



Tosoh HPLC G11 instrument

for the diagnosis of haemoglobinopathies



Rome 02/07/2018
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Manager Clinical Affairs



TOSOH

Outcome form the evaluation



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Outcome form the evaluation

- General performances characteristics
- Comparison of HBA2/ HbF between:
 - Tosoh HLC-723G11 (G11)
 - Tosoh HLC-723G8 (G8)
 - Bio-Rad Variant II (V II)
- Comparison of some Hb Variants on all systems



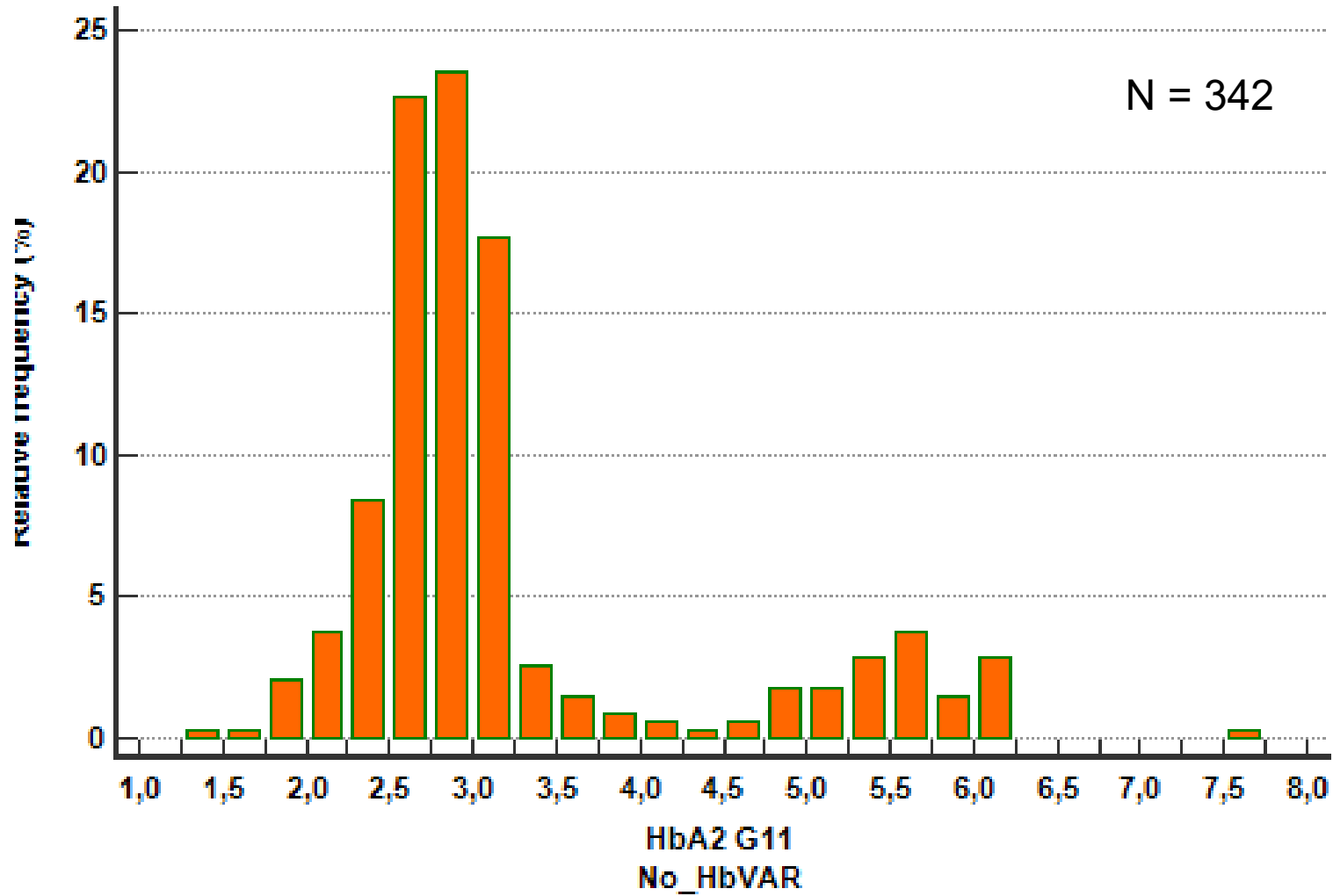
Intra-Assay

<u>HbA2</u>					
Run	Calibrator	Control Level 1	Control Level 2	Normal Patient Whole blood	Thal Trait patient Whole blood
Mean	4,87	2,66	5,94	2,81	6,48
SD	0,01	0,02	0,02	0,01	0,03
CV	0,19	0,57	0,25	0,42	0,50

<u>HbF</u>					
Run	Calibrator	Control Level 1	Control Level 2	Normal Patient Whole blood	Thal Trait patient Whole blood
Mean	5,07	2,45	6,89	0,41	5,60
SD	0,02	0,02	0,04	0,01	0,02
CV	0,35	1,00	0,51	2,43	0,43



Distribution of HbA2 on G11





Comparison HbA2 :G11-G8

Sample size	342
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	Variable X	Variable Y
Lowest value	1,4000	1,4000
Highest value	8,7000	7,7000
Arithmetic mean	3,3211	3,1971
Median	2,9000	2,9000
Standard deviation	1,2486	1,0871
Standard error of the mean	0,06752	0,05878

