	26.27	14.11	10.43	8.99	13.13	8.99
9	6.29	35.91	19.13	25.02	12.13	8.54	7.98	10.94	8.81
10	5.04	34.08	16.59	22.20	10.25	7.06	5.38	8.30	6.11
11	3.73	30.60	15.79	21.57	10.05	6.50	4.91	7.46	5.55
12	3.72	30.58	15.82	20.67	9.66	6.93	5.24	7.90	5.94
13	3.72	30.64	15.79	21.07	10.08	7.42	5.54	8.15	6.24
14	3.82	30.54	15.70	20.92	9.89	7.28	5.53	8.22	6.18
15	3.70	30.55	15.78	20.99	9.97	7.27	5.47	8.02	5.87
16	3.90	30.76	15.89	21.37	10.03	7.56	5.66	8.54	6.59
17	3.98	30.86	15.97	21.28	10.04	7.46	5.52	8.38	6.56
21	4.73	34.63	18.25	24.09	11.58	8.75	6.92	9.76	7.40
24	4.90	34.61	17.47	24.45	11.66	9.16	6.86	9.52	7.31
Decrease	2.928	0.672	0.016	-0.624	0.632	-0.072	0.576	1.216	1.304
Average	0.74								
Decrease/day	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1
(%)	1.56	0.08	0.00	-0.11	0.21	-0.03	0.32	0.47	0.63
Average (%)	0.35								

Hb stability in Sample Collection Tube 2-8ºC

CUMULATIVE DECREASE AT 2-8ºC (%)									
(µg/l)									
SAMPLE	S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8	S-9
Initial Conc (µg/l)	87.8	441	228.6	297.8	183.4	133.6	92.9	155.6	127.7
Initial Conc (µg Hb/ g faeces)	7.82	35.28	17.49	23.82	12.30	9.09	7.43	10.74	8.62
DAY									
1	4.0	1.7	-8.1	-5.9	-18.2	-17.3	-18.6	-12.0	-21.0
2	2.4	-0.5	-8.6	-5.3	-11.5	-9.4	-12.3	-9.0	-3.0
3	-17.5	-8.0	-20.9	-19.1	-19.8	-36.4	-9.7	-25.4	-36.0
6	-11.3	-5.5	-17.8	-13.9	-22.3	-19.9	-20.5	-19.5	-19.4
7	13.7	-6.8	-19.0	-13.5	-23.4	-16.4	-27.7	-27.1	-9.7
8	16.1	-6.0	-17.8	-10.3	-14.8	-14.8	-21.0	-22.3	-4.4
9	19.6	-1.8	-9.4	-5.0	1.4	6.1	-7.4	-1.9	-2.2
10	35.6	3.4	5.1	6.8	16.7	22.3	27.6	22.7	29.1
11	52.3	13.3	9.7	9.5	18.3	28.5	33.9	30.5	35.6
12	52.5	13.3	9.5	13.2	21.4	23.8	29.4	26.4	31.0
13	52.5	13.2	9.7	11.6	18.0	18.4	25.4	24.1	27.5
14	51.1	13.4	10.2	12.2	19.6	19.9	25.6	23.4	28.3
15	52.7	13.4	9.7	11.9	18.9	20.0	26.4	25.3	31.9
16	50.1	12.8	9.1	10.3	18.4	16.8	23.9	20.5	23.5
17	39.5	12.5	8.7	10.7	18.4	17.9	25.7	22.0	23.8
21	37.0	0.1	3.5	4.5	20.4	17.9	21.5	18.9	29.0
24	37.4	1.9	0.1	-2.6	5.1	-0.8	7.8	11.3	15.1
RECOVERY (%)	62.6	98.1	99.9	102.6	94.9	100.8	92.2	88.7	84.9

16-B. Sample stability (Continuation) in the Sample Collection Tube (-20ºC, 30ºC and 35ºC)

New stability study has been run at -20ºC and 29-35ºC.

