Tecniche di laboratorio per la determinazione delle varianti emoglobiniche.

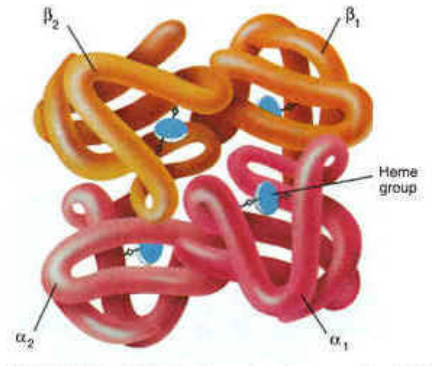
Giuseppe Lippi

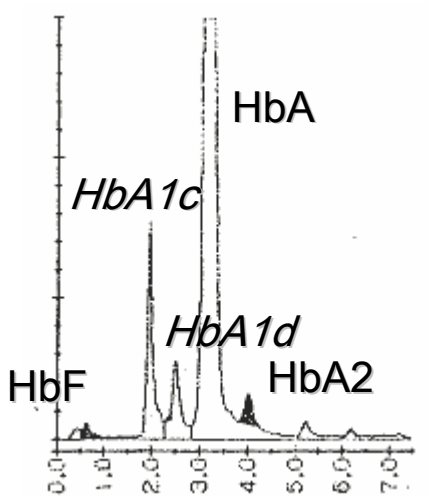
Università degli Studi di Verona

"*Emoglobinopati*" non ci s'inventa…

Si diventa!

Hemoglobin (Hb;[1] M_r 68 000) is the oxygen-carrying moiety of erythrocytes. It is a polypeptide tetramer, globular in structure, and consisting of two pairs of unlike globin chains (i.e., α plus β, δ, or γ), which form a shell around a central cavity containing four oxygen-binding heme groups each covalently linked to a globin chain. In healthy adults, ~95% of the Hb is Hb A ($\alpha_2\beta_2$) with small amounts (<3.5%) of Hb A_2 ($\alpha_2\delta_2$) and Hb F ($\alpha_2\gamma_2$) present. During embryonic development, "pre alpha" ζ globin chains contribute to embryonic Hb. During fetal development, β-like globin chains ϵ and γ contribute to the Hb *(1)*.





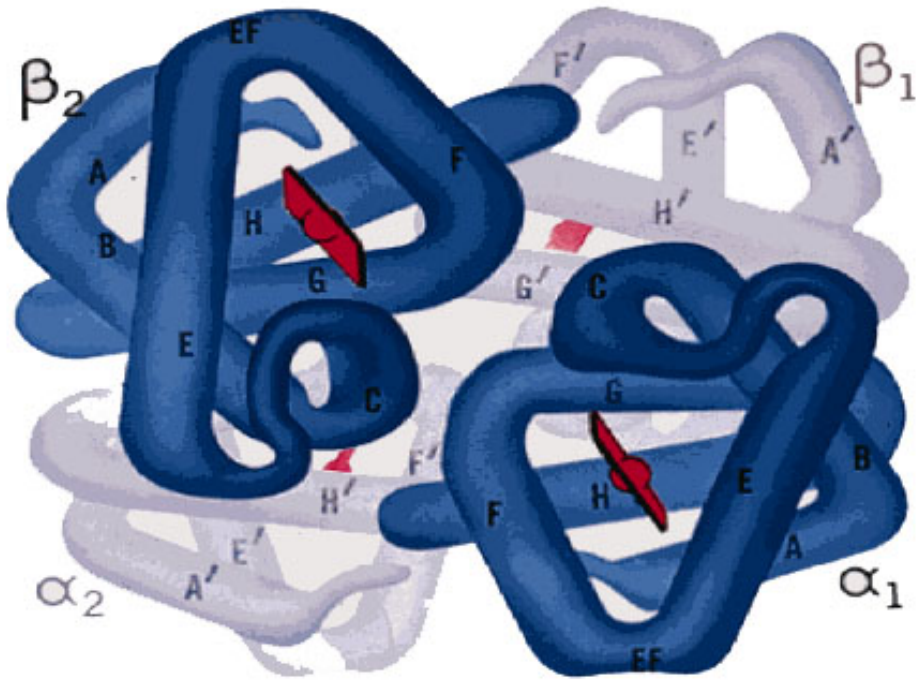
Clinical Chemistry 46:8(B) 1284–1290 (2000)

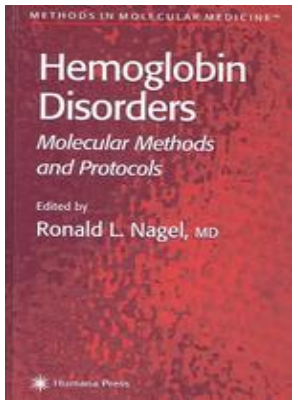
Laboratory Investigation of Hemoglobinopathies and Thalassemias: Review and Update

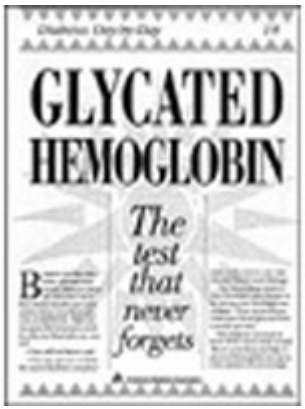
Gwendolyn M. Clarke and Trefor N. Higgins*

Cismed

Clinical applications of automated hemoglobin testing







HbA$_{1c}$

HbA$_{1d3a}$

HbA$_{1d3b}$

HbF

HbA$_2$

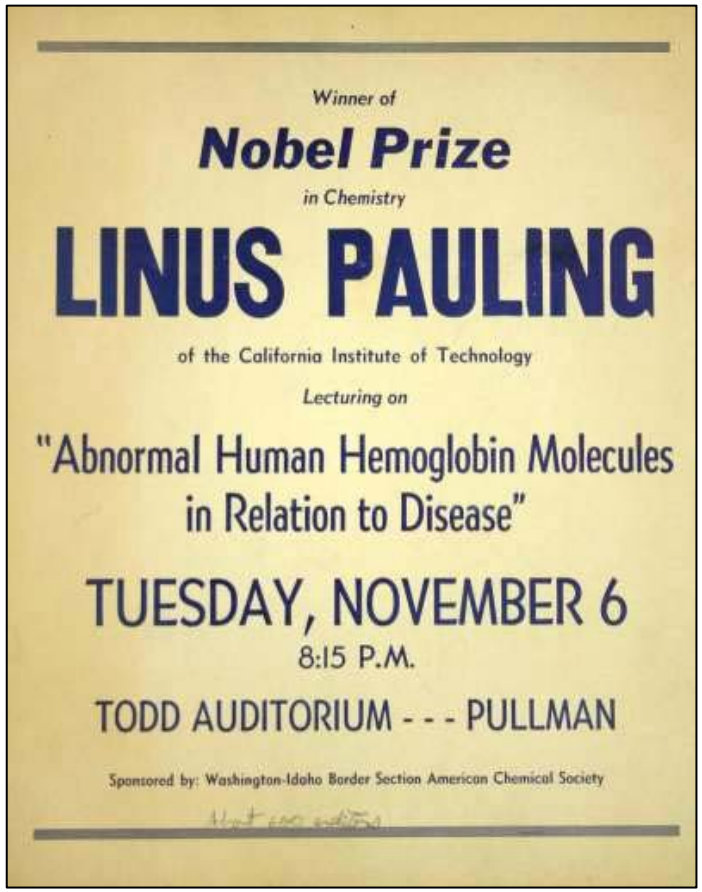
The exact quantification of minor Hbs has important diagnostic implications. For example, HbA$_{1c}$ is a marker for diabetes mellitus *(1, 17)*; HbA$_{1d3a}$ and HbA$_{1d3b}$ are increased in uremic and diabetic patients, respectively *(4)*; and the controversial influence of Hb–acetaldehyde adducts on HbA$_{1d3}$ in female heavy drinkers has been reported *(4, 11)*. HbF is increased in hereditary persistence of fetal Hb, β-thalassemia intermedia, β-thalassemia major, and specific drug treatments *(18)*. Increased HbF is known to inhibit the polymerization of HbS, and the monitoring of HbF concentrations during the follow-up and treatment of patients with sickle cell anemia is mandatory. HbA$_2$ is increased in β-thalassemia and in megaloblastic anemia *(1)* but is decreased in α-thalassemia, iron deficiency, and sideroblastic anemia *(1, 18)*.

Clinical Chemistry 51:6
980–996 (2005)

Analysis of Minor Hemoglobins by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry

Karin Zurbriggen,[1] Markus Schmugge,[1] Marlis Schmid,[1] Silke Durka,[2] Peter Kleinert,[2] Thomas Kuster,[2] Claus W. Heizmann,[2] and Heinz Troxler[2]*

Cismel



Abnormal Human Hemoglobin Molecules in Relation to Disease.

Northwest United States Section of the American Chemical Society, Pullman, Washington, November 5, 1956.

Disorders of globin chain synthesis, both thalassaemias and structurally abnormal haemoglobins, are common in the U.K. and constitute a significant public health problem. Diagnosis may be required:

1) To confirm a provisional diagnosis, such as sickle cell disease or beta-thalassemia major;

2) To explain a hematologic abnormality, such as anemia or microcytosis;

3) To identify an abnormality in the presymptomatic phase, as in neonatal screening;

4) To predict serious disorders of globin chain synthesis in the fetus and offer the option of termination of pregnancy;

5) To permit genetic counselling of prospective parents;

6) As preoperative screening for the presence of sickle cell disease.

British Journal of Haematology, 1998, 101, 783–792
Guideline
THE LABORATORY DIAGNOSIS OF HAEMOGLOBINOPATHIES*,†

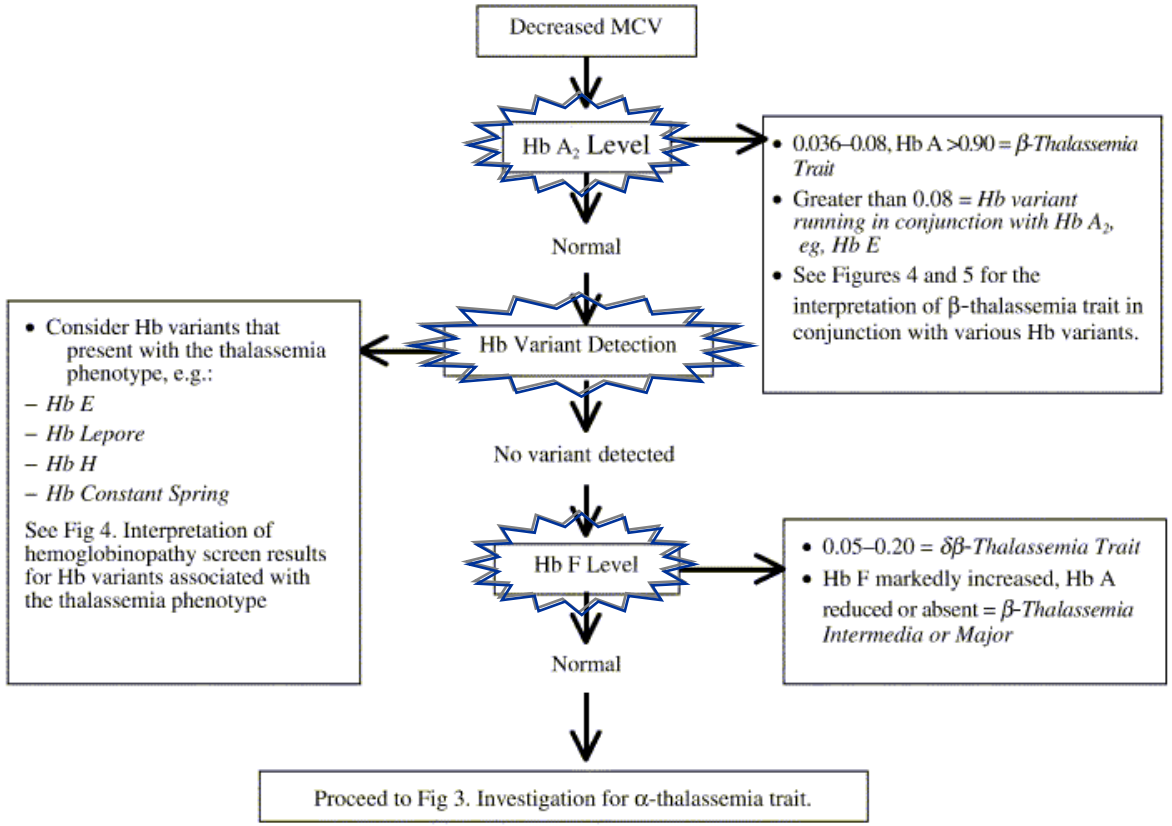
Improved fully automated systems and reagents for techniques such as high-performance liquid chromatography (HPLC) and isoelectric focusing (IEF) have led to their introduction in many laboratories. Immunological methods for the identification of variant haemoglobins have also become available. There is therefore a need for an updated guideline defining the role of new techniques in relation to traditional techniques.

British Journal of Haematology, 1998, **101**, 783–792

Guideline

THE LABORATORY DIAGNOSIS OF HAEMOGLOBINOPATHIES*,†

FIGURE 1. Preliminary hemoglobinopathy considerations based on complete blood cell count (CBC) [7,10-14,16,18]. MCV indicates mean cell volume; Hb, hemoglobin.



Laboratory Hematology 9:237-245

Good Practice Guidelines for Laboratory Investigation of Hemoglobinopathies

John D. Lafferty,[1,2] John S. Waye,[1,3] David H. K. Chui,[1,3] Linda Crawford,[4] Anne Raby,[4] Harold Richardson[4]

Further Investigation of Abnormal Findings [7,9,10,17-19]*

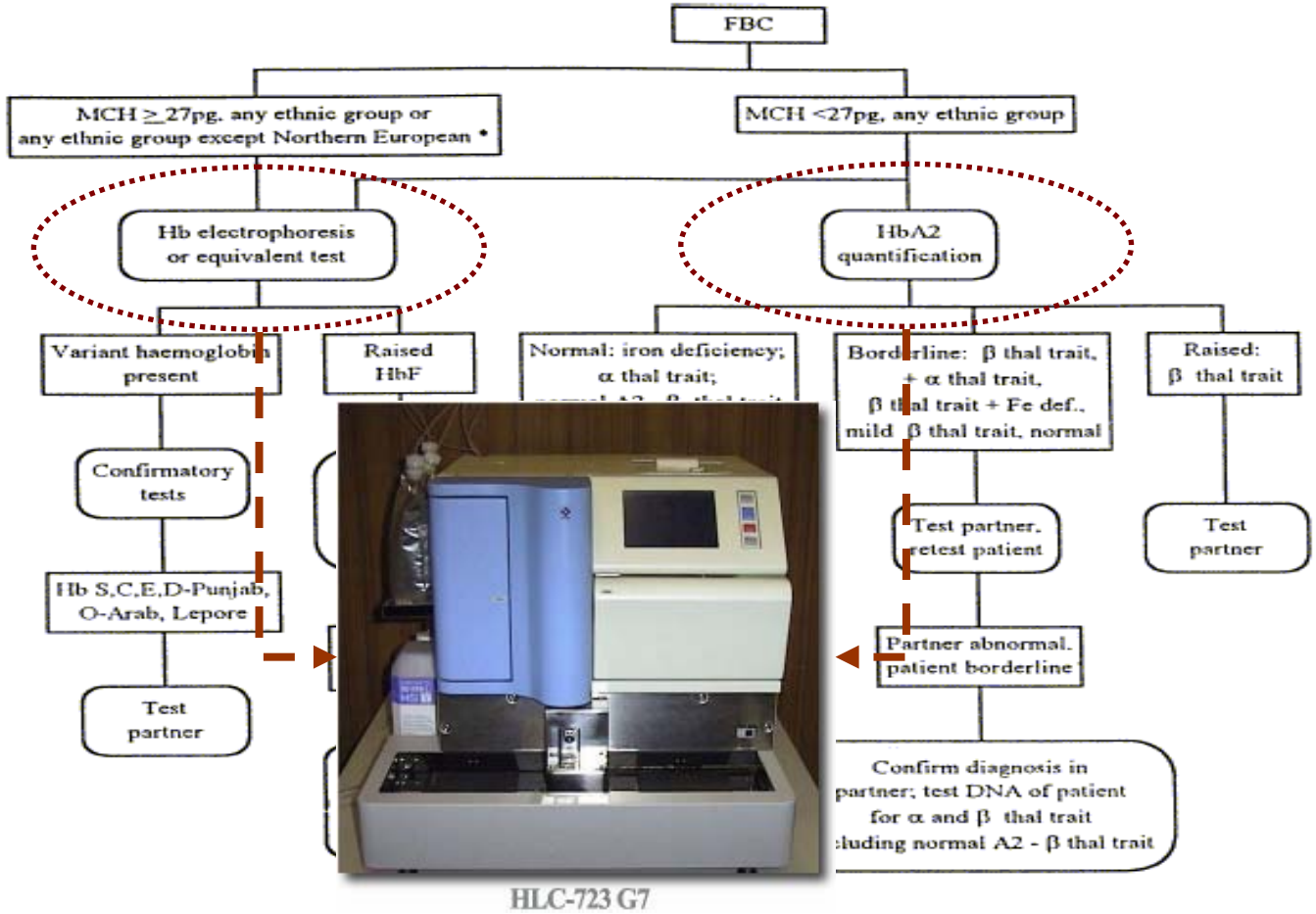
Hemoglobinopathy Investigation Finding	Confirmatory Investigative Technique
Hb S	Hb S solubility screen† Alternative Hb variant detection technique can be used but is not required.
Hb C	Hb S solubility screen† Alternative Hb variant detection technique‡
Other common Hb variants, eg, Hb E, Hb D Punjab, Hb Lepore	Alternate Hb variant detection technique‡
α-Thalassemia (Hb H inclusion body positive)	Genotype confirmation by DNA investigation if patient is involved in a pregnancy or planned pregnancy
Suspect α-thalassemia	DNA investigation if patient is involved in a pregnancy or planned pregnancy.
Rare Hb variant	Alternative Hb variant detection technique‡ Functional testing and subsequent DNA investigation if the patient is involved in a pregnancy or planning pregnancy or there is evidence of clinical significance, ie, Heinz body–induced hemolysis, abnormal oxygen affinity, unexplained cyanosis

Laboratory Hematology 9:237-245

Good Practice Guidelines for Laboratory Investigation of Hemoglobinopathies

John D. Lafferty,[1,2] John S. Waye,[1,3] David H. K. Chui,[1,3] Linda Crawford,[4] Anne Raby,[4] Harold Richardson[4]