Regression Equation

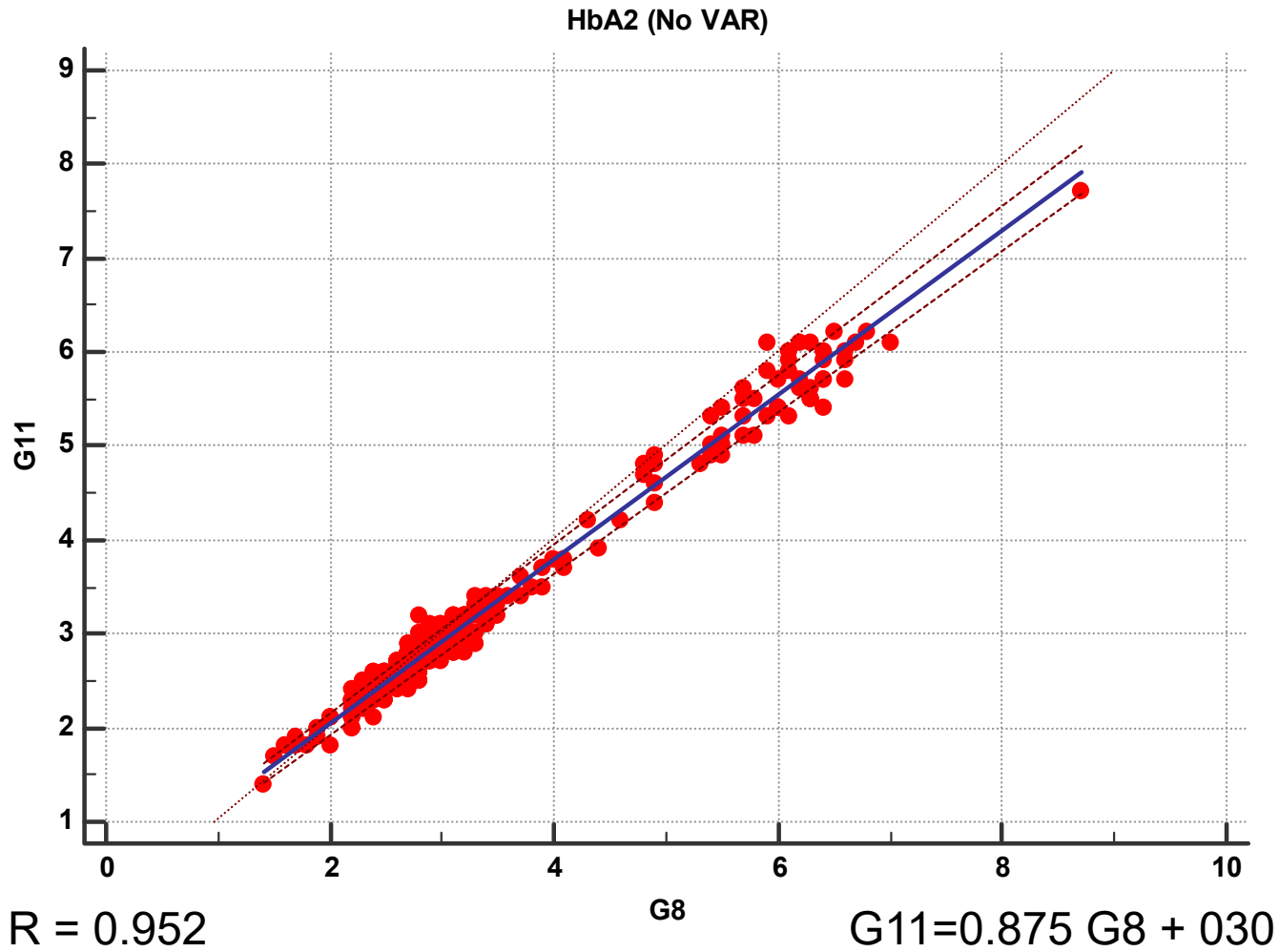
y = 0,300000 + 0,875000 x	
Systematic differences	
Intercept A	0,3000
95% CI	0,2200 to 0,3571
Proportional differences	
Slope B	0,8750
95% CI	0,8571 to 0,9000
Random differences	
Residual Standard Deviation (RSD)	0,1136
± 1.96 RSD Interval	-0,2226 to 0,2226
Linear model validity	
Cusum test for linearity	Significant deviation from linearity (P=0,01)

Spearman rank correlation coefficient

Correlation coefficient	0,952
Significance level	P<0,0001
95% CI	0,941 to 0,961



Comparison HbA2 :G11-G8





Comparison HbA2 :G11-Variant II

Sample size	341	
	Variant II	G11
Lowest value	1,6000	1,4000
Highest value	6,7000	7,7000
Arithmetic mean	3,0979	3,1974
Median	2,8000	2,9000
Standard deviation	0,8790	1,0887
Standard error of the mean	0,04760	0,05895