16-B.1. Stability at -20ºC

Faecal samples were collected from healthy voluntary individuals and resulted negative for blood. These samples were then pooled and divided into 3 fractions. Each fraction was mixed with different amounts of fresh human haemoglobin (high, medium and low level). 3 plastic tubes were filled with 5 mL of extraction buffer and each tube spiked with *62 µg of each fecal-haemoglobin sample. Tubes were stored at -20ºC.
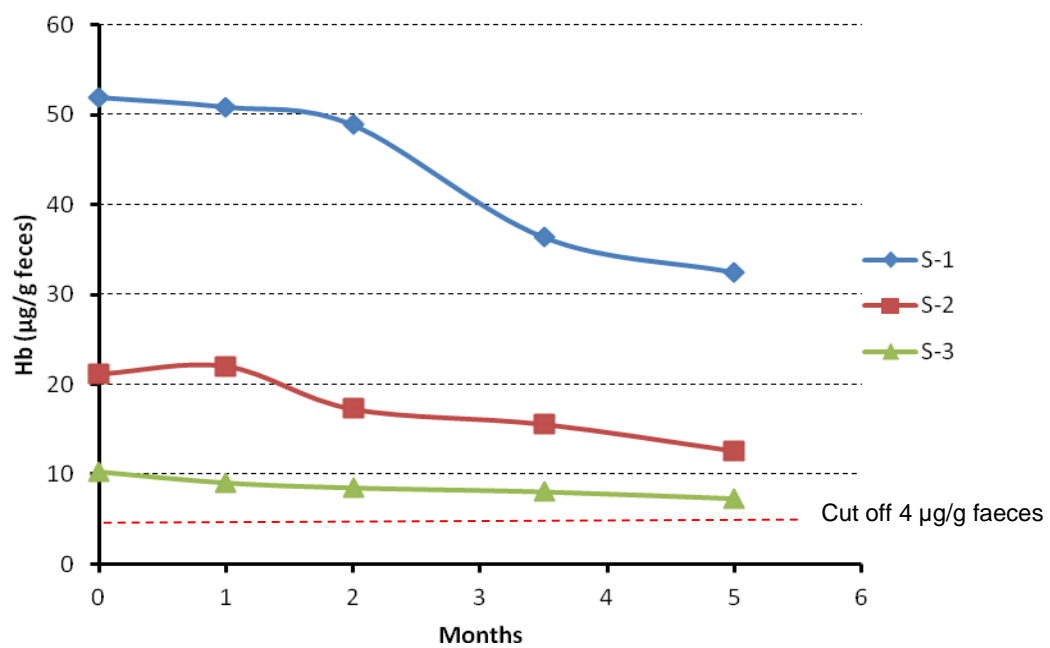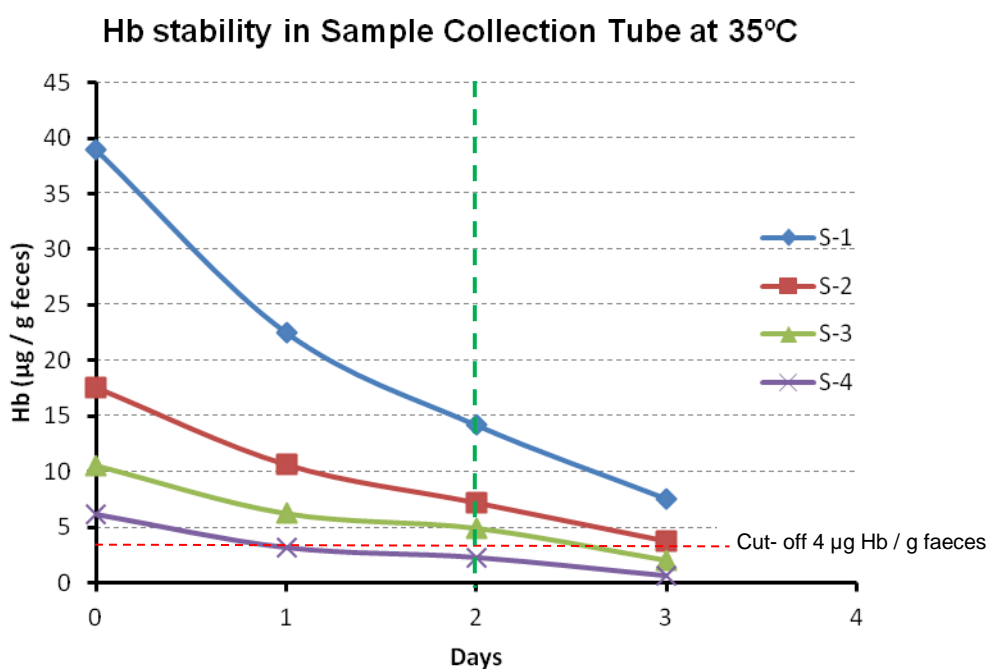Tubes were monthly tested for haemoglobin using I-FOB Turbidimetric kit from ST0200. Results are shown below.
(*): This figure corresponds to the amount of faeces dosed for each Sample Collection Tube (20 µg/1.6 ml)

