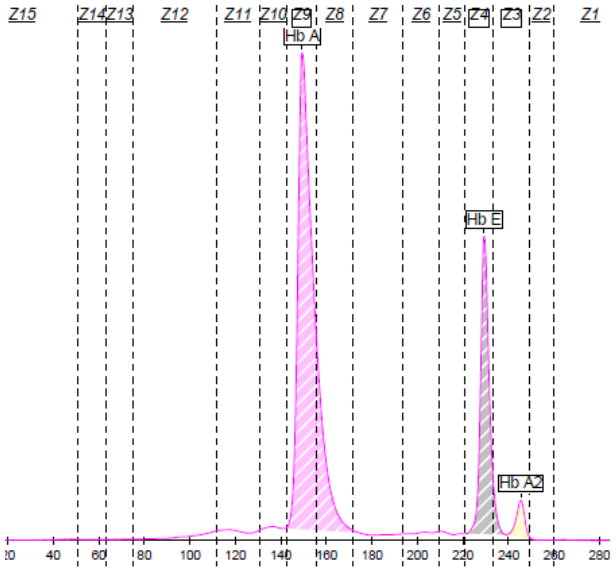
Leids Universitair
Medisch Centrum

External Quality Assessment in Hemoglobinopathies in The Netherlands



Cornelis L. Harteveld

Hemoglobinopathy Expert Centre LUMC

LEIDEN UNIVERSITY MEDICAL CENTRE , LEIDEN

Stichting Kwaliteitsbewaking
Medische Laboratoriumdiagnostiek

Disclosure

The speaker is coördinator of the Hb variant assessment organized by the SKML (Foundation for quality assessment Medical Labortories)

No financial relationships with industry

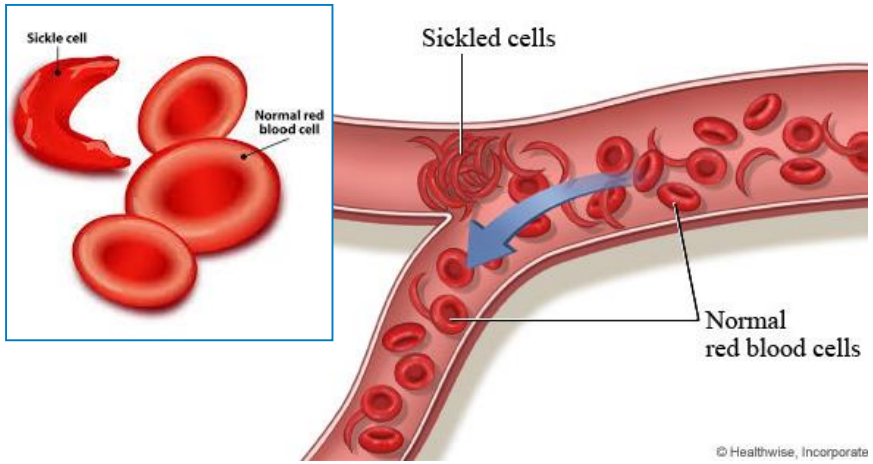
No sponsoring by industry

No shareholder in companies

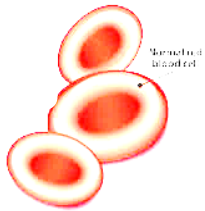
Introduction

The Hemoglobinopathies

Sickle Cell Disease:
HbS/S, HbS/C, HbS/D etc.

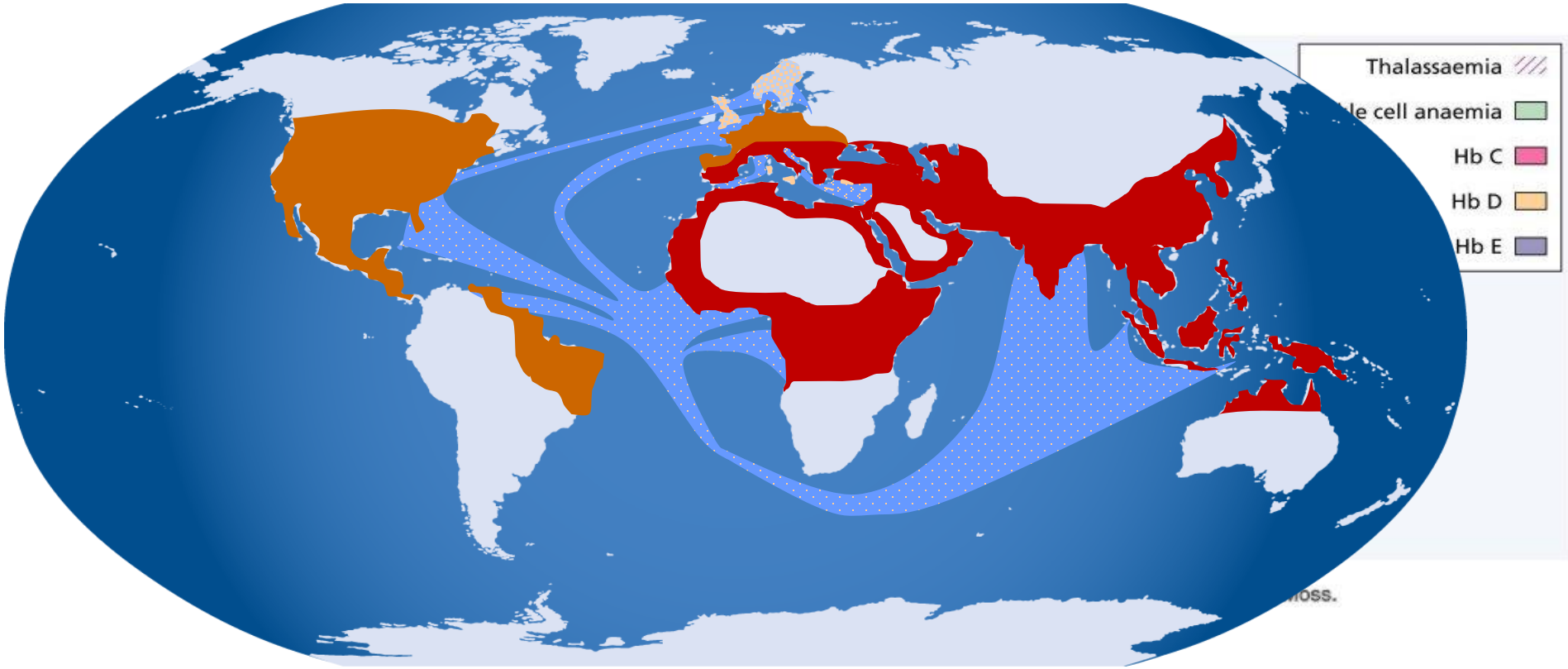


Thalassemia:
α-thalassemia
β-thalassemia



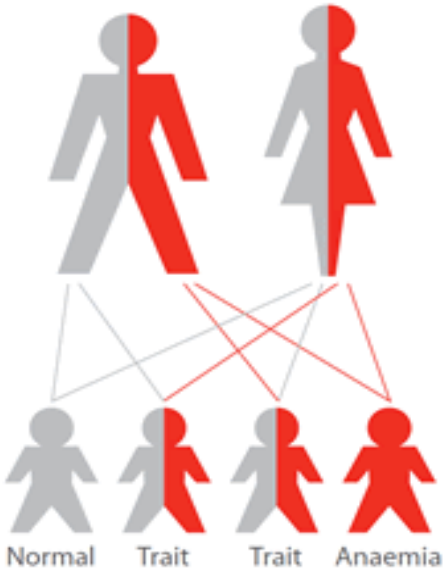
Microcytic
Hypochromic
Low Hb level

Spreading of hemoglobinopathy syndromes



- 7% of the world's population is a carrier of HbP leading to the birth of approximately 300,000 sickle cell- and 40,000 transfusion dependent beta-thalassemia major /yr