Cismel



British Journal of Haematology, 1998, 101, 783–792

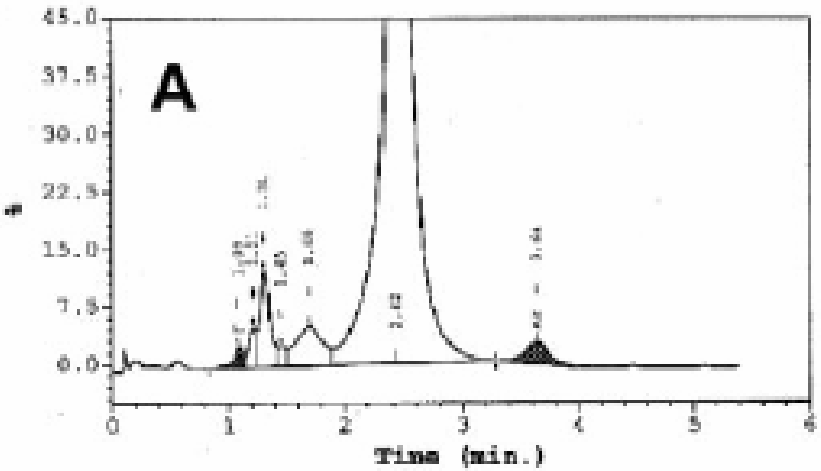
Guideline

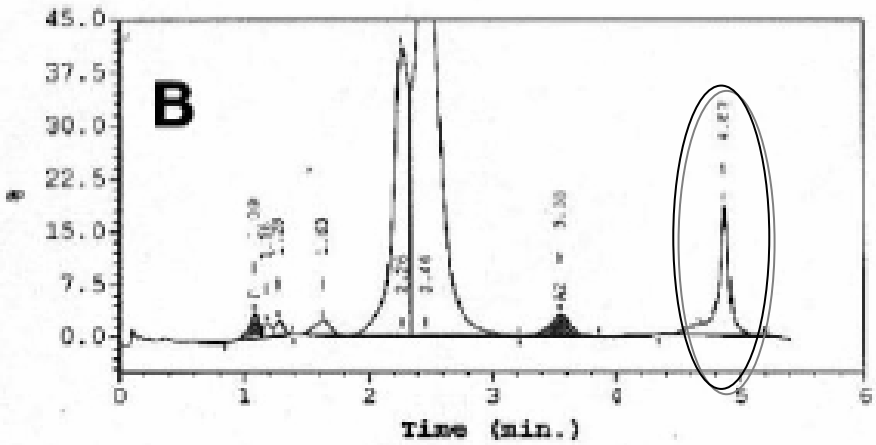
THE LABORATORY DIAGNOSIS OF HAEMOGLOBINOPATHIES*,†

Cismel

Emoglobine patologiche

- *HbS* -





Isoelectric focusing (IEF)
The haemoglobins which can be distinguished from each other by isoelectric focusing differ between different instrument/reagent systems (Table I). In addition, an increased percentage of haemoglobin A2 may be observed but this technique has not been validated for haemoglobin A2 quantification.

Variant haemoglobins which can be distinguished from each other by isoelectric focusing.*

Instrument/reagent system	Distinguished from each other
Isolab	A, F, S, C, D-Punjab, G-Philadelphia/Lepore, E/A2/O-Arab
Helena Rapid Electrophoresis	A, F, S. C, D-Punjab, G-Philadelphia, E/A2†, O-Arab
Pharmacia Phast	Information not available

British Journal of Haematology, 1998, 101, 783–792
Guideline

THE LABORATORY DIAGNOSIS OF HAEMOGLOBINOPATHIES*,†

High-performance liquid chromatography (HPLC)
HPLC can be used for the quantification of haemoglobins A2 and F and the detection, provisional identification and quantification of variant haemoglobins. The haemoglobins which can be distinguished from each other vary somewhat between different instruments and reagent systems.

Variant haemoglobins which can be distinguished from each other by high-performance liquid chromatography.*

Instrument/reagent system	Distinguished from each other
Primus Variant System 99	A, F, S, C, E/A2, D-Punjab, G-Philadelphia, O-Arab
Kontron Instruments Haemoglobin System PV	A, F, S, C, E/A2†, D-Punjab, G-Philadelphia
BioRad Variant ('β thal short program')	A, F, S, C, E/A2, D-Punjab, G-Philadelphia, O-Arab
Glycomat 765 'Green' Kit	
In Hb A2 mode	A, F‡, S, C, D-Punjab/G-Philadelphia/E/A2
In variant mode	A, F, S, C, D-Punjab, G-Philadelphia, E/A2
Glycomat 'Gold' Kit (also Biomen Gold Kit)	
In HbA2 mode	A, F, S, C, D-Punjab/G-Philadelphia, E/A2
In variant mode	A, F, S, C, D-Punjab, G-Philadelphia, E/A2
Shimadzu Industry Standard HPLC	S, D-Punjab, G-Philadelphia, C, E/A2, O-Arab
Protech Scientific Ltd, HaemaChrom	Information not available

British Journal of Haematology, 1998, 101, 783–792

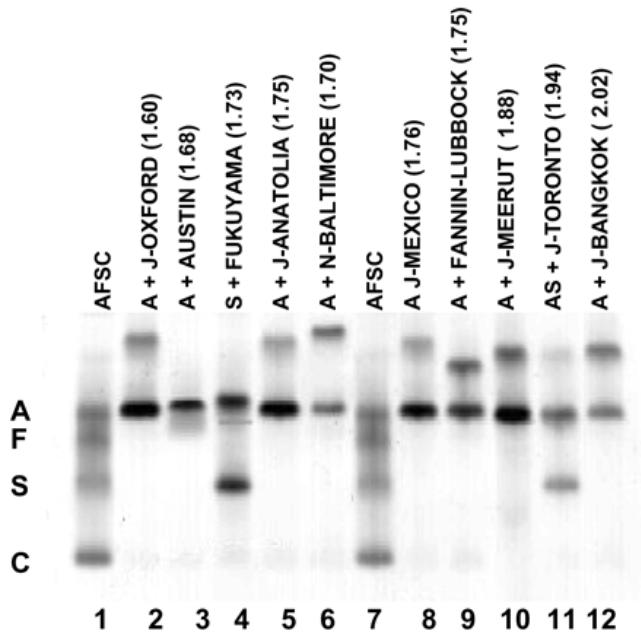
Guideline

THE LABORATORY DIAGNOSIS OF HAEMOGLOBINOPATHIES*,†

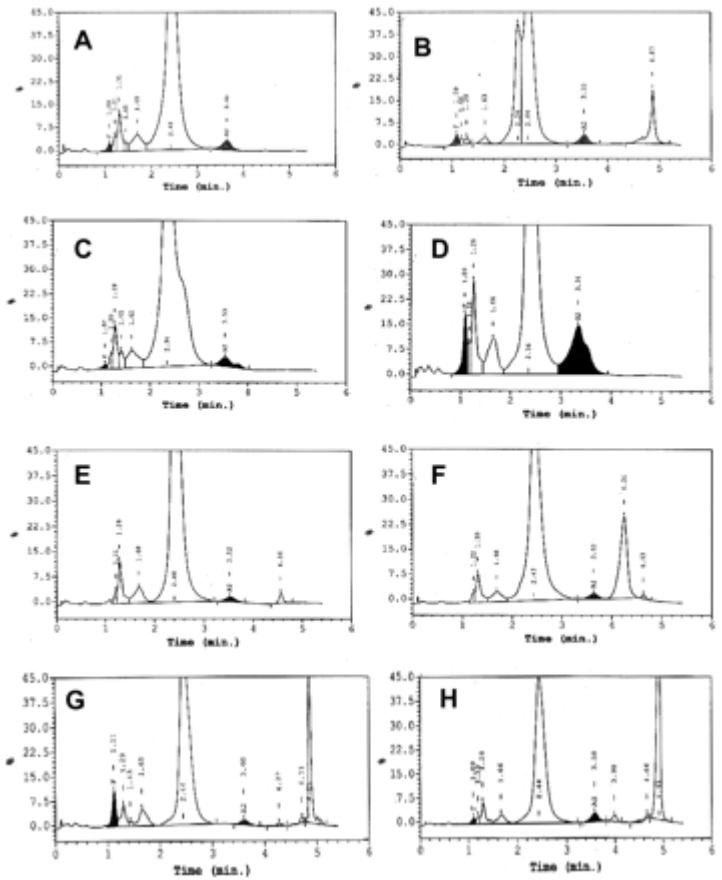
Clinical Chemistry 50:10
1736–1747 (2004)

HPLC Retention Time as a Diagnostic Tool for Hemoglobin Variants and Hemoglobinopathies: A Study of 60 000 Samples in a Clinical Diagnostic Laboratory

Alla Joutovsky,[1] Joan Hadzi-Nesic,[1] and Michael A. Nardi[2*]



Alkaline hemoglobin electrophoresis



Elution chromatograms

Clinical Chemistry 50:10
1736–1747 (2004)

HPLC Retention Time as a Diagnostic Tool for Hemoglobin Variants and Hemoglobinopathies: A Study of 60 000 Samples in a Clinical Diagnostic Laboratory