RESULTS	STABILITY AT -20ºC			STABILITY AT -20ºC		
	SAMPLES (µg/l)			SAMPLES (µg Hb/ g faeces) 1 µg Hb/l = 0.08 µg Hb / g faeces		
MONTHS	S-1	S-2	S-3	S-1	S-2	S-3
0	649.5	264.6	128.9	51.96	21.17	10.3
1	636.1	275.3	113.4	50.9	22.02	9.06
2	611	216	106.1	48.88	17.28	8.49
3,5	455	195	100.5	36.4	15.6	8.04
5	406	158	90.8	32.48	12.64	7.26
Decrease	243.5	106.6	38.1	19.5	8.5	3
Decrease/month	48.7	21.3	7.6	3.9	1.7	0.6
%	7.5	8.05	5.9	7.5	8.05	5.9
Average (%)	7.15			7.15		

Hb Stability in Sample Collection Tube at -20ºC

Y-axis: Hb (μg/g feces) — 0, 10, 20, 30, 40, 50, 60

X-axis: Months — 0, 1, 2, 3, 4, 5, 6

Legend: S-1, S-2, S-3

Cut off 4 μg/g faeces

16-B.2. Stability at 34-35ºC

Faecal samples were collected from healthy voluntary individuals and resulted negative for blood.
These samples were then pooled and divided into 4 fractions. Each fraction was mixed with different amounts of fresh human haemoglobin. 4 plastic tubes were filled with 5 mL of ST0200 extraction buffer and each tube spiked with *62 µg of each fecal-haemoglobin fraction.

- Tubes are icubated at (35ºC).

Tubes were daily tested for haemoglobin using I-FOB turbidimetric kit from ST0200. Results are shown below.

(*): This quantity corresponds to the quantity of faeces dosed for each Sample Collection Tube (20 µg/1.6 ml)

RESULTS	Stability at 34 ºC				Stability at 34 ºC			
	sample (µg Hb/l)				sample (µg Hb / g faeces)			
Days	1	2	3	4	1	2	3	4
0	486.9	218.9	132.3	76.4	38.9	17.5	10.6	6.1
1	281.1	132.8	77.8	39.2	22.5	10.6	6.2	3.1
2	177	90	61	28	14.1	7.2	4.9	2.2
3	94	47	24.5	7.5	7.5	3.8	2.0	0.6
Decrease	392.9	171.9	107.8	68.9	31.4	13.8	8.6	5.5
Decre / day (%)	131.0	57.3	35.9	23.0	10.5	4.6	2.9	1.8
	26.9	26.2	27.2	30.1	26.9	26.2	27.2	30.1
Average (%)	27.6				27.6			

Hb stability in Sample Collection Tube at 35ºC

16-B.3. Sample stability in the Sample Collection Tube (30⁰C)

New stability study has been run at 30ºC.

Faecal samples were collected from healthy voluntary individuals and resulted negative for blood.
These samples were then pooled and divided into 6 fractions. Each fraction was mixed with different amounts of fresh human haemoglobin. 6 plastic tubes were filled with 5 mL of ST0200 extraction buffer and each tube spiked with *62 μg of each fecal-haemoglobin fraction.