Laboratory Approach to Hb Disorders

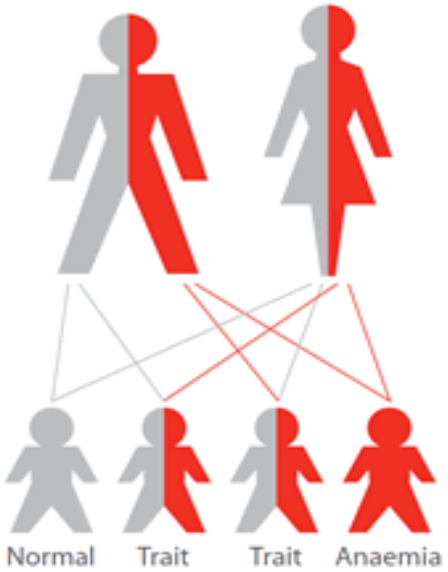


Carrier detection

To prevent:

1. Unnecessary iron treatment
2. Prenatal Diagnosis

Laboratory Assessment



Complete Blood Count

Hemocytometric assessment scheme

Hb-electro-phoresis

This tal

DNA analysis

Hb electrophoresis in the diagnosis of hemoglobinopathies

High Pressure Liquid Chromatography (HPLC)
Capillary Electrophoresis (CE)
Iso Electric Focussing (IEF)

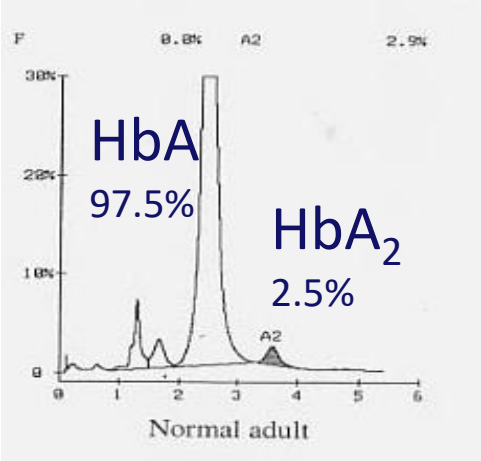
Abnormal separation .....

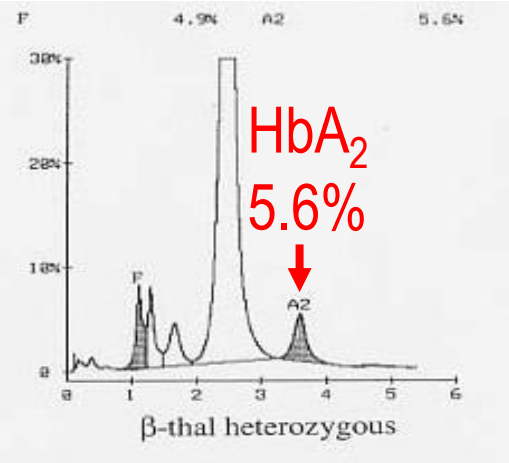
- Hb variants (HbS, HbC, HbD, HbE, Hb O-Arab etc.) at fixed positions

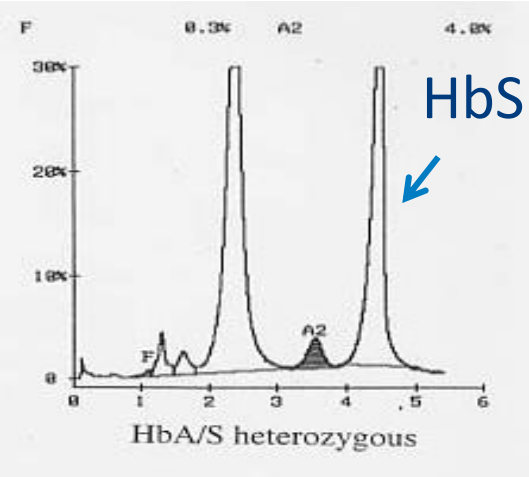
...and quantitation of hemoglobin fractions

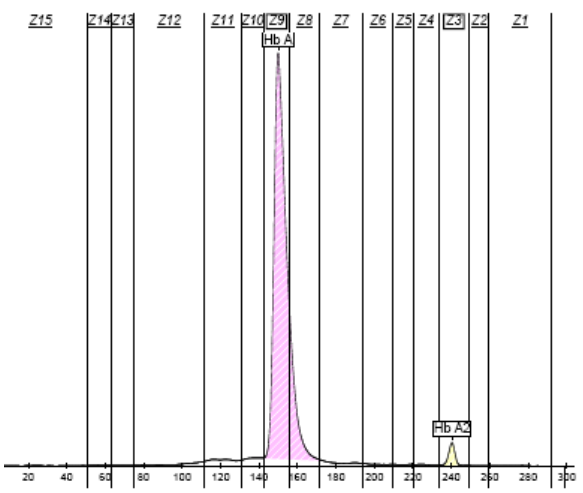
- Elevated HbA_2 indicative for β-thalassemia carrier
- Reduced HbA_2 level might be indicative of α-thal
- Amount is indicative of co-existing α- or β-thalassemia

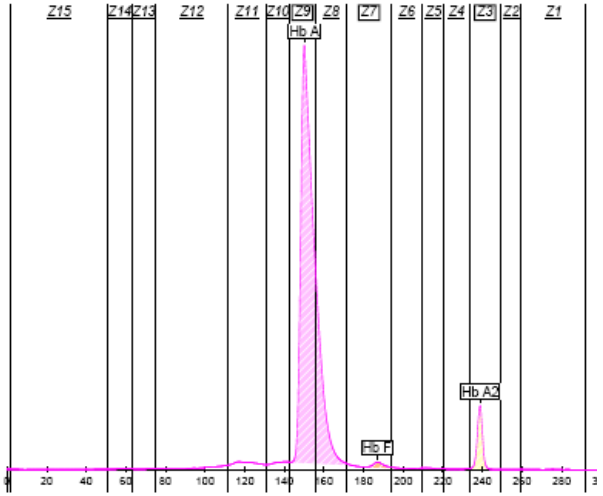
Normal and abnormal patterns on HPLC and CE

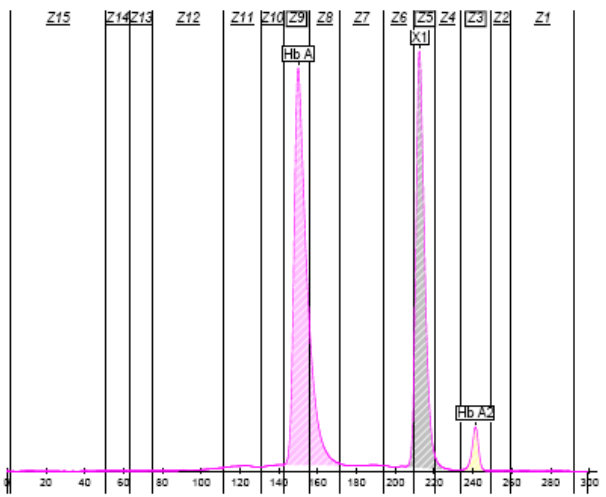












Normal β-thalassemia trait HbS trait

Stichting Kwaliteitsbewaking Medische Laboratoria

Stichting Kwaliteitsbewaking
Medische Laboratoriumdiagnostiek

- SKML has a national QA scheme for Hb variants
- spare EDTA-blood after HbP analysis and informed consent
- 12 samples/yr with case description
- 52 participants (only NL)
- Interpretation of HPLC or CE patterns (qualitative/quantitative)
- Percentages of Hb fractions compared to those of expert lab and mean consensus
- Questions need to be answered and presumptive diagnosis

What is the main purpose?

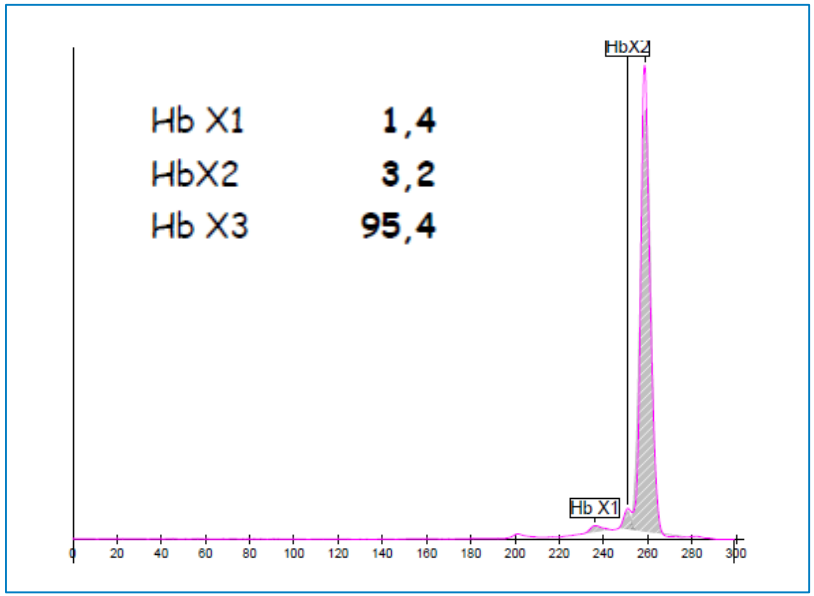
The participant has to give the correct diagnosis

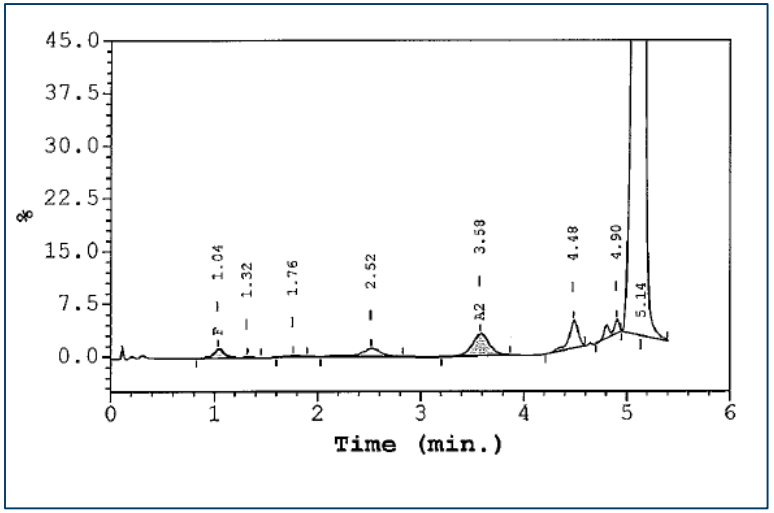
(using their own test results and information given)

Case 1

African lady,
normocytic normochromic, no anemia
mild splenomegaly,

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
F	0.9	---	1.04	12076
P2	---	0.1	1.32	1006
P3	---	0.1	1.76	1768
Ao	---	1.6	2.52	22247
A2	3.2	---	3.58	51711
S-window	---	2.5	4.48	34804
Unknown	---	1.3	4.90	18465
C-window	---	90.0	5.14	1276821





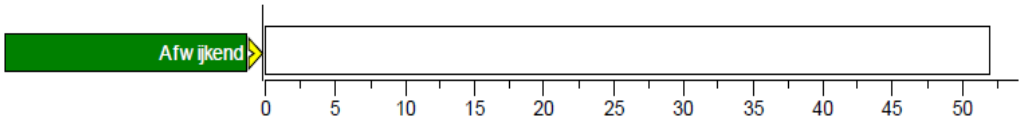
HbC/C disease

Case 1

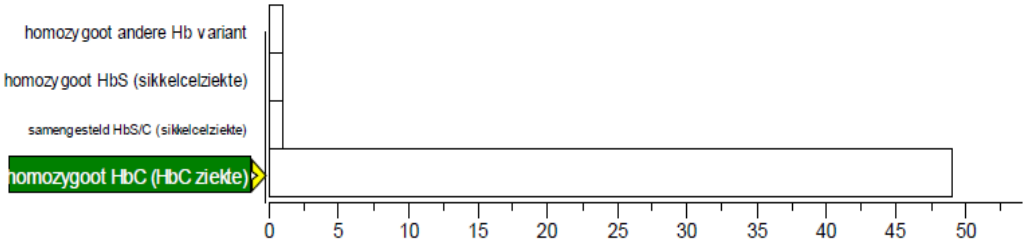
Monster : 2015.1 C

Correct

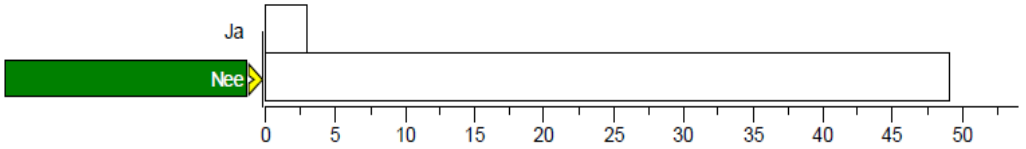
Hb separation



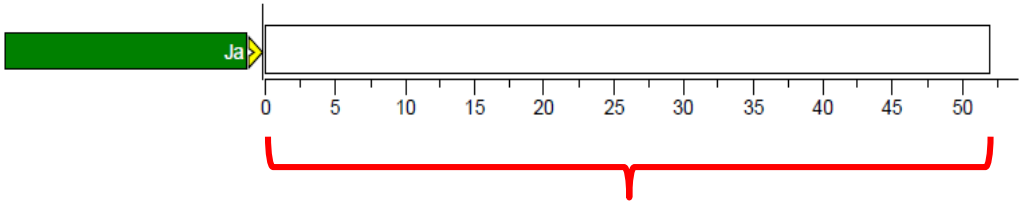
Most likely diagnosis



Clues for coexisting alpha-thal?



Family analysis important?

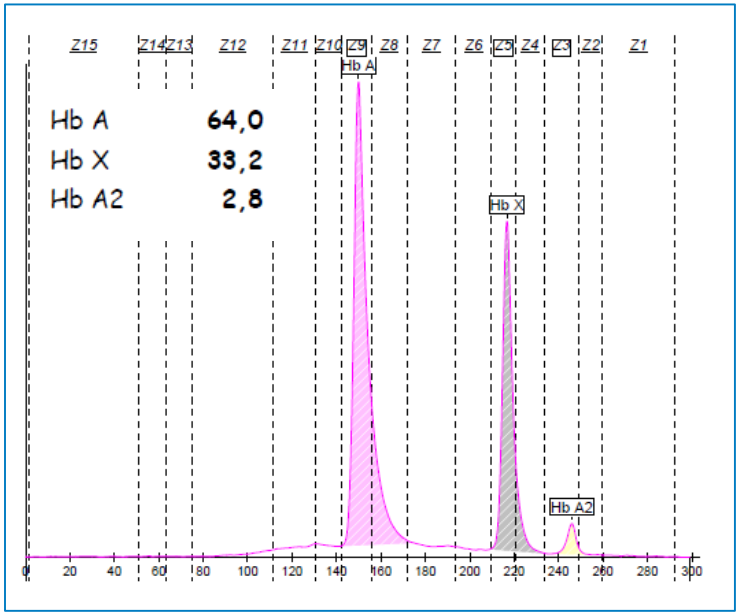


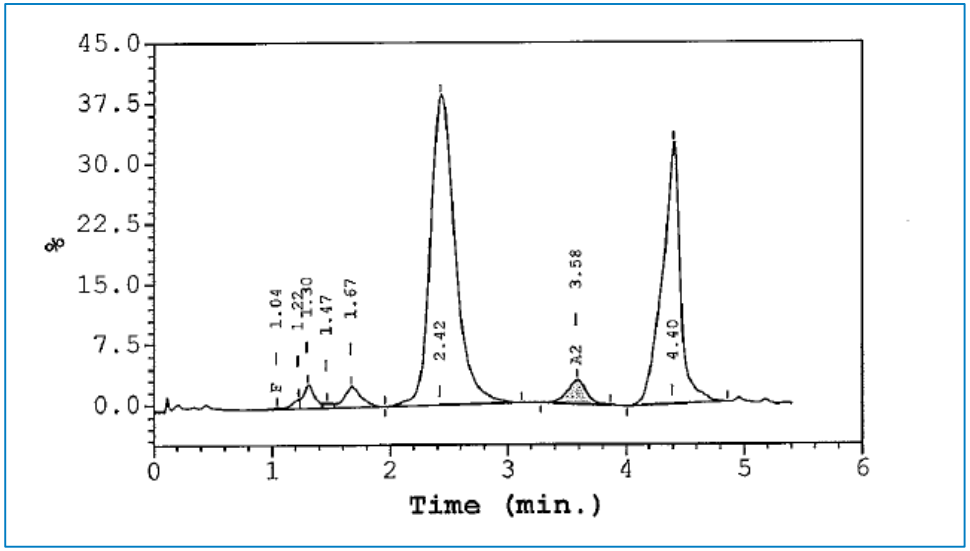
Nr of partipants giving the correct answers

Case 2

African man,
microcytic hypochromic,
normal iron

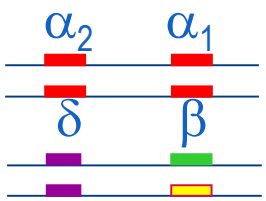
Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
F	0.2	---	1.04	2812
Unknown	---	0.6	1.22	11057
P2	---	1.9	1.30	34412
Unknown	---	0.3	1.47	6194
P3	---	2.6	1.67	46593
Ao	---	56.5	2.42	1007563
A2	3.0	---	3.58	60585
S-window	---	34.4	4.40	613045





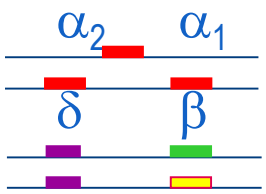
HbS in combination with α-thalassemia

HbS carrier



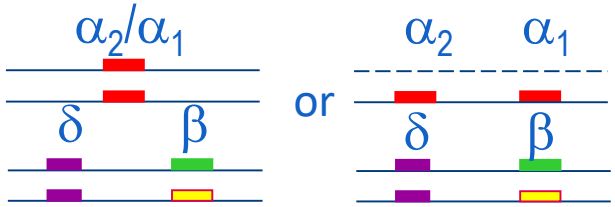
HbS
40.6%

HbS/α+-thal

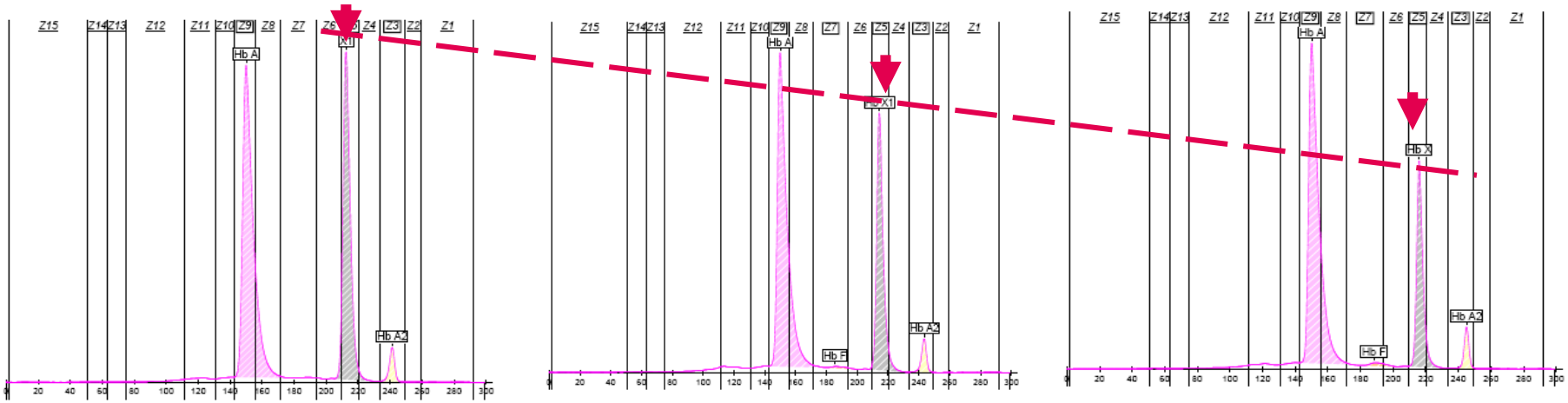


HbS
34.3%

HbS/ hom α+- or/ α0-thal



HbS
26.7%

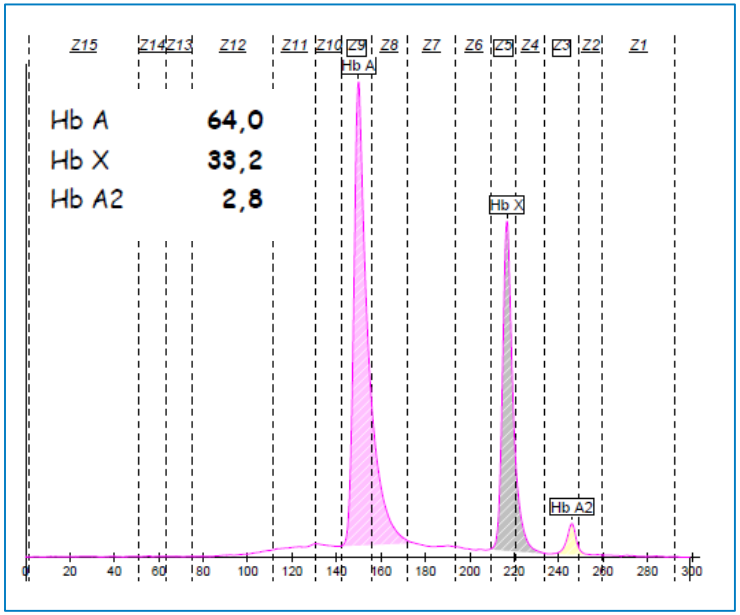


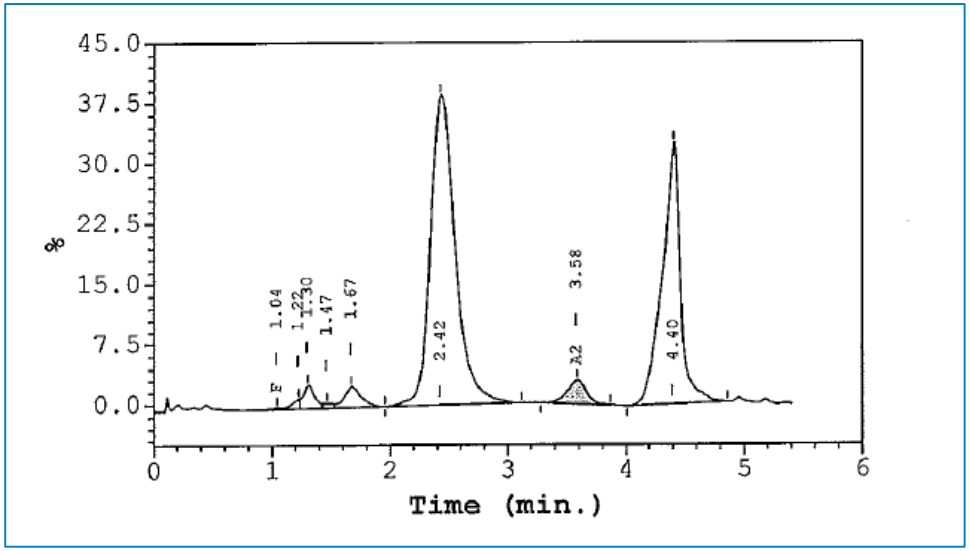
Case 2

African man,
microcytic hypochromic,
normal iron,

HbS carrier with alpha^{+}-thal

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
F	0.2	---	1.04	2812
Unknown	---	0.6	1.22	11057
P2	---	1.9	1.30	34412
Unknown	---	0.3	1.47	6194
P3	---	2.6	1.67	46593
Ao	---	56.5	2.42	1007563
A2	3.0	---	3.58	60585
S-window	---	34.4	4.40	613045

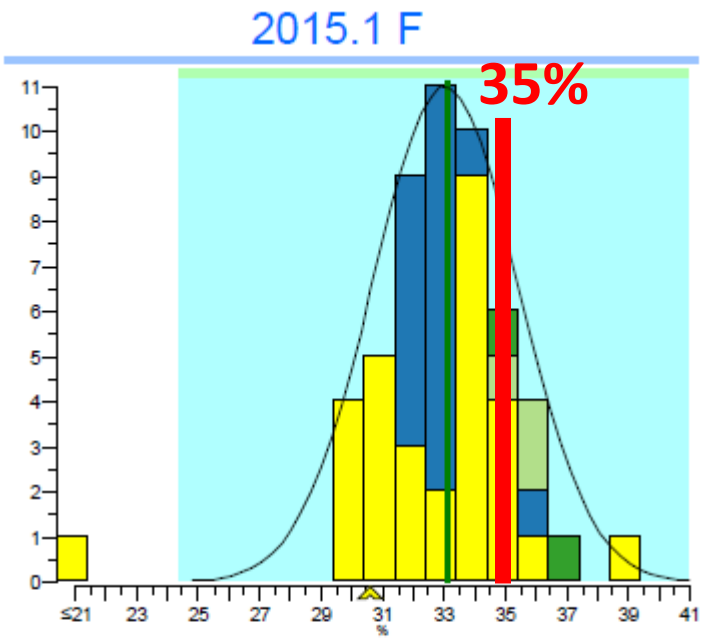




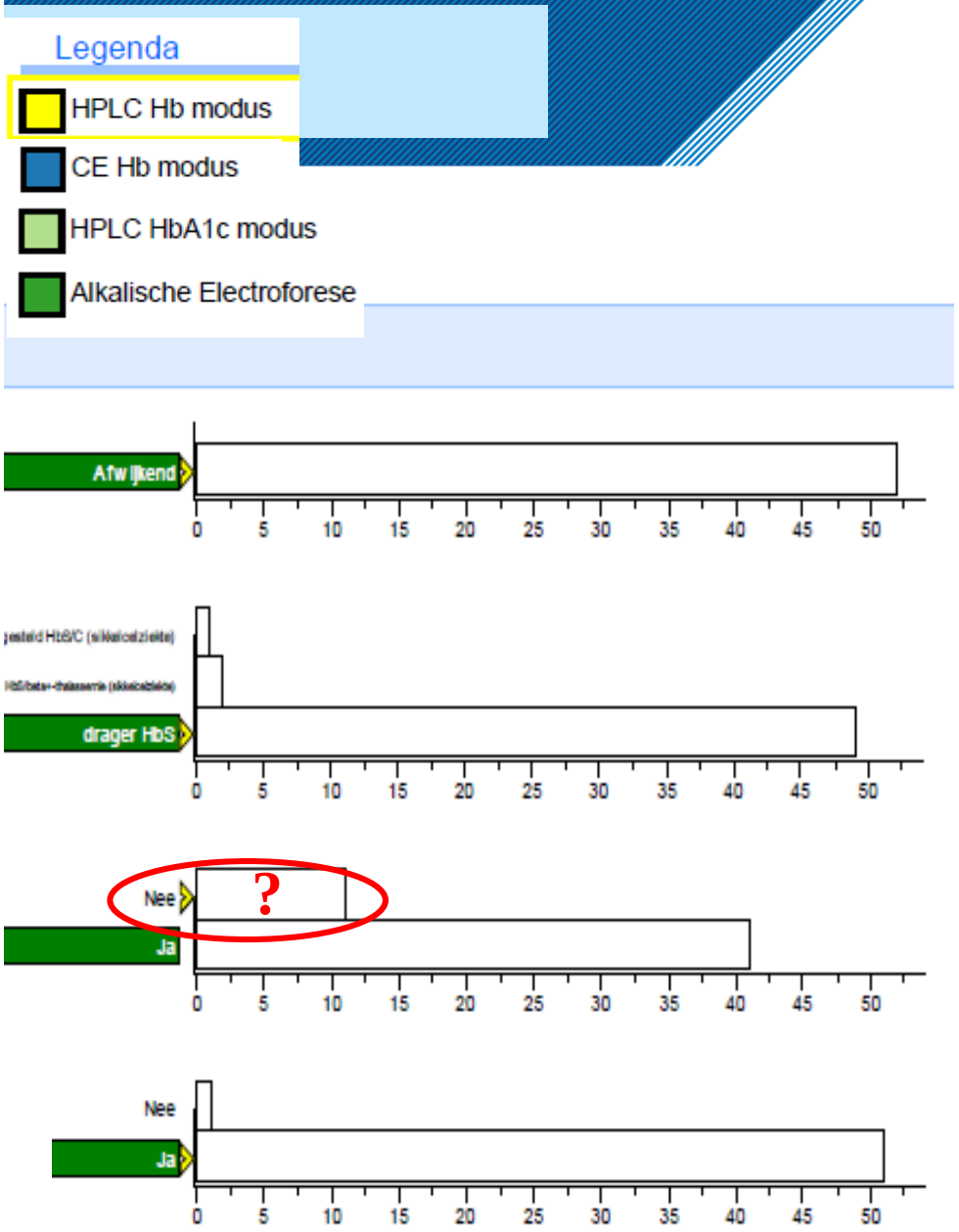
$\alpha\alpha/\alpha\alpha$ β^A/β^S } 36-42% HbS

$-\alpha/\alpha\alpha$ β^A/β^S } 30-35% HbS

$-\alpha/-\alpha$ or $--/\alpha\alpha$ β^A/β^S } 22-29% HbS



	cons.	meth.	exp.	lab
gem.	33.0	33.0	33.1	30.6
SD	2.4	2.4		
n	30	30		
nu	1	1		



12 participants don't measure decreased HbS

They fail to diagnose co-existing alpha-thal in spite of the microcytic hypochromic blood picture

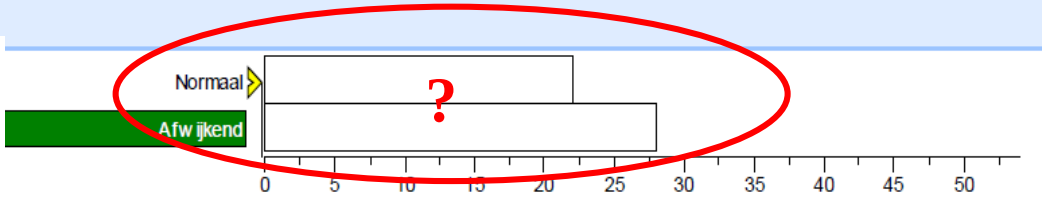
Case 3

woman, Near-East,
microcytic hypochromic anemia
She wants to get pregnant

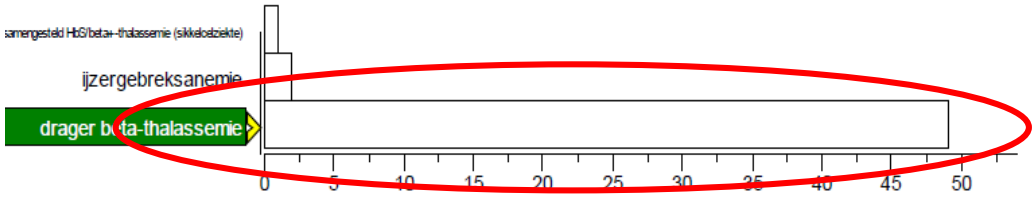
Just a matter of definition:
Elevated HbA_2, do you report that as normal or abnormal Hb separation?

Monster : 2015.1 A

Hb separation

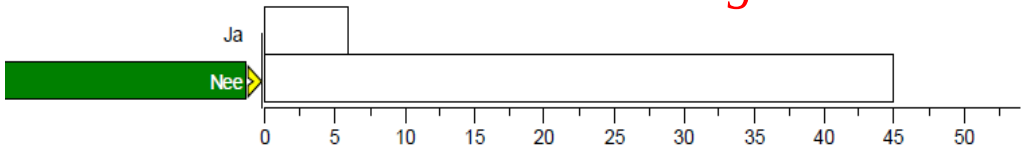


Most likely diagnosis

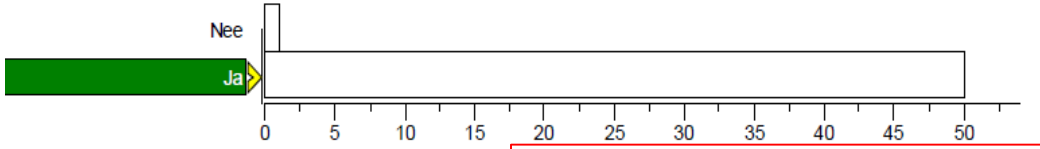


Almost all have the correct diagnosis!!

Clues for coexisting alpha-thal?



Family analysis important?



beta-thalassemia carrier

The diagnosis of beta-thalassemia trait

- Microcytic hypochromic anemia
- Elevated HbA_2

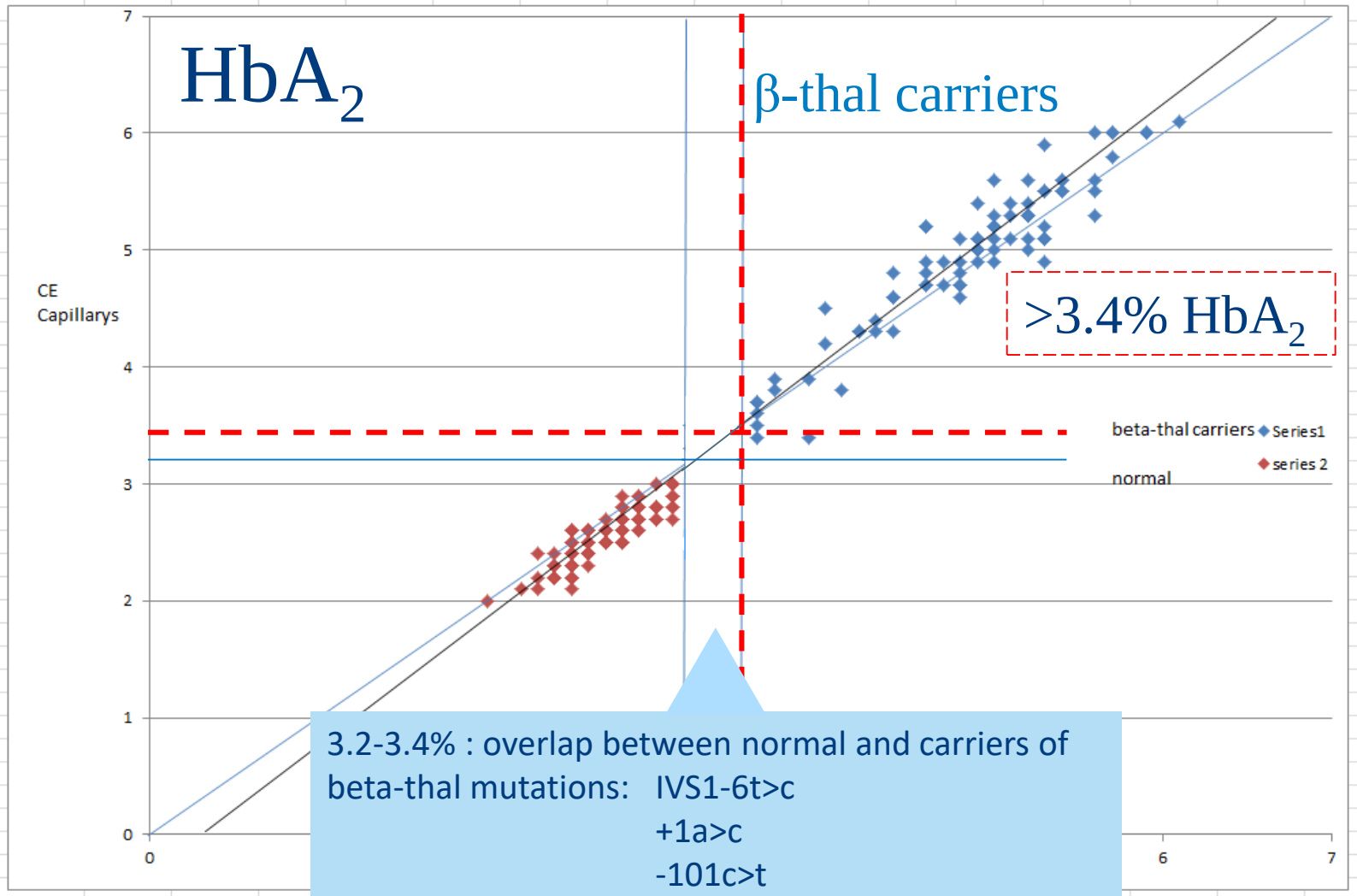
  (cut-off usually higher than 3.2-3.4%, but may vary for different devices)

An international HbA_2 standard may help to decrease the variety between different devices;
There is a WHO standard available, but a new IFCC/ICSH standard for HbA_2 is in development

In the absence of standardization each device is calibrated locally with controls delivered by the manufacturer

=> cut-off values may differ between labs

…however, biology doesn't believe in 'cut-off values'



3.2-3.4% : overlap between normal and carriers of beta-thal mutations:
- IVS1-6t>c
- +1a>c
- -101c>t
- +1570t>c
- polyA (AATGAA)
- IVS2-844c>a

..and sometimes serious problems may occur!

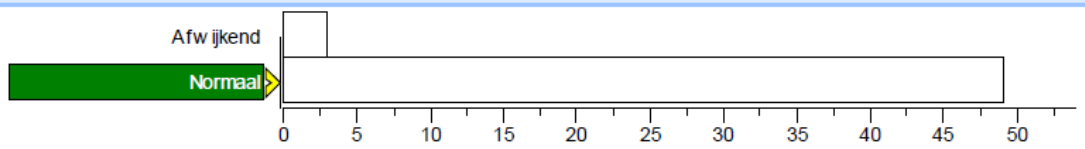
Normal samples are also taken along in assessment scheme:

Monster : 2015.1 B

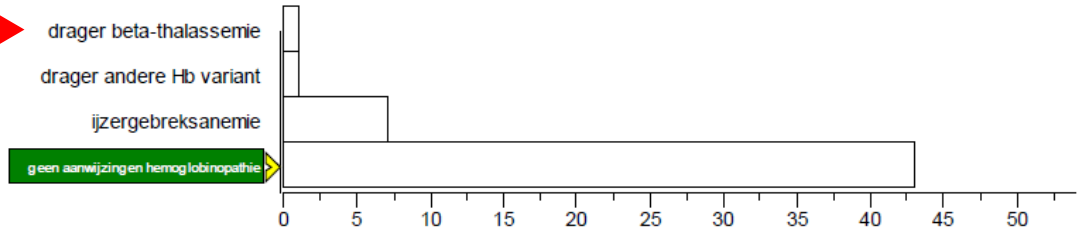
Monster : 2015.1 D

Monster : 2015.1 E

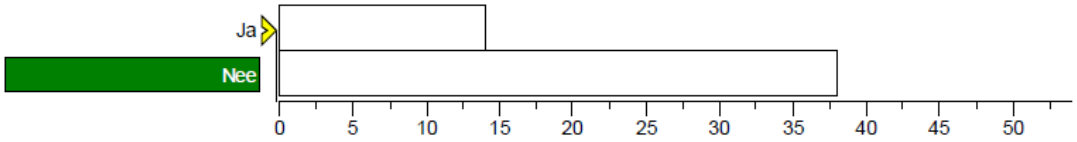
Rapportage Hb scheiding.



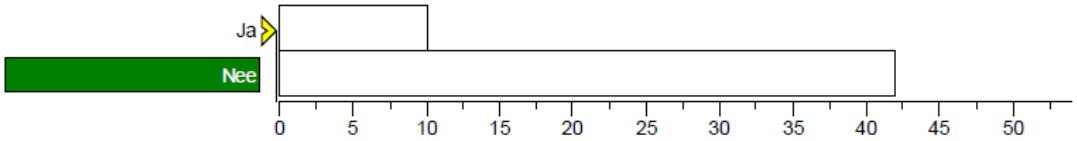
M *carrier beta-thalassemia?*



Zijn er aanwijzingen voor de aanwezigheid van alfa-thalassemie?

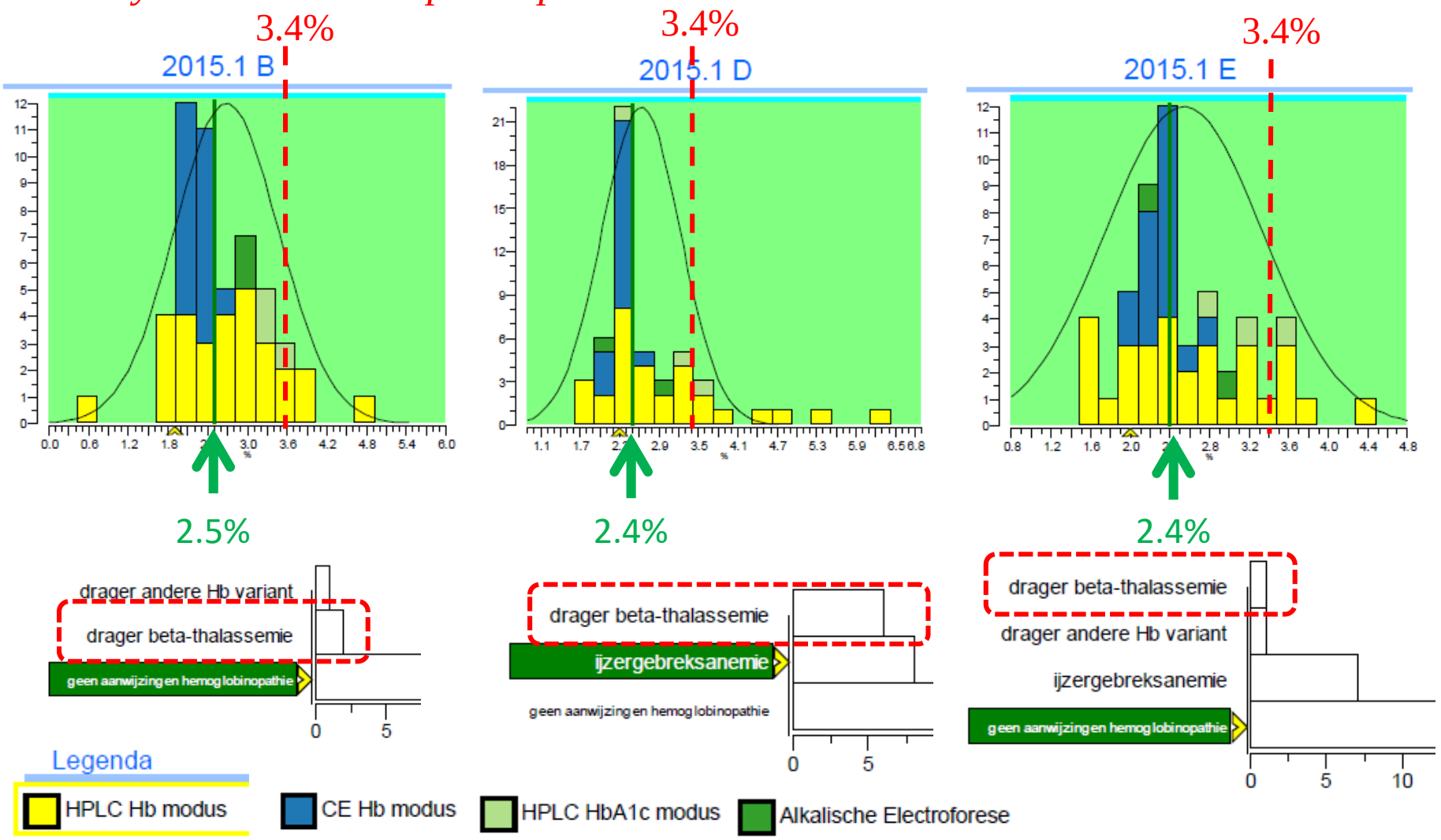


familieonderzoek ouders/partner/kinderen/familieleden?

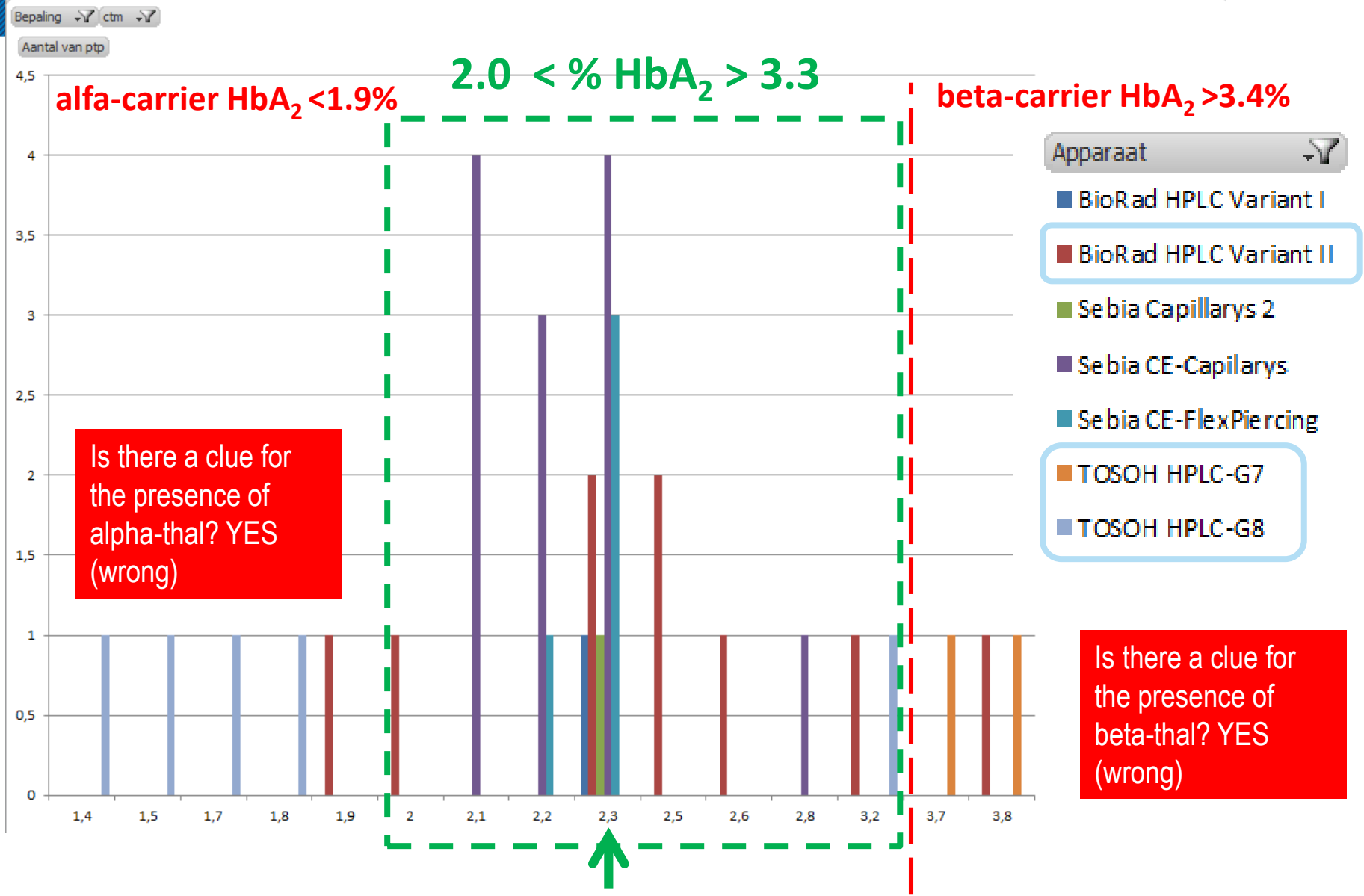


How come?

Why are normal samples reported as beta-thal carrier?



Sample 2015.2A: normal, HbA_2 measurement