Regression Equation

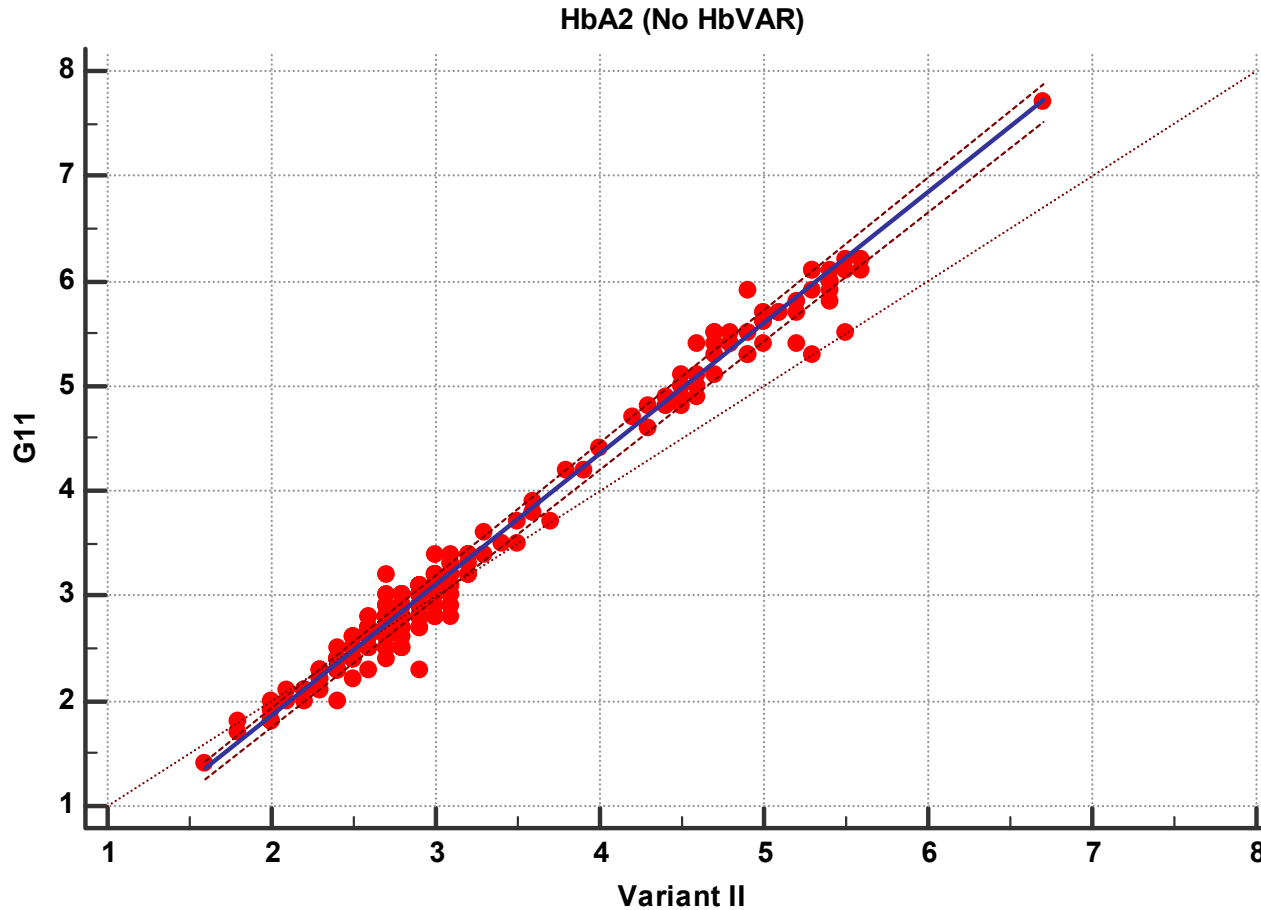
$y = -0,650000 + 1,250000 x$	
Systematic differences	
Intercept A	-0,6500
95% CI	-0,7071 to -0,5909
Proportional differences	
Slope B	1,2500
95% CI	1,2273 to 1,2619
Random differences	
Residual Standard Deviation (RSD)	0,08989
± 1.96 RSD Interval	-0,1762 to 0,1762
Linear model validity	
Cusum test for linearity	No significant deviation from linearity (P=0,17)

Spearman rank correlation coefficient

Correlation coefficient	0,949
Significance level	P<0,0001
95% CI	0,937 to 0,959



Comparison HbA2 :G11-Variant II



R = 0.949

$$G11 = 1.25 \text{ V II} - 0.65$$



Comparison HbF :G11- G8

Sample size	381	
	G8	G11
Lowest value	0,2000	0,0000
Highest value	23,2000	17,3000
Arithmetic mean	1,2121	0,8717
Median	0,6000	0,4000
Standard deviation	2,5509	1,9713
Standard error of the mean	0,1307	0,1010

Regression Equation

$$y = -0,0285714 + 0,714286 x$$

Systematic differences

Intercept A	-0,02857
95% CI	-0,04681 to -0,01250

Proportional differences

Slope B	0,7143
95% CI	0,6875 to 0,7447

Random differences

Residual Standard Deviation (RSD)	0,1793
± 1.96 RSD Interval	-0,3514 to 0,3514

Linear model validity

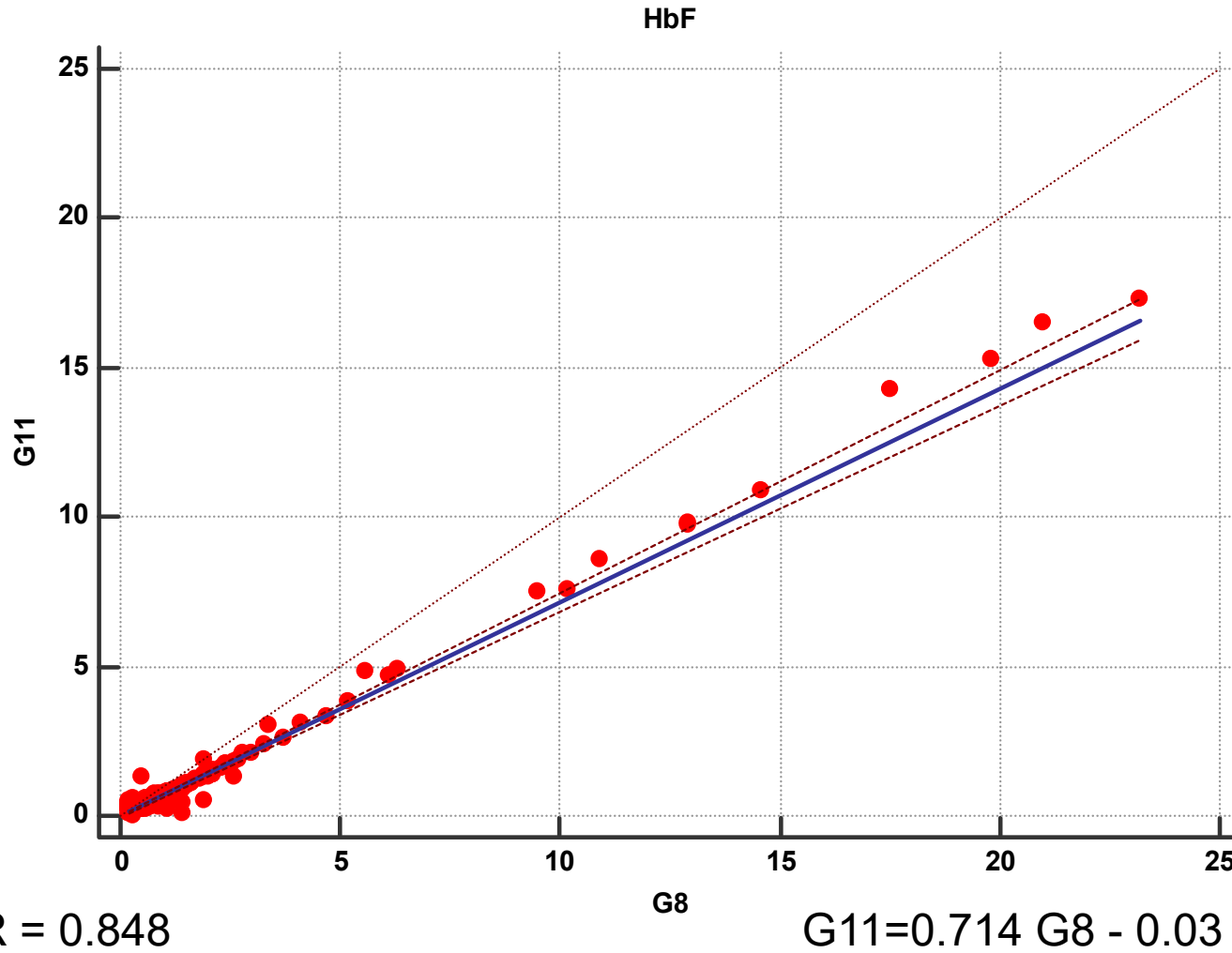
Cusum test for linearity	Significant deviation from linearity (P<0,01)
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Spearman rank correlation coefficient