- Tubes are icubated to the incubator at 30ºC

Tubes were daily tested for haemoglobin using I-FOB turbidimetric kit from ST0200. Results are shown below.

(*): This quantity corresponds to the quantity of faeces dosed for each Sample Collection Tube (20 μg/1.6 ml)

RESULTS	STABILITY AT 30ºC					
	Samples (μg/l)					
DAYS	S-1	S-2	S-3	S-4	S-5	S-6
0	244	293.4	797	455	261	335
1	283.6	319.3	670	232.4	264.8	279.1
2	278.5	265	528.7	197	200	273
3	223.4	227.1	469	168.3	186.4	249.7
4	186.5	169.4	321	139.9	157.8	212.6
5	140.0	118.0	251.0	98.0	116.0	180.0
Decrease	104.0	175.4	546.0	357.0	145.0	155.0
Average	247.07					
Decrease / day	20.8	35.1	109.2	71.4	29.0	31.0
(%)	8.52	11.96	13.70	15.69	11.11	9.25
Average (%)	11.71					

RESULTS	STABILITY AT 30ºC					
	Samples (μg Hb/g faeces)					
DAYS	S-1	S-2	S-3	S-4	S-5	S-6
0	19.520	23.472	63.760	36.400	20.880	26.800
1	22.688	25.544	53.600	18.592	21.184	22.328
2	22.280	21.200	42.296	15.760	16.000	21.840
3	17.872	18.168	37.520	13.464	14.912	19.976
4	14.920	13.552	25.680	11.192	12.624	17.008
5	11.200	9.440	20.080	7.840	9.280	14.400
Decrease	8.3	14.0	43.7	28.6	12	12
Average	19.77					
Decrease / day	1.7	2.8	8.7	5.7	2.3	2.5
(%)	8.52	11.96	13.70	15.69	11.11	9.25
Average (%)	11.71					

Hb Stability in iFOB-Tube (30ºC)
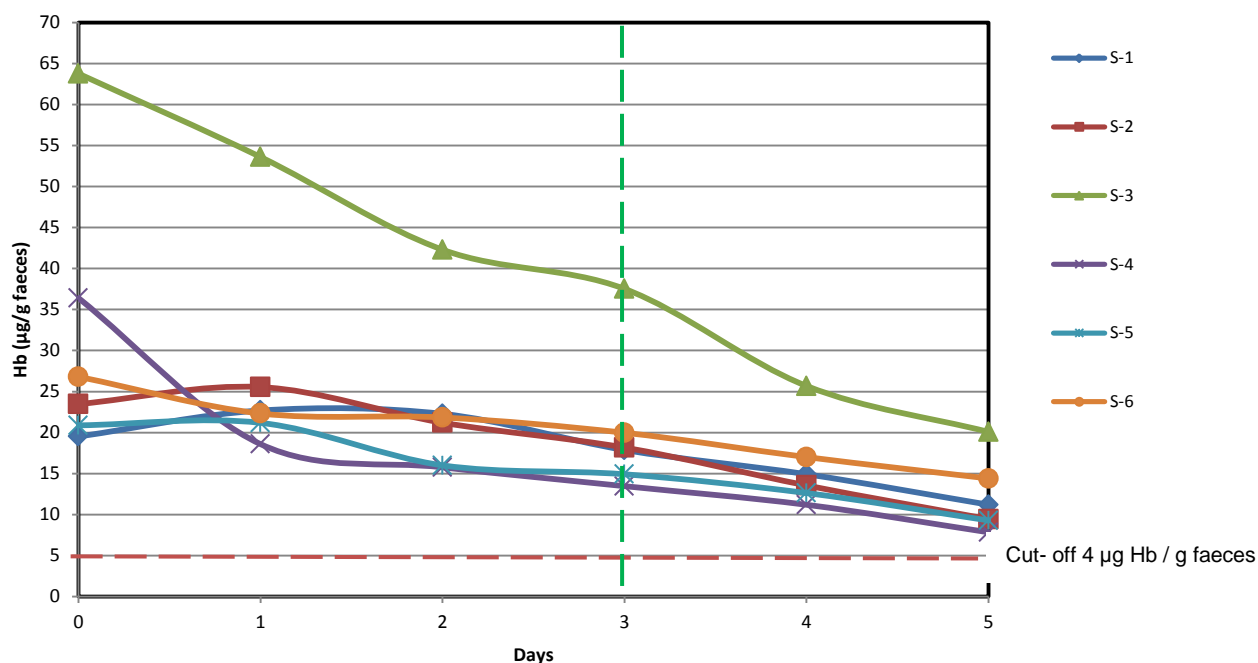
From the results it can be concluded that haemoglobin in the collection tube is stable, at least:

- 10 days at 2-8ºC
- 7 at room temperature (20-25ºC)
- 3 days at 25-30ºC

Temperatures (30ºC and over) and time can affect the test result.

Positivity results may depend on initial Hb concentration and the selected "cut off".

17. Carryover Test

Sample carryover was assessed according the following procedure: Solutions of human Hb were prepared using the collection tube buffer. 1 sample (A) with high Hb concentrations (> 5000 µg/l) and 1 sample (B) with low Hb concentrations (300 µg/l) were prepared. Sample (A) was aliquoted in 5 aliquots (A1, A2, A3, A4, A5) and sample (B) was aliquoted in 5 aliquotes (B1, B2, B3, B4, B5). Samples were tested in Kroma IT instrument with the following sequence: B1 (A1, A2, A3, A4, B1, B2, B3, B4) and repeated 3 times. The carry over factor K was calculated as follow:

$$K \text{ (carry over)} = \left[\frac{B1}{B_{average}} - 1 \right] \times 100 \qquad B_{average} = (B2 + B3 + B4 + B5) / 4$$

SAMPLE	Run 1	Run 2	Run 3
A1	> 1000	> 1000	> 1000
A2	> 1000	> 1000	> 1000
A3	> 1000	> 1000	> 1000
A4	> 1000	> 1000	> 1000
A5	> 1000	> 1000	> 1000
B1	309	319	323
B2	314	319	318
B3	307	317	323
B4	310	315	324
B5	316	310	309
K	**-0.90 %**	**-1.17 %**	**1.57 %**

Tolerance: K < 5%. There is no carry over effect.

18. Inter-Lot (Reagents) variability

Inter-lot variability has been studied in regard to the accuracy variability of different manufactured lots against 8 reference samples with values assigned with an Internal Hb Calibrator traceable to Primary Reference Material CRM 522 (IRMM) and stored freeze-dried at -70ºC.

Reference samples are aliquoted and stored at -70ºC. One aliquot of each sample is thawed at room temperature for each manufactured lot before to run a test.

Reagents of each manufactured lot are calibrated with the Internal Hb calibrator. Two levels of iFOB controls are included in each run.

# 31414 Samples	Expected (E) µg Hb/l	Obtained (O) µg Hb/l	Rate O/E	Average	Tolerance range
1	540	567	1.05		
2	423	434	1.03		
3	112	121	1.08		
4	0	2	--	**1.06**	0.9 - 1.1
5	76	83	1.09		
6	1896	> 1000	--		
7	26	27	1.04		
8	139	145	1.04		
Ctrol L-I	89.6	86.15	0.96	Tolerance Range	
Ctrol L-II	380.8	353	0.93	+/- 20%	

# 31450 Samples	Expected (E) µg Hb/k	Obtained (O) µg Hb/k	Rate O/E	Average	Tolerance range
1	540	554	1.03		
2	423	419	0.99		
3	112	115	1.03		
4	0	0	--	**1.01**	0.9 - 1.1
5	76	71	0.93		
6	1896	>1000	--		
7	26	29	1.12		
8	139	129	0.93		
Ctrol L-I	89.6	118	1.32	Tolerance Range	
Ctrol L-II	38.,8	397	1.04	+/- 20%	