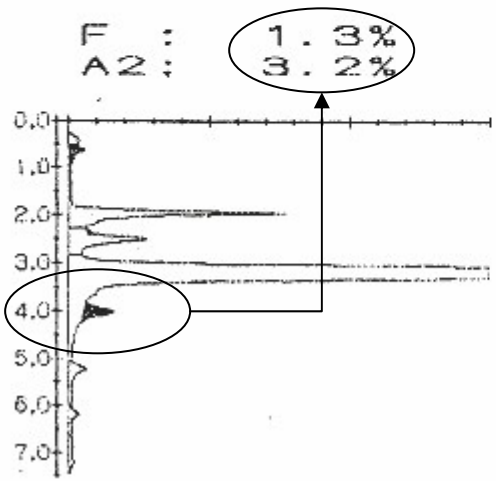
Alla Joutovsky,[1] Joan Hadzi-Nesic,[1] and Michael A. Nardi[2*]

Cismel

β-thalassaemia (7.5 minute) mode examples

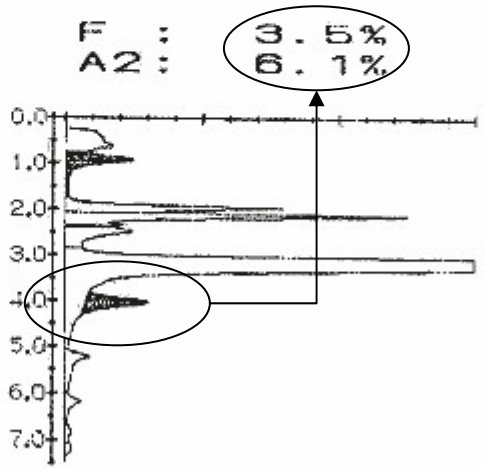
Normal Patient Example

NAME	%	TIME	AREA
F	1.3	0.83	21.47
A0	83.5	3.17	1366.22
A2	3.2	4.03	23.59
D+	0.0	0.00	0.00
S+	0.0	0.00	0.00
C+	0.0	0.00	0.00
	TOTAL	AREA	1635.80



β-thal-Trait Patient Example

NAME	%	TIME	AREA
F	3.5	0.83	57.42
A0	71.3	3.17	1182.42
A2	6.1	4.05	45.67
D+	0.0	0.00	0.00
S+	0.0	0.00	0.00
C+	0.0	0.00	0.00
	TOTAL	AREA	1659.00



Screening for Hemoglobinopathies During Routine Hemoglobin A1c Testing Using the Tosoh G7 Glycohemoglobin Analyzer

L. Brannon Thomas,[1,2] Steven J. Agosti,[1,2] Mary A. Man,[1] and Stephen M. Mastorides[1,2]
[1] Department of Pathology and Laboratory Medicine, James A. Haley VA Medical Center, Tampa, Florida;
[2]Department of Pathology and Cell Biology, University of South Florida, Tampa, Florida

The retention times (RT) (min) observed for various Hb variants and hemoglobinopathies.

Hb variant	N	Mutation (7)(8)	Migration pattern (7)(8)	Sickle test	Mean RT	SD
Hb AS	252	β 6Glu→Val	Alkaline/Acid: A + S position	+	1.44	0.02
Hb SS	2	β 6Glu→Val	Alkaline/Acid: S position	+	1.43	-
Hb AC	82	β 6Glu→Lys	Alkaline: A + A_2 Acid: A + C	-	1.66	0.03
Hb CC	2	β 6Glu→Lys	Alkaline: A_2 Acid: C	-	1.67	-
Hb SC	1	β 6Glu→Val 6Glu→Lys	Alkaline: S + A_2 Acid: S + C	+	1.40, 1.62	-
Hb C Harlem trait	2	β 6 Val, 73Asn	Alkaline: C + S Acid: S	+	1.51	-
α chain variant 1[a]	1	α 75Asp→Tyr	Alkaline: anodal to S Acid: A	-	1.32	-
α chain variant 2[b]	4	Various	Alkaline: A + S Acid: anodal to S	-	1.52	0.02
D trait[c]	9	β 121Glu→Gln	Alkaline: A + S Acid: A	-	1.26	0.05
G trait[c]	4	α 68Asn→Lys	Alkaline: A + S Acid: A	-	1.27	0.05
Hb J variant (Baltimore)	4	β 16Gly→Asp	Alkaline: Fast migration Acid: A	-	1.01	0.01
Hb Raleigh	2	β 1Val→ac-Ala	Alkaline: A Acid: A + F	-	0.66	-
Hb Lepore	1	Fusion, β and δ genes	Alkaline: S Acid: A	-	Not detected	-

Hemoglobins seen.

Variant name	n[a]	Retention time,[b] min	%Hb[c]	ΔTime,[d] months	Variant[e]
Hb Barts	ND[f]	0.2	ND	32	γ4
Hb H	12	0.2	12.5 (4.0)	32	β4
Hb F_1	ND	0.5	ND	32	–
Hb F	160[g]	1.10 (0.017)	1.0 (0.5)	32	–
Hb Hope	11	1.39 (0.007)	45.9 (2.2)	32	β 136Gly→Asp
Hb Camden	2	1.50; 1.48	52.4; 49.3	5	β 131Gln→Glu
Hb J-Oxford	1	1.60	24.7	–	α 15Gly→Asp
Hb Austin	3	1.68 (0.017)	47.1 (0.4)	12	β 40Arg→Ser
Hb N-Baltimore	6	1.70 (0.031)	47.8 (0.9)	21	β 95Lys→Glu
Hb Fukuyama	2	1.72; 1.73	–[h]	0	β 77His→Tyr
Hb Fannin-Lubbock	7	1.75 (0.024)	35.0 (3.0)	21	β 119Gly→Asp
Hb J-Anatolia	2	1.75; 1.75	19.9; 21.2	0.5	α 61Lys→Thr
Hb J-Mexico	2	1.74; 1.78	22.7; 22.3	10	α 54Gln→Glu
Hb J-Meerut	2	1.88; 1.88	25.4; 25.2	11	α 120Ala→Glu
Hb J-Toronto	1	1.94	–[i]	–	α 5Ala→Asp
Hb J-Bangkok	1	2.02	43.6	–	β 56Gly→Asp
Hb Ty Gard	1	2.20	34.1	–	β 124Pro→ Gln
Hb Köln[j]	2	2.26; 2.26 (4.93; 4.87)[j]	26.8; 23.5 (7.0; 7.3)[j]	24	β 98Val→Met
Hb A_0	160[g]	2.43 (0.041)	86.3 (1.5)	32	–
Hb New York	4[k]	2.43 (0.010)	Does not separate	12	β 113Val→Glu
Hb Twin Peaks	3	Appears as hump	Does not separate	11	α 113Leu→His
Hb Lepore	3	3.37 (0.019)	12.1 (1.5)	24	δβ-hybrid
Hb D-Iran	1	3.49	47.7	–	β 22Glu→Gln
Hb A_2	160[g]	3.63 (0.035)	2.7 (0.4)	32	–
Hb E	83[l]	3.69 (0.069)	30.3 (4.0)[m]	32	β 26Glu→Lys
Hb Osu-Christiansborg	1	3.77	44.0	–	β 52Asp→Asn
Hb G-Honolulu	1	3.86	27.4	–	α 30Glu→Gln
Hb Korle-Bu	8	3.92 (0.050)	46.5 (3.7)	16	β 73Asp→Asn
Hb D-Punjab	7	4.18 (0.007)	33.1 (1.8)	24	β 121Glu→Gln
Hb G-Philadelphia	8	4.22 (0.037)	26.4 (6.6)	29	α 68Asn→Lys
Hb E-Saskatoon	2	4.34; 4.32	39.3; 40.4	2	β 22Glu→Lys
Hb S	3587[n]	4.51 (0.030)	34.9 (4.1)[m]	32	β 6Glu→Val
Hb Manitoba	1	4.58	16.5	–	α 102Ser→Arg
Hb Montgomery	7	4.58 (0.020)	15.7 (2.2)	32	α 48Leu→Arg
Hb A_2'	81[o]	4.59 (0.030)	1.2 (0.1)	2	δ 16Gly→Arg
Hb Q-Thailand	1	4.67	29.3	–	α 74Asp→His
Hb Hasharon	7	4.83 (0.016)	17.9 (1.5)	32	α 47Asp→His
Hb O-Arab	6	4.91 (0.008)	35.9 (3.0)	24	β 121Glu→Lys
Hb G-Siriraj	1	5.08	24.2	–	β 7Glu→Lys
Hb C	962[p]	5.18 (0.013)	35.6 (4.0)[m]	32	β 6Glu→Lys

Clinical Chemistry 50:10
1736–1747 (2004)

HPLC Retention Time as a Diagnostic Tool for Hemoglobin Variants and Hemoglobinopathies: A Study of 60 000 Samples in a Clinical Diagnostic Laboratory

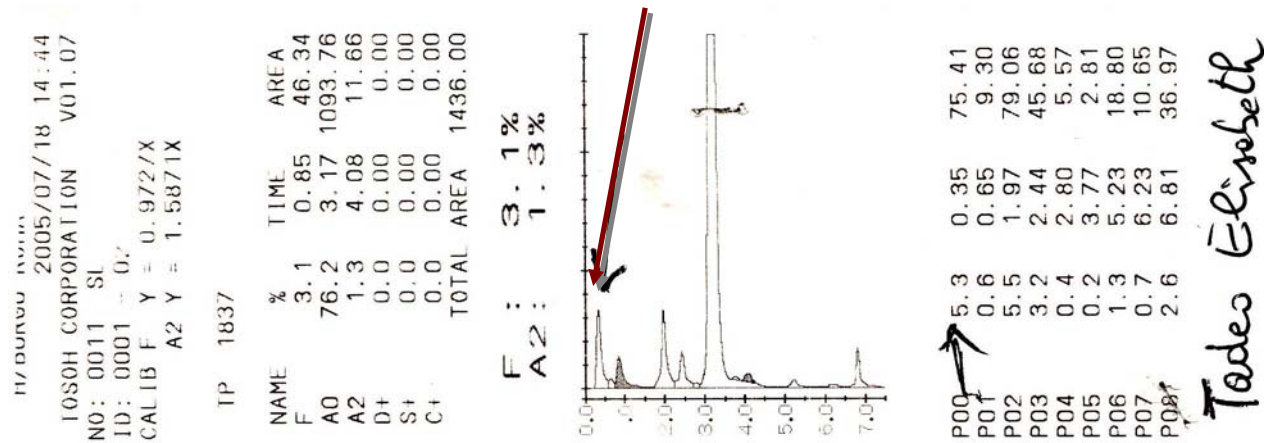
Alla Joutovsky,[1] Joan Hadzi-Nesic,[1] and Michael A. Nardi[2*]

Table 1. Manufacturer-assigned windows for Bio-Rad Variant II HPLC system.

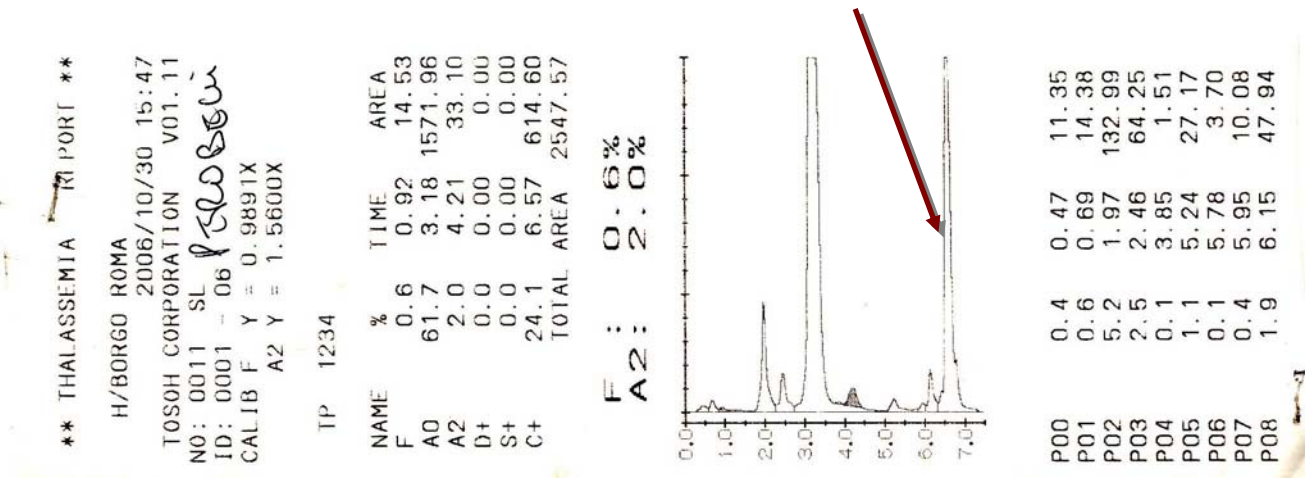
Peak name	Retention time, min
P1 window	0.63–0.85
F window	0.98–1.20
P2 window	1.24–1.40
P3 window	1.40–1.90
A_0 window	1.90–3.10
A_2 window	3.30–3.90
D window	3.90–4.30
S window	4.30–4.70
C window	4.90–5.30

Emoglobine instabili

- *HbH* -



Emoglobine instabili
- *Hb Hasaron* -



** THALASSEMIA REPORT **

H/BORGO ROMA
2006/10/30 15:47
TOSOH CORPORATION V01.11
NO: 0011 SL
ID: 0001 - 06
CALIB F Y = 0.9891X
A2 Y = 1.5600X

TP 1234

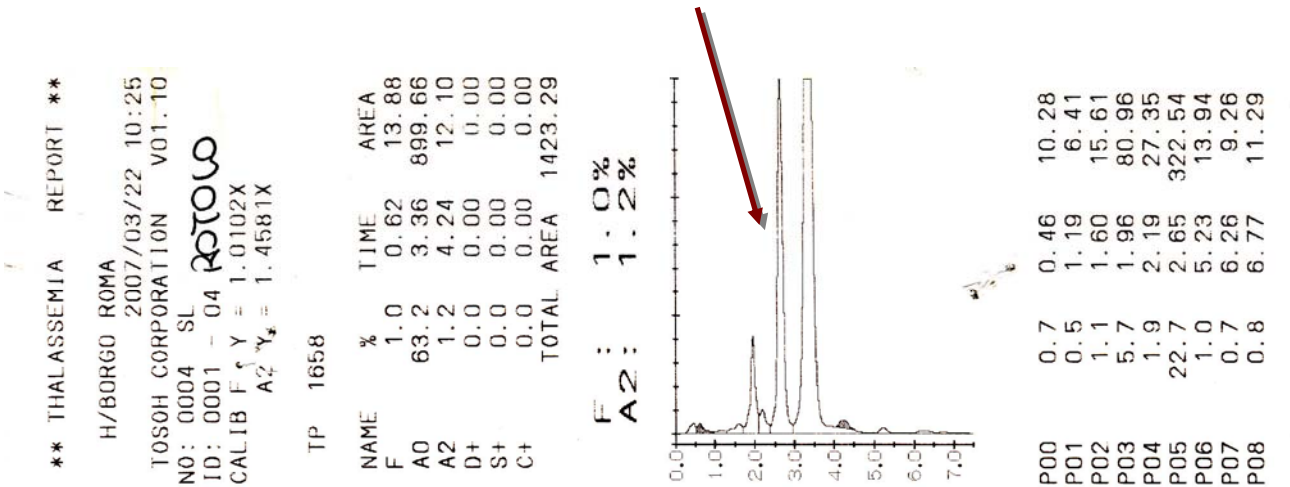
NAME	%	TIME	AREA
F	0.6	0.92	14.53
A0	61.7	3.18	1571.96
A2	2.0	4.21	33.10
D+	0.0	0.00	0.00
S+	0.0	0.00	0.00
C+	24.1	6.57	614.60
	TOTAL AREA		2547.57

F : 0.6%
A2 : 2.0%

0.0 1.0 2.0 3.0 4.0 5.0 6.0 7.0

P00	0.4	0.47	11.35
P01	0.6	0.69	14.38
P02	5.2	1.97	132.99
P03	2.5	2.46	64.25
P04	0.1	3.85	1.51
P05	1.1	5.24	27.17
P06	0.1	5.78	3.70
P07	0.4	5.95	10.08
P08	1.9	6.15	47.94

Emoglobine instabili
- *HbJ*-



** THALASSEMIA REPORT **

H/BORGO ROMA
2007/03/22 10:25
TOSOH CORPORATION V01.10
NO: 0004 SL
ID: 0001 - 04 ROTOLO
CALIB F Y = 1.0102X
A2 Y = 1.4581X

TP 1658

NAME	%	TIME	AREA
F	1.0	0.62	13.88
A0	63.2	3.36	899.66
A2	1.2	4.24	12.10
D+	0.0	0.00	0.00
S+	0.0	0.00	0.00
C+	0.0	0.00	0.00
	TOTAL AREA		1423.29

F : 1.0%
A2 : 1.2%

0.0 1.0 2.0 3.0 4.0 5.0 6.0 7.0

P00	0.7	0.46	10.28
P01	0.5	1.19	6.41
P02	1.1	1.60	15.61
P03	5.7	1.96	80.96
P04	1.9	2.19	27.35
P05	22.7	2.65	322.54
P06	1.0	5.23	13.94
P07	0.7	6.26	9.26
P08	0.8	6.77	11.29

Conclusions: The retention time on HPLC is reliable, reproducible, and in many cases superior to conventional hemoglobin electrophoresis for the detection and identification of hemoglobin variants. Confirmatory testing by electrophoresis can be eliminated in the majority of cases by use of retention time, proportion of total hemoglobin, and peak characteristics of HPLC.

Clinical Chemistry 50:10
1736–1747 (2004)

HPLC Retention Time as a Diagnostic Tool for Hemoglobin Variants and Hemoglobinopathies: A Study of 60 000 Samples in a Clinical Diagnostic Laboratory

Alla Joutovsky,[1] Joan Hadzi-Nesic,[1] and Michael A. Nardi[2*]

Summary of Hb variant retention times by HPLC.

Peak	Retention time on the Bio-Rad Variant, min	No. of variants	
		Mayo	Joutovsky et al. *(1)*
P1 window	0.63–0.85	1	0
Between P_1 and F	0.85–0.93	0	0
F window	0.93–1.25	8	0
P_2 window	1.25–1.43	9	1
Between P_2 and P_3	1.43–1.51	7	0
P_3 window	1.51–1.71	23	9
Between P_3 and A_0	1.71–1.91	17	0
A_0 window	1.91–3.03	76	6
Between A_0 and A_2	3.03–3.39	7	0
A_2 window	3.39–3.89	27	5
D window	3.89–4.31	26	3
S window	4.31–4.63	30	5
Between S and C	4.63–4.90	29	1
C window	4.90–5.30	8	3
Total		268	33

A recent search of the hemoglobin variant database (http://globin.cse.psu.edu) showed that there are currently 482 known β-chain variants and 297 variants of either the α_1- or α_2-globin chain.

In summary, we agree with the authors that the retention time obtained by HPLC, as well the percentage of the variant and the appearance of the chromatogram, are very useful pieces of information that can help in the identification of many Hb variants. However, the overlap of retention times of variants precludes definitive identification of Hb variants by HPLC alone. HPLC should not be used as the sole means of identification of Hb variants, particularly for laboratories that analyze only small numbers of samples.

Clinical Chemistry 51, No. 7, 2005
Identification of Hemoglobin Variants by HPLC
James D. Hoyer*
Regina M. Scheidt

- Hb E elutes in the A_2 window (range, 3.61–3.70 min). We have identified 18 other Hb variants, including 13 β-chain variants, that have a retention time that overlaps with the retention time range of Hb E.

- Hb S elutes in the S window (range, 4.38–4.50 min). We have observed 28 variants that have a retention time that overlaps with the retention time range of Hb S, including 10 other β-chain variants.

28!!!!!!

18!!!!!!

British Journal of Haematology, 1998, **101**, 783–792

Guideline

THE LABORATORY DIAGNOSIS OF HAEMOGLOBINOPATHIES*,†

Automated HPLC systems

When are further tests indicated? The nature of any variant haemoglobin detected by HPLC which is of potential clinical relevance (e.g. for genetic counselling) should be confirmed by an alternative technique.

Clinical Chemistry 51, No. 7, 2005

Identification of Hemoglobin Variants by HPLC

James D. Hoyer*
Regina M. Scheidt

Cismel

TECHNICAL REPORT INTERNATIONAL JOURNAL OF LABORATORY HEMATOLOGY

Separation of haemoglobin HbE and HbA_2 by the fully automated, high-pressure liquid chromatography Tosoh HLC-723 G7 analyzer

G. LIPPI*, M. R. CARTA†, G. L. SALVAGNO*, F. BELLORIO*, M. MONTAGNANA*, G. SOFFIATI†, G. C. GUIDI*

Figure 1. Electrophoretogram of hemoglobins on agarose gel at (a) alkaline (8.6) and (b) acid (6.0) pH. Lane 1: haemolysate from a normal subject; lane 2, haemolysate from a subject with double HbS and HbC heterozygosis; lane 3, haemolysate from a subject HbE heterozygosis.

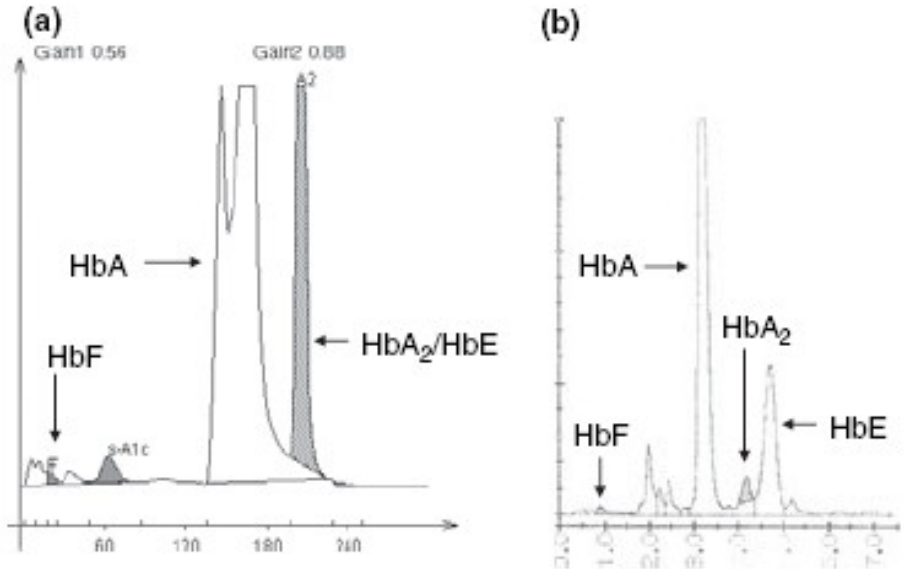
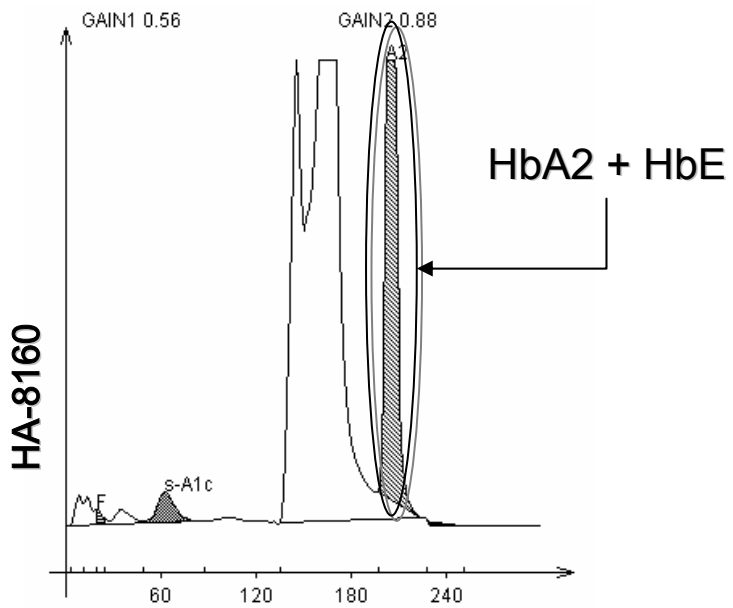


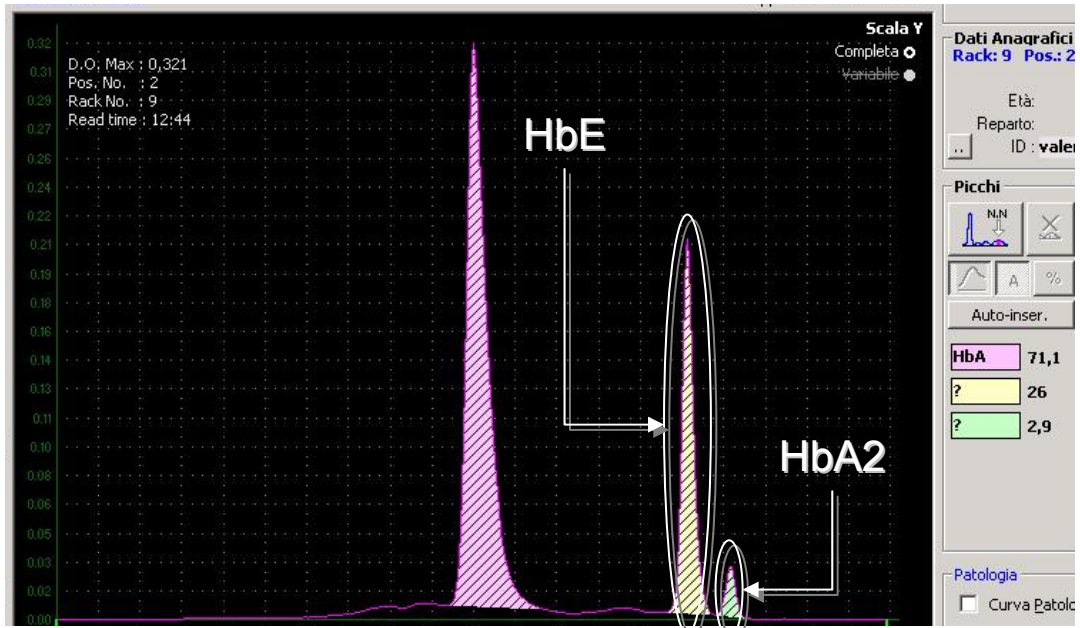
Figure 2. Elution patterns of a patient heterozygous for the HbE hemoglobin variant. (a) Elution pattern on the Menarini HA-8160. The HbA_2 peak is superimposed with that of HbE and it is indicated as 'A2/Var'. The quantification of the peaks is 1.0% HbF,

TECHNICAL REPORT INTERNATIONAL JOURNAL OF LABORATORY HEMATOLOGY

Separation of haemoglobin HbE and HbA_2 by the fully automated, high-pressure liquid chromatography Tosoh HLC-723 G7 analyzer

G. LIPPI*, M. R. CARTA†, G. L. SALVAGNO*, F. BELLORIO*, M. MONTAGNANA*, G. SOFFIATI†, G. C. GUIDI*

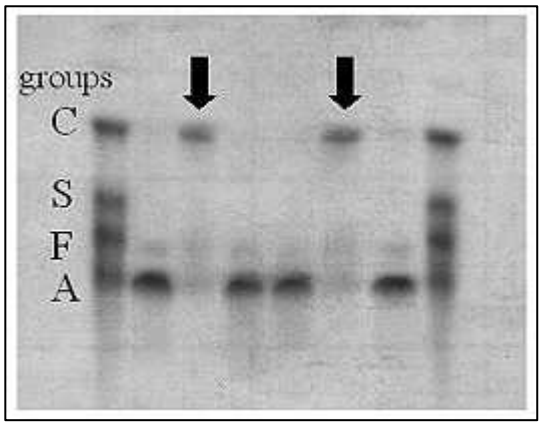




Capillary electrophoresis

Haemoglobin electrophoresis on cellulose acetate at alkaline pH (pH 8·2–8·6)

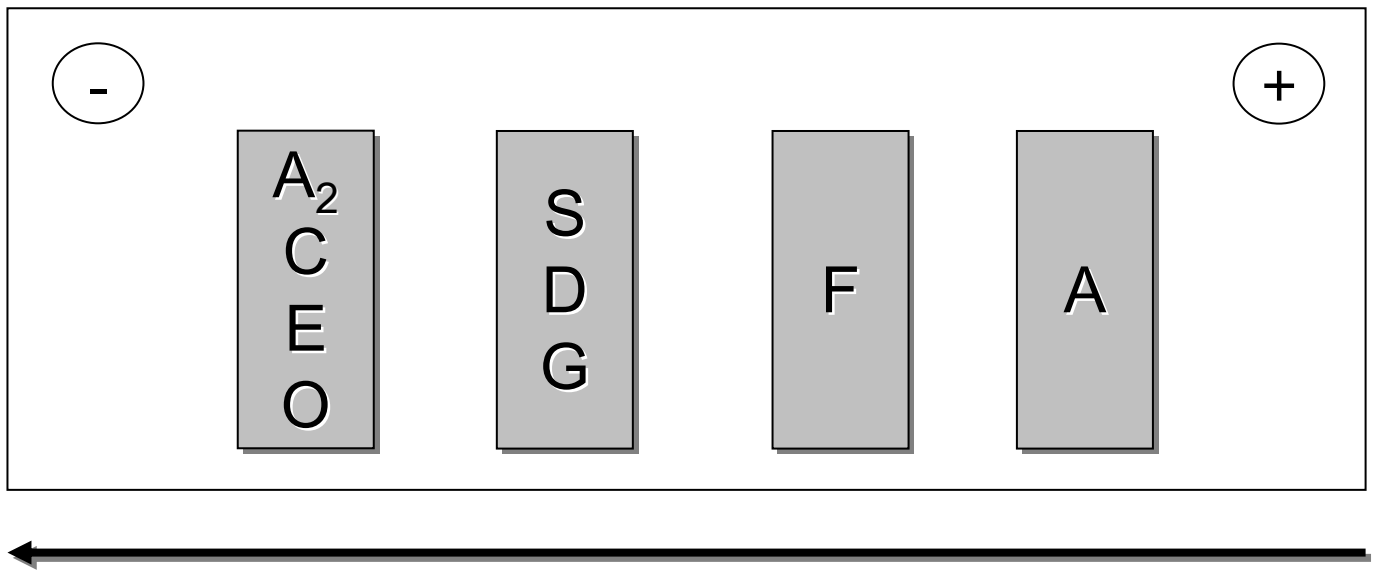
Cellulose acetate electrophoresis enables the provisional identification of haemoglobins A, F, S/G/D, C/E/O-Arab, H and a number of less common variant haemoglobins. With good electrophoretic techniques, haemoglobin F levels >2% can be recognized visually; when an increased level is detected, quantification is required. Good techniques also enable a split A2 band to be recognized. This is useful in helping to distinguish α-chain variants, e.g. haemoglobin G Philadelphia, from β-chain variants, e.g. haemoglobin D Punjab. It is also essential if β-thalassaemia trait is to be diagnosed in individuals who also have a δ-chain variant (see below).



1) Hb A, F,
2) Hb H,
3) Hb S/G/D,
4) Hb C/E/O-Arab

British Journal of Haematology, 1998, 101, 783–792
Guideline
THE LABORATORY DIAGNOSIS OF HAEMOGLOBINOPATHIES*,†

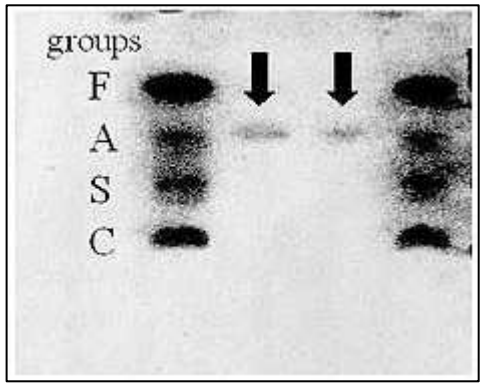
Elettroforesi a pH 8.2 – 8.6 (ALCALINA)



Haemoglobin electrophoresis on citrate agar or agarose gel at pH 6·0–6·2

Electrophoresis at acid pH on citrate agar or appropriate agarose gel is usually used as a supplement to cellulose acetate electrophoresis at alkaline pH. There are differences in the relative mobilities of variant haemoglobins between citrate agar and agarose gel. Both techniques distinguish haemoglobin S from D/G but do not distinguish between most types of D and G. They will distinguish haemoglobin C from E, C-Harlem and O-Arab.

Electrophoresis at acid pH is indicated in the investigation of suspected high-affinity haemoglobins even when electrophoresis at alkaline pH is normal

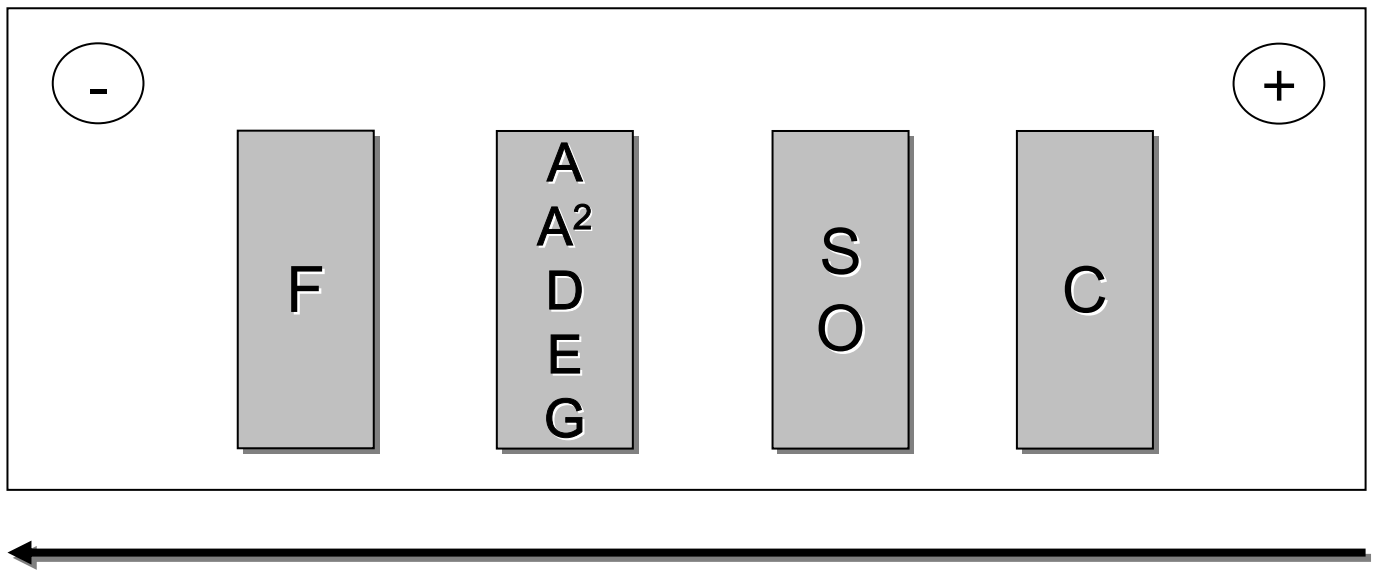


1) Hb D & G
2) Hb E, Harlem, O-Arab
3) Hb High affinity

British Journal of Haematology, 1998, 101, 783–792
Guideline
THE LABORATORY DIAGNOSIS OF HAEMOGLOBINOPATHIES*,†

Cismel

Elettroforesi a pH 6.0 – 6.2 (ACIDA)



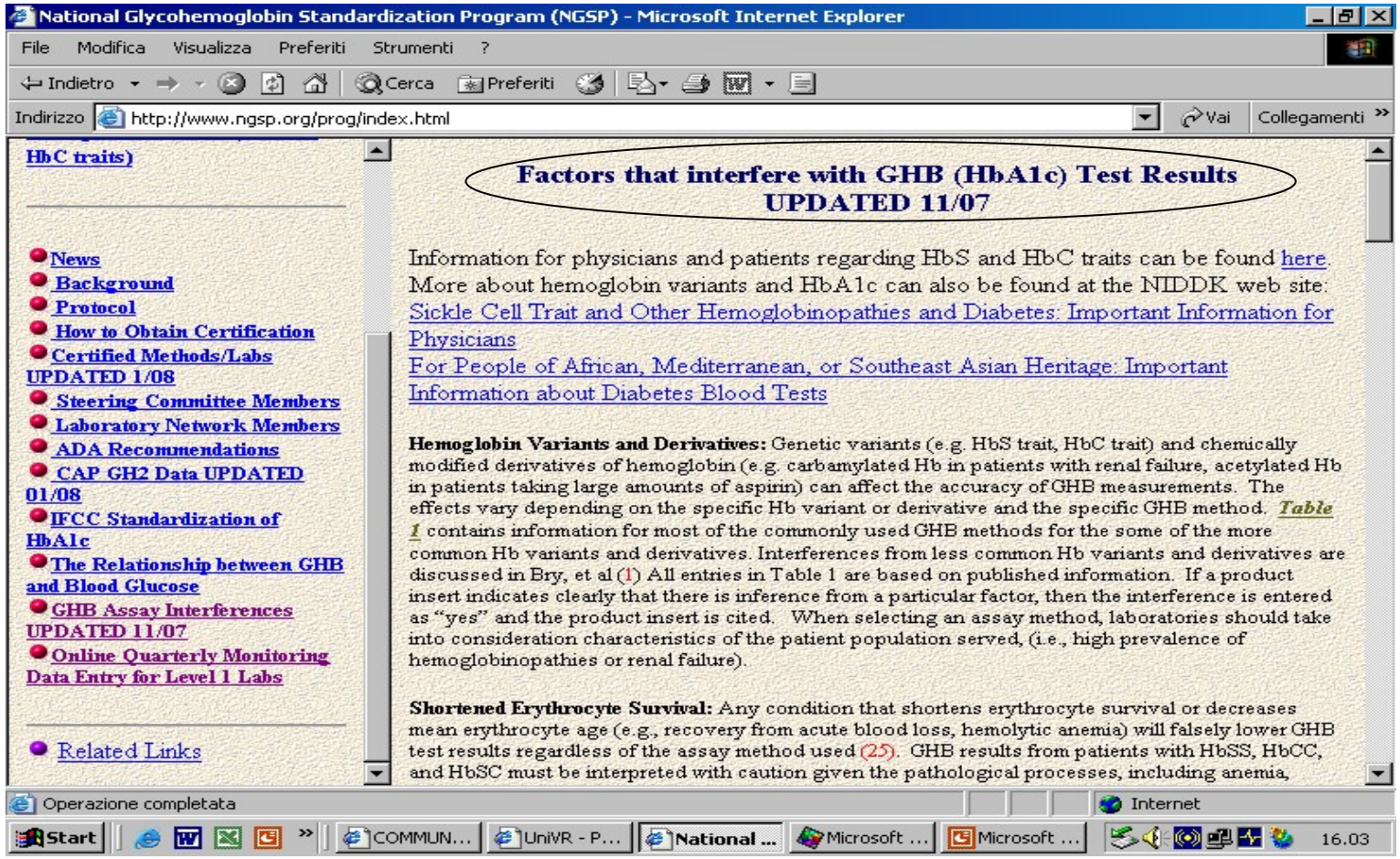

National Glycohemoglobin Standardization Program (NGSP) - Microsoft Internet Explorer
File Modifica Visualizza Preferiti Strumenti ?
Indietro Cerca Preferiti
Indirizzo http://www.ngsp.org/prog/index.html
Vai Collegamenti
HbC traits)
News
Background
Protocol
How to Obtain Certification
Certified Methods/Labs UPDATED 1/08
Steering Committee Members
Laboratory Network Members
ADA Recommendations
CAP GH2 Data UPDATED 01/08
IFCC Standardization of HbA1c
The Relationship between GHB and Blood Glucose
GHB Assay Interferences UPDATED 11/07
Online Quarterly Monitoring Data Entry for Level 1 Labs
Related Links
Factors that interfere with GHB (HbA1c) Test Results UPDATED 11/07
Information for physicians and patients regarding HbS and HbC traits can be found here.
More about hemoglobin variants and HbA1c can also be found at the NIDDK web site:
Sickle Cell Trait and Other Hemoglobinopathies and Diabetes: Important Information for Physicians
For People of African, Mediterranean, or Southeast Asian Heritage: Important Information about Diabetes Blood Tests
Hemoglobin Variants and Derivatives: Genetic variants (e.g. HbS trait, HbC trait) and chemically modified derivatives of hemoglobin (e.g. carbamylated Hb in patients with renal failure, acetylated Hb in patients taking large amounts of aspirin) can affect the accuracy of GHB measurements. The effects vary depending on the specific Hb variant or derivative and the specific GHB method. Table 1 contains information for most of the commonly used GHB methods for the some of the more common Hb variants and derivatives. Interferences from less common Hb variants and derivatives are discussed in Bry, et al (1) All entries in Table 1 are based on published information. If a product insert indicates clearly that there is inference from a particular factor, then the interference is entered as "yes" and the product insert is cited. When selecting an assay method, laboratories should take into consideration characteristics of the patient population served, (i.e., high prevalence of hemoglobinopathies or renal failure).
Shortened Erythrocyte Survival: Any condition that shortens erythrocyte survival or decreases mean erythrocyte age (e.g., recovery from acute blood loss, hemolytic anemia) will falsely lower GHB test results regardless of the assay method used (25). GHB results from patients with HbSS, HbCC, and HbSC must be interpreted with caution given the pathological processes, including anemia,
Operazione completata
Internet
Start
COMMUN...
UniVR - P...
National ...
Microsoft ...
Microsoft ...
16.03

Table 1: Effects of frequently encountered Hb variants and derivatives on GHB measurement

Method (listed in alphabetical order by manufacturer)	Interference (Yes/No)				
	Hb C trait	*Hb S trait*	*Hb E trait*	*Elevated HbF*	*Carbamyl-Hb*
***Abbott Architect (Seradyn Reagents)**	Yes 37	Yes 37	-	-	-
***Axis-Shield Nycocard** (Primus Nycocard)	No 5	No 5	-	-	-
***Bayer Advia**	Yes 36	Yes 36	-	-	-
***Bayer DCA 2000**	No 1, 6, 7	No 1, 6	No 1, 8-9	Yes 35	No 4, 10
Beckman Diatrac	Yes 1,2,11	Yes 1,2,11	-	Yes 11	Yes 1,11
***Beckman Synchron CX7**	No 5	No 5	-	-	-
***Bio-Rad D-10 Dual Short Program**	Yes 33	No 33	-	-	-
Bio-Rad DiaSTAT	No 33	Yes 33	-	-	-
***Bio-Rad Variant A1c**	No 1-2	Yes 1,2	No 1,8	-	Yes 1,4,12,13,15
***Bio-Rad Variant GHb**	No 5	No 5	-	-	-
***Bio-Rad Variant II A1c**	Yes 33/No 5,34	Yes 33/No 5,34	-	No 35	No 12
***Dade Dimension RxL**	No 33	No 33	-	-	-
***Drew Scientific DS5**	No 33	Yes 33	-	-	-
Helena Glyco-Tek	Yes 5,6	No 5,6	-	-	-
***Menarini HA8140**	No 5	Yes 5	Yes 1,17	No 1,17	**Yes**/No 1,4,18,19
***Menarini HA8160**	No 33	No 33	-	-	-
***Metrika A1c Now**	Yes 33	Yes 33	-	-	-
***Olympus**	Yes 37, 38	Yes 37, 38	-	Yes (>10%) 38	-
***Ortho-Clinical Vitros**	No 34	No 34	-	-	-
***Pointe Scientific Hemoglobin A1c**	No 34	No 34	-	-	-
***Primus Boronate Affinity HPLC**	No 1-2,33	No 1-2,33	No 1,8	Yes 35	No 1,4,10,20
***Provalis Glycosal** (Bio-Rad MicroMat)	Yes5	No5	-	-	-
Randox Haemoglobin A1c	Yes 39	Yes 39	-	Yes (>10%) 39	-
***Roche Cobas Integra**	Yes 5	Yes 5	-	-	-
***Roche Cobas Integra Gen. 2**	No 40	No 40	No 40	-	-
***Roche Tina-quant II**	No 1,2,21	No 1,2,21	No 1,21	No (≤30% HbF) 21	No 1,4,18
***Roche Unimate**	Yes 1,22	Yes 1,22	-	-	No 1,4,12
***Tosoh A1c 2.2 Plus**	No 1,2,33	No 1,2,33	Yes 1,8	Yes 35	Yes/**No** 1,4,12,15, 18,23,24
***Tosoh G7**	No 33	No 33	-	No 35	-

* indicates an NGSP certified method (as of April 2007)

Learning points

- Haemoglobin variants can interfere with most HbA1c methods and cause problems with interpretation.
- The possibility of haemoglobin variants should be considered when HbA1c results do not concur with clinical expectations.
- Haemoglobin variants may not always be revealed by electrophoresis.
- Analysis of HbA1c by alternative methods, in particular by mass spectrometry may help to elucidate the nature of confounding variants.
- It is occasionally necessary to consider alternative measures of glycation than HbA1c.

Haemoglobin Marseille-Long Island and interpretation of HbA1c: which HbA1c result is the "right answer"?

C M Florkowski, T A Walmsley, S O Brennan, P M George

Postgrad Med J 2003;79:174–175

Suggerimenti "operativi"

(1) Dotarsi di una tecnica di screening:
- Automatizzata
- In grado di processare provetta primaria
- In grado di processare HbA1c e/o Varianti
- Dotata di software di archiviazione delle varianti

(2) Analizzare visivamente (sistematicamente ed accuratamente) tutti i tracciati, anche quelli per cui è richiesta solo quantificazione HbA1c.

(3) Mai porre diagnosi solo sulla base del tracciato HPLC/CE(IEF).

(4) In presenza di variante trasmettere il campione con la maggior parte dei dati clinici / laboratoristici disponibili ad un *centro di riferimento… prosegue…*

"*Emoglobinopati*" non ci s'inventa…

Si diventa!