Correlation coefficient	0,848
Significance level	P<0,0001
95% CI	0,817 to 0,874



Comparison HbF :G11- G8





Comparison HbF :G11-Variant II

Sample size	379	
	Variant II	G11
Lowest value	0,1000	0,0000
Highest value	22,4000	17,3000
Arithmetic mean	1,0063	0,8747
Median	0,4000	0,4000
Standard deviation	2,4942	1,9760
Standard error of the mean	0,1281	0,1015

Regression Equation

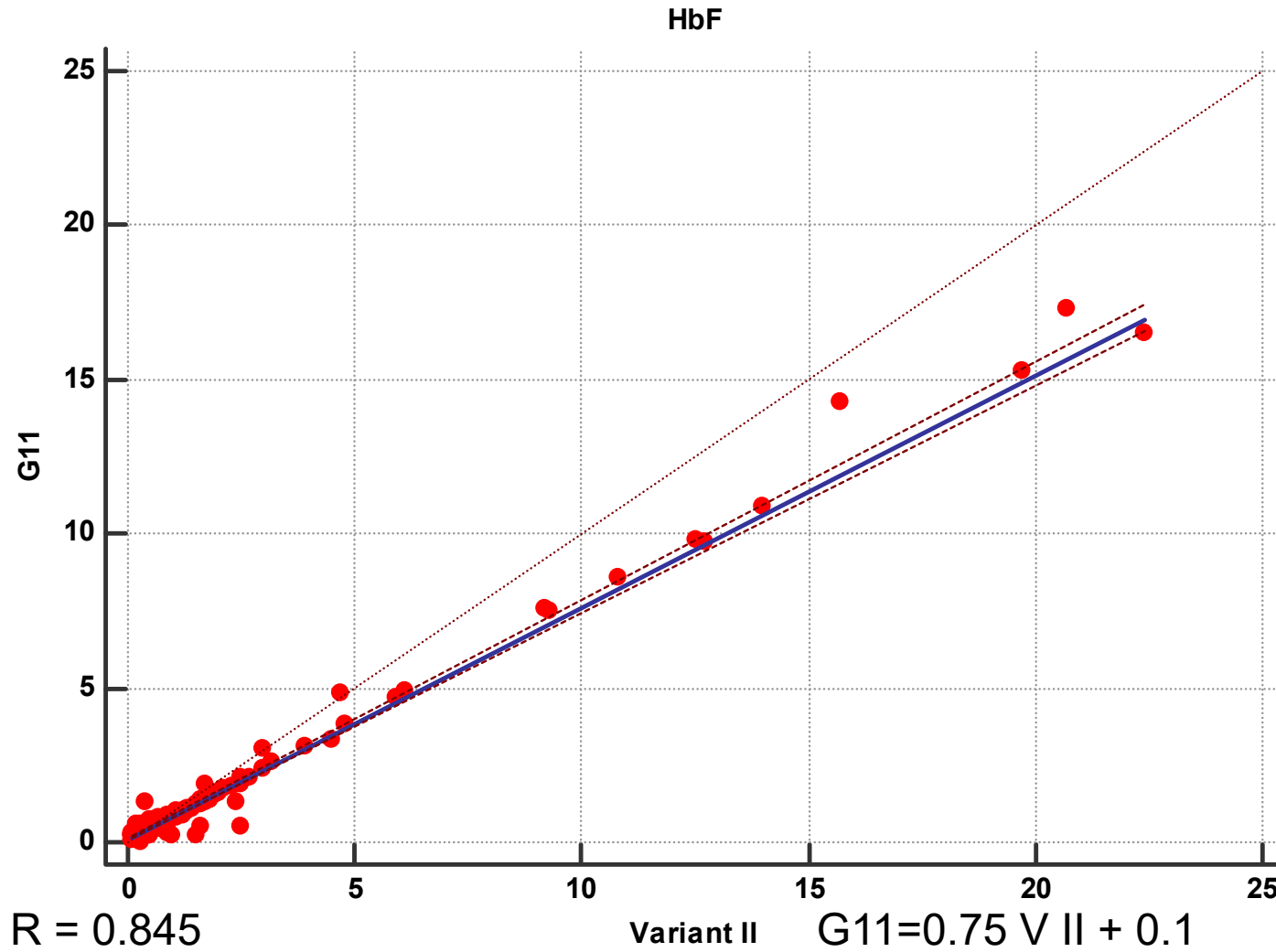
$y = 0,100000 + 0,750000 x$	
Systematic differences	
Intercept A	0,1000
95% CI	0,09119 to 0,1105
Proportional differences	
Slope B	0,7500
95% CI	0,7368 to 0,7720
Random differences	
Residual Standard Deviation (RSD)	0,1870
± 1.96 RSD Interval	-0,3665 to 0,3665
Linear model validity	
Cusum test for linearity	Significant deviation from linearity (P<0,01)

Spearman rank correlation coefficient

Correlation coefficient	0,845
Significance level	P<0,0001
95% CI	0,814 to 0,872



Comparison HbF :G11-Variant II





TOSOH

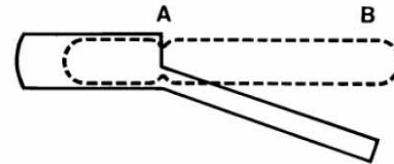
Standardisation on the NIBSC



Medicines & Healthcare products
Regulatory Agency



WHO Reference Reagent
International Reference Reagent for Haemoglobin A2
NIBSC code: 89/666
Instructions for use
(Version 3.0, Dated 04/04/2008)

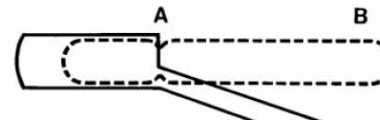


Medicines & Healthcare products
Regulatory Agency



WHO Reference Reagent
Haemoglobin F Lysate, Raised
NIBSC code: 85/616
Instructions for use
(Version 4.0, Dated 04/04/2008)

cuts and projectile glass fragments that enter eyes. Take care that no material is lost from the ampoule and that no glass falls into the ampoule.





HbAS Patient

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
F	1.1	---	1.07	28425
Unknown	---	0.5	1.23	13668
P2	---	2.2	1.33	56298
P3	---	2.4	1.70	62096
Ao	---	53.2	2.39	1360654
A2	3.7*	---	3.60	94857
S-window	---	36.8	4.32	940608

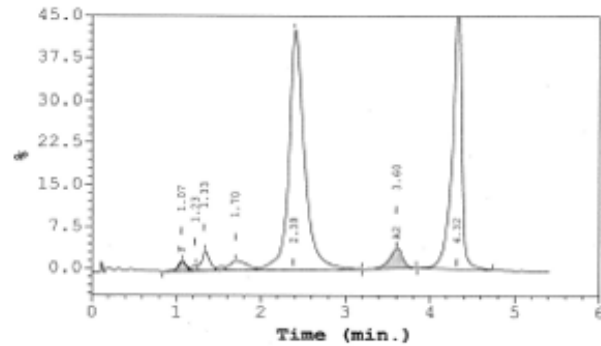
Total Area: 2,556,605

F Concentration = 1.1 %

A2 Concentration = 3.7*%

*Values outside of expected ranges

Analysis comments:



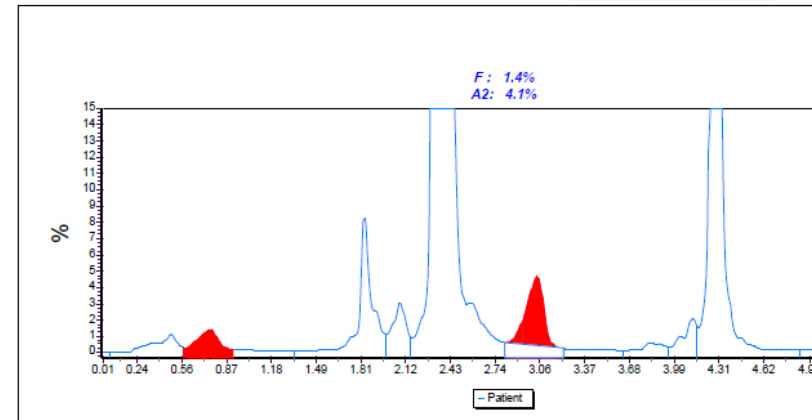
Parameter	Value %	Time min.	Area	Total Area
P00	1.1%	0.47	41.87	3,945.2
F	1.4%	0.75	50.09	
P01	0.2%	0.82	9.82	
P02	4.1%	1.83	162.93	
P03	1.7%	2.08	68.4	
A0	51.1%	2.33	2,014.98	
A2	4.1%	3.03	116.42	
P04	0.6%	3.82	21.81	
P05	1.1%	4.12	44.64	
S+	35.3%	4.28	1,391.7	
P06	0.3%	4.94	11.27	
P07	0.2%	5.34	7.32	
P08	0.1%	5.75	4.39	

Y=(Ax+B)

Element	Factor-A	Factor-B
1	1.0829	0.0000
2	1.4051	0.0000

Analyzer: G8
Serial Nb.: 11819110
Soft. Version: 5.24
UIN: Analyzer UIN

B-Thalassemia





Comparison HbS :G11-Variant II

Sample size	34	
	Variant II	G11
Lowest value	17,2000	16,2000
Highest value	72,3000	80,7000
Arithmetic mean	41,5618	39,0765
Median	38,0000	34,1000
Standard deviation	14,3178	14,2077
Standard error of the mean	2,4555	2,4366

Regression Equation

$$y = 1,362863 + 0,862510 x$$

Systematic differences

Intercept A	1,3629
95% CI	-1,4294 to 3,0128

Proportional differences

Slope B	0,8625
95% CI	0,8187 to 0,9412

Random differences

Residual Standard Deviation (RSD)	4,1275
± 1.96 RSD Interval	-8,0899 to 8,0899

Linear model validity

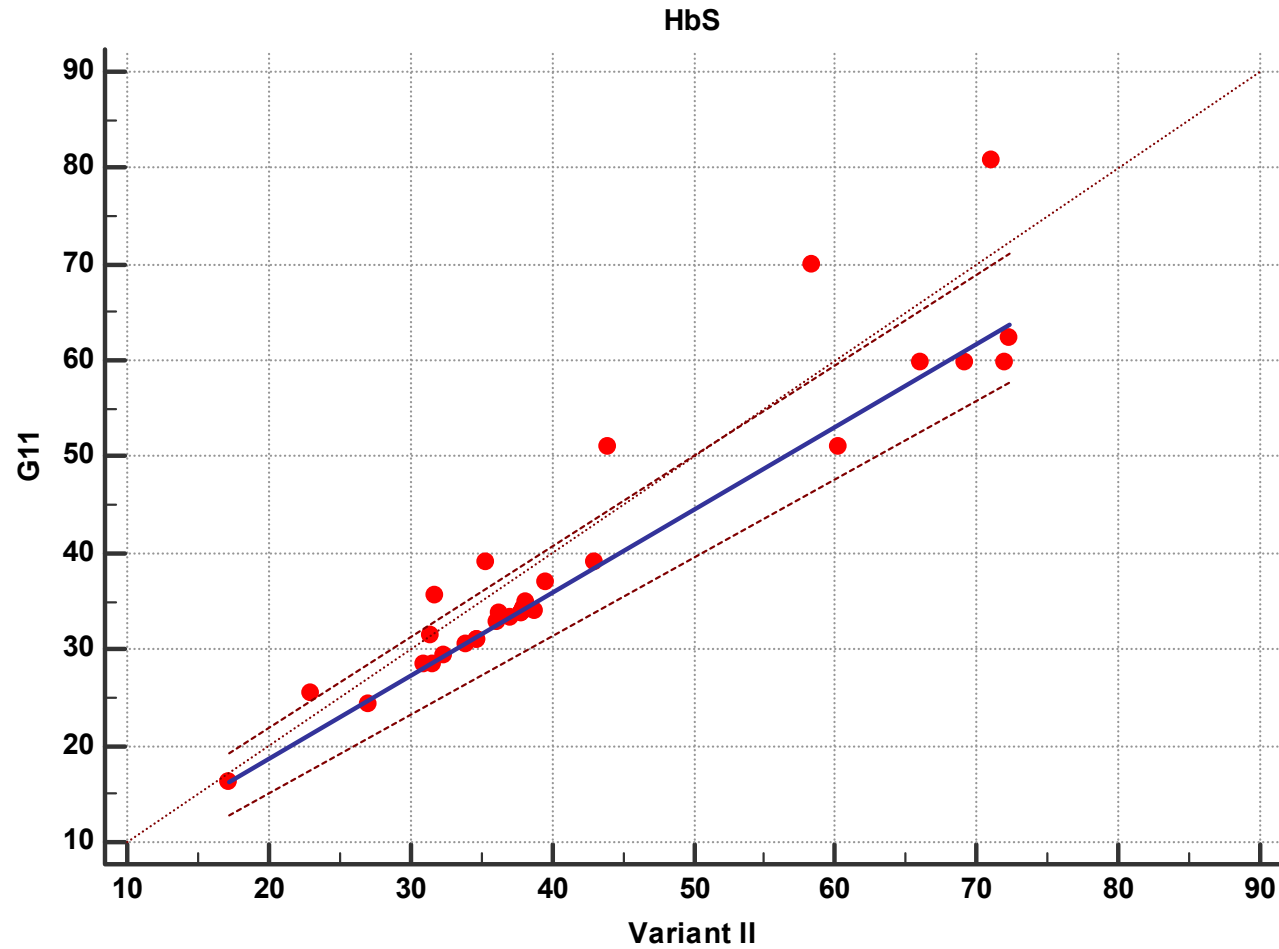
Cusum test for linearity	No significant deviation from linearity (P=0,71)
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Spearman rank correlation coefficient

Correlation coefficient	0,901
Significance level	P<0,0001
95% CI	0,809 to 0,950



Comparison HbS :G11-Variant II



R = 0.901

$$G11 = 0.86 \text{ V II} + 1.4$$



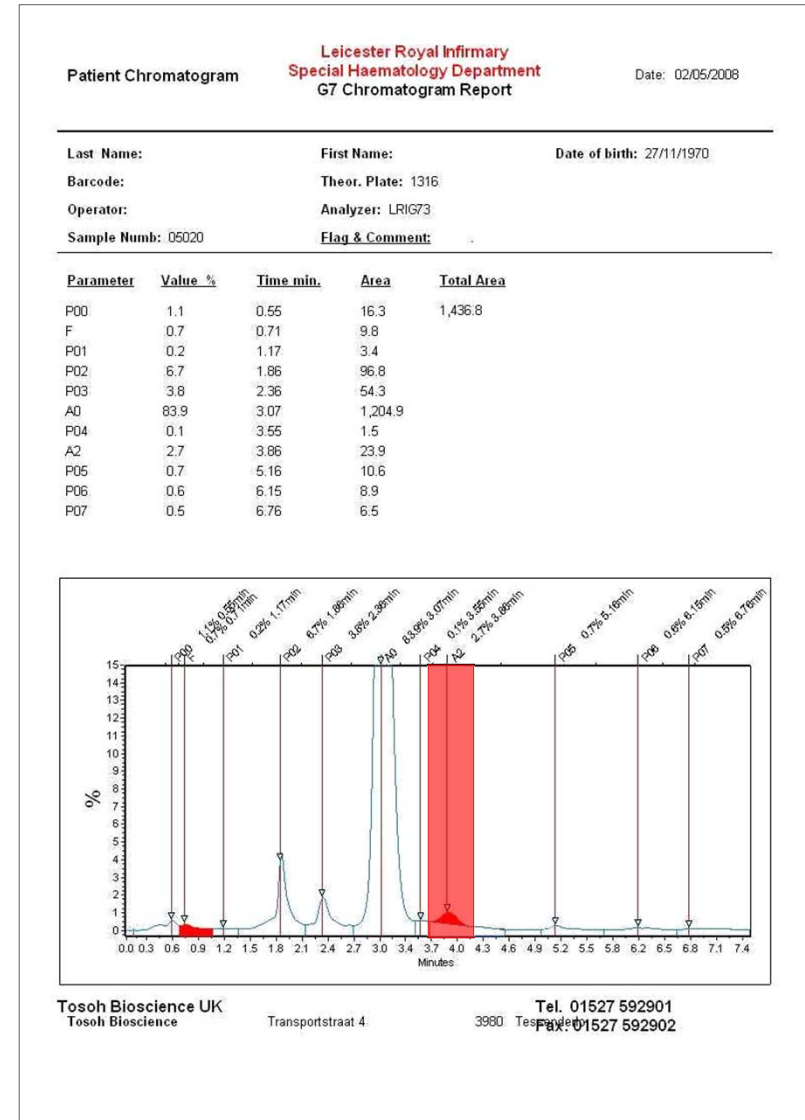
The G8 HbA₂ "window"

HbA₂

HbE

HbD Iran

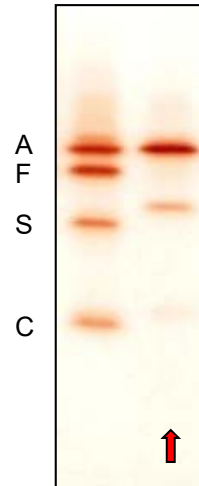
HbLepore





Hb Lepore trait
♂ (Greek Cypriot) Age 70

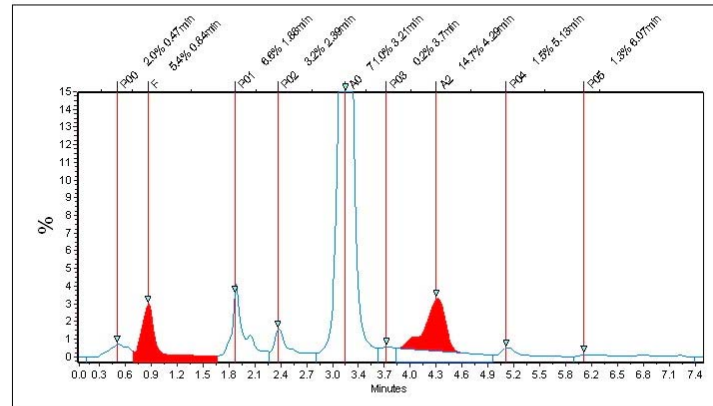
RBC 5.45
Hb 12.9
MCV 74
MCH 23.7



Leicester Royal Infirmary
Special Haematology Department
G7 Chromatogram Report
Date: 18/05/2007

Last Name: First Name: Date of birth: 14/05/1961
Barcode: 468628 Theor. Plate: 2611
Operator: SUPERUSER Analyzer: LRIG73
Sample Num: 05010 Flag & Comment: 23 HbE Suspected

Parameter	Value %	Time min.	Area	Total Area
P00	2.0	0.47	13.8	706.1
F	5.4	0.84	38.5	
P01	6.6	1.88	46.3	
P02	3.2	2.39	22.4	
A0	71.0	3.21	501.5	
P03	0.2	3.7	1.1	
A2	14.7	4.29	62.7	
P04	1.5	5.13	10.4	
P05	1.3	6.07	9.3	



Tosoh Bioscience UK Tel. 01527 592901
Tosoh Bioscience Transportstraat 4 3980 Tesselt Fax: 01527 592902



TOSI

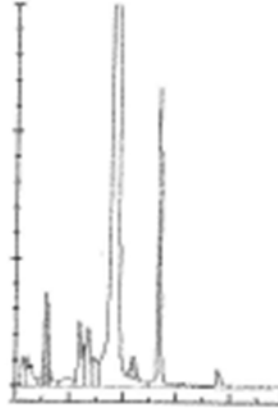
Hb Lepore

** THALASSEMIA REPORT **

2017/09/22 10:53
 OP:Root
 NO: 0003 SL 0002 - 01
 V03.00
 ID: 15526
 CAL F Y= 0.9552X - 0.0536
 A2 Y= 1.3852X + 0.3157

NAME	%	TIME	AREA
F	4.1	0.60	50.36
A0	69.1	1.88	800.66
A2	2.4	2.21	17.26
E+	0.0	0.00	0.00
D+	0.0	0.00	0.00
S+	0.0	0.00	0.00
C+	0.0	0.00	0.00
TOTAL AREA			1159.01

F : 4.1%
 A2: 2.4%



P00	1.2	0.17	13.93
P01	1.9	0.24	22.38
P02	0.4	0.43	4.74
P03	0.4	0.67	4.98
P04	1.2	1.02	13.45
P05	3.5	1.21	40.20
P06	3.3	1.37	38.69
P07	1.9	1.47	21.45
P08	8.9	2.66	103.65
P09	0.4	3.14	5.20
P10	0.1	3.49	1.73
P11	1.0	3.78	12.10
P12	0.6	4.07	6.56
P13	0.1	4.68	1.64



Relative frequency of Hb VAR (I)

Frequenze relative delle principali varianti emoglobiniche riscontrate presso i laboratori degli autori

Variante emoglobinica	Frequenza	
	1995	2010
HbS	61,0%	67,0%
HbC	3,5%	5,3%
HbD Punjab (Los Angeles)	5,9%	4,5%
Hb Lepore Boston ^a	4,5%	4,0%
HbE ^a	0,4%	3,3%
Hb Hasharon	3,1%	2,0%
HbJ Oxford	2,5%	1,5%
HbJ Sardegna	1,4%	1,2%
HbG Copenhagen	1,0%	0,5%
HbG St. Josè	0,8%	0,4%
Hb Camperdown	0,4%	0,3%
Altre rare varianti	15,5%	10,0%

^a*Varianti associate a fenotipo talassemico.*

biochimica clinica, 2016, vol. 40, n. 2



TOSOH

Haemoglobin AE Variant

Patient Chromatogram

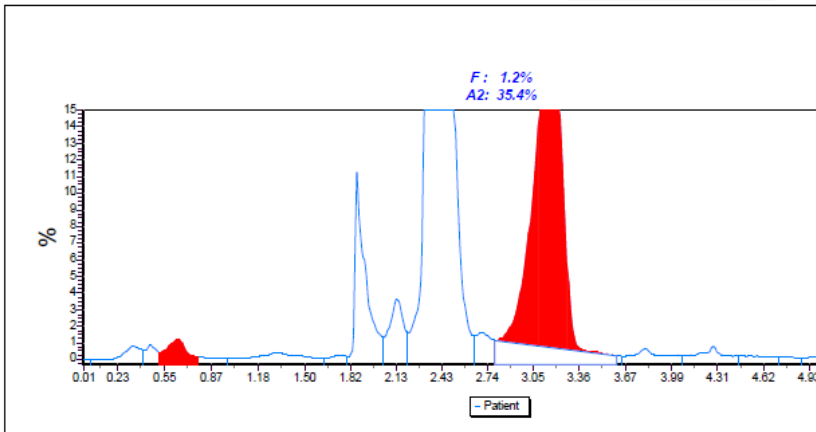
Date: 18/06/2018

Last Name: Result First Name: Unknown Date of birth:
 Barcode: BEGUM RIPA Theor. Plate: 1547 PUI:
 Rack: 0005 Operator: SUPERUSER Version: 4.41.0.0 Rev. I
 Position: 08 Analyzer: G8 Date of analysis: 19/09/2017
 Sample Numb: 09190 Flag & Comment: Time of analysis: 17.42.49

Parameter	Value %	Time min.	Area	Total Area	Y=(Ax+B)
P00	0.6%	0.33	29.53	4.981.8	
P01	0.4%	0.45	21.04		
F	1.2%	0.63	50.89		
P02	0.1%	0.78	5.94		
P03	0.8%	1.32	38.92		
P04	0.2%	1.73	8.94		
P05	5.5%	1.85	271.87		
P06	2.4%	2.13	119.04		
A0	57.0%	2.36	2.841.39		
P07	4.6%	2.7	307.68		
A2	35.4%	3.16	1.224.97		
P08	0.8%	3.8	39.61		
P09	0.8%	4.27	39.09		
P10	0.3%	4.5	13.95		
P11	0.1%	4.75	5.97		
P12	0.1%	4.98	7.12		
P13	0.2%	5.18	8.03		
P14	0.3%	5.33	15.87		
P15	0.2%	5.75	11.95		

Analyzer: G8
 Serial Nb.: 11819110
 Soft. Version: 5.24
 UIN: Analyzer UIN

B-Thalassemia



Patient Chromatogram

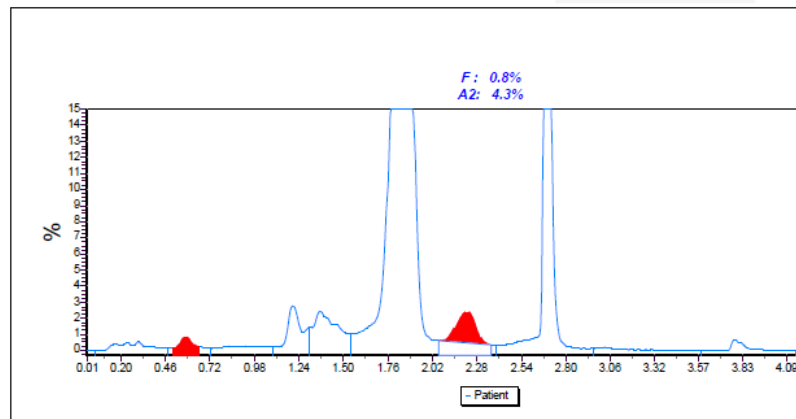
Date: 18/06/2018

Last Name: Result First Name: Unknown Date of birth:
 Barcode: 15536 Theor. Plate: 1130 PUI:
 Rack: 0005 Operator: SUPERUSER Version: 4.41.0.0 Rev. I
 Position: 08 Analyzer: G11 Date of analysis: 19/09/2017
 Sample Numb: 15490 Flag & Comment: Time of analysis: 15.37.08

Parameter	Value %	Time min.	Area	Total Area	Y=(Ax+B)
P00	1.4%	0.23	44.48	3.192.2	
P01	0.1%	0.48	1.88		
F	0.8%	0.57	27.88		
P02	0.1%	0.66	4.09		
P03	1.0%	0.87	31.69		
P04	3.2%	1.2	103.51		
P05	4.9%	1.36	155.49		
A0	60.9%	1.84	1.944.04		
A2	4.3%	2.22	94.88		
E+	23.0%	2.69	733.56		
P06	0.5%	3.02	17.38		
P07	0.8%	3.81	26.01		
P08	0.2%	4.23	7.56		

Analyzer: G11
 Serial Nb.: demo
 Soft. Version: demo
 UIN: Analyzer UIN

B-Thalassemia



TOSOH EUROPE Transportstraat 4 3980 Tessenderlo



Haemoglobin D Iran

** THALASSEMIA REPORT **

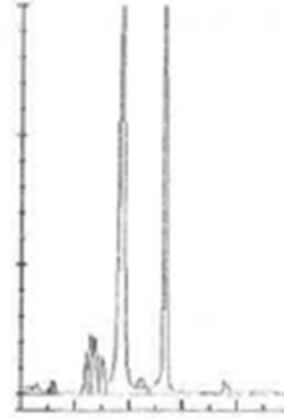
OP:ROOT 2017/02/02 12:26
 NO: 0024 SL 0003 - 02
 ID: 3.2 V03.00
 CAL F Y= 0.9207X + 0.0184
 A2 Y= 1.3911X + 0.2689

TP 1897

Column: ---/---/---
 Buff.1: ---/---/---
 Buff.2: ---/---/---
 Buff.3: ---/---/---
 H&W : ---/---/---
 CalSet: 2017/01/27

NAME	%	TIME	AREA
F	1.01	0.61	9.92
A0	44.82	1.90	412.91
A2	1.22	2.24	6.36
E+	35.85	2.69	330.26
D+	0.00	0.00	0.00
S+	0.00	0.00	0.00
C+	0.00	0.00	0.00
TOTAL		AREA	921.35

F : 1.01%
 A2 : 1.22%



P00	0.80	0.17	7.36
P01	1.05	0.31	9.66
P02	0.22	0.45	2.03
P03	0.26	0.69	2.42
P04	0.63	1.03	5.79
P05	2.45	1.23	22.56
P06	2.74	1.31	25.28
P07	3.43	1.39	31.63
P08	2.82	1.53	25.95
P09	2.01	3.78	18.49
P10	0.95	4.35	8.72



Tosoh HPLC G11 instrument

- Quick system
- Low CV
- Easy manipulation
- Separation from A2 of
HbE, Hb Lepore and HBD Iran



Thanks for your attention

Any Questions??