# 31500 Samples	Expected (E) µg Hb/l	Obtained (O) µg Hb/l	Rate O/E	Average	Tolerance range
1	540	550	1.02		
2	423	426	1.01		
3	112	108	0.96		
4	0	1	--	**1.02**	0.9 - 1.1
5	76	79	1.04		
6	1896	> 1000	--		
7	26	29	1.12		
8	139	131	0.94		
Ctrol L-I	114	120	1.05	Tolerance Range	
Ctrol L-II	470	426	0.91	+/- 20%	

Inter-Lot Variability: 1.03 %

19.   Sample collection tube imprecision

3 fecal samples spiked with different concentrations of human haemoglobin were prepared from a fecal pool.
5 Collection Tubes were loaded onto each of the 3 fecal samples. Tubes were mixed on a roller for 30 minutes prior to analysis. Hb on each tube is determined with repeated measurements (n=2) following the manufacturer's package insert in Kroma IT instrument.

Results are shown below:

Tubes	SAMPLES (µg Hb/g faeces)		
	1	2	3
1	48.8	7.8	32.6
2	58.9	8.2	35.2
3	76.3	10.1	24.9
4	68.5	10.9	26.0
5	44.9	10.7	26.9
AVERAGE	59.5	9.5	29.1
SD	13.2	1.4	4.5
CV (%)	22.1	15.1	15.6

DIAGNOSTIC CHARACTERISTICS

Table 3 shows the Faecal Occult Blood Levels detected according to Colonoscopy and Pathology findings. (Vilkin Alex. et al. *American Journal of Gastroenterology.* 2005; 100 (11): 2519-2525)

Table 3

Diagnosis	No.	Faecal Hb (µg/l) (mean +/- SD)	Significance*
Normal[†]	381	29.1 +/- 103.8	
Adenomas			
All	113	231.9 +/- 567.5	NS
Non-AAP	85	58.0 +/- 156.5	NS
AAP	28	759.8 +/- 935.7	$p < 0.01$
Cancer	6	1154.3 +/- 793.0	$p < 0.01$
CRC+AAP	34	829.4 +/- 913.8	$p < 0.01$

AAP = advanced adenomatous polyps; CRC = colorectal cancer
* As compared to the normal group
† Includes 64 patients with only hyperplasic polyps

Haemoglobin quantification allows selection of a suitable threshold level for fellow-up colonoscopy. Table 4 shows the sensitivity and specificity for Significant Colorectal Neoplasia at differing faecal levels. (Vilkin Alex. et al. *American Journal of Gastroenterology*. 2005; 100 (11): 2519-2525).

Table 4

Faecal Hb (µg/l)	Faecal Hb (µg/ g faeces)	Sensitivity (%)	Specificity (%)
50	4	79.4	89.7
75	6	76.5	93.3
100	8	76.5	95.3
125	10	70.6	95.7
150	12	70.6	95.9
200	16	64.7	96.3

Number of Significant Colorectal Neoplasia = 34 (6 CRC and 28 AAP)
Number of Differing Faecal Hb Levels = 500 (utilizing the highest of the three I-FOBT measurements in each patient.

With this study, we can confirm that I-FOBT threshold of 100 µg/l (8 µg Hb / g faeces) allow detecting all the cancers and the majority of advanced adenomas, with a sensitivity of 76.5% and acceptable specificity of 95.3%. This means that in this group of high risk and asymptomatic patients, all cases of cancer and the most advances adenomas would be detected and a negative test would provide a very high degree of certainty that there was no clinically significant colorectal neoplasia at this round of screening.

Physiological faecal occult blood has been estimated to be 0.31 +/- 0.09 mg/g stool (*Schwartz S. et al. Gastroenterology 1985. 89: 19-26*). 0.1 – 0.2 mg/g stool (*Yoshida Y. et al. Gastroenterology 1986; 90: abstr: 1699)* Equivalence between faces and haemoglobin: 2 nL of blood ≈ 0.3 µg haemoglobin / g stool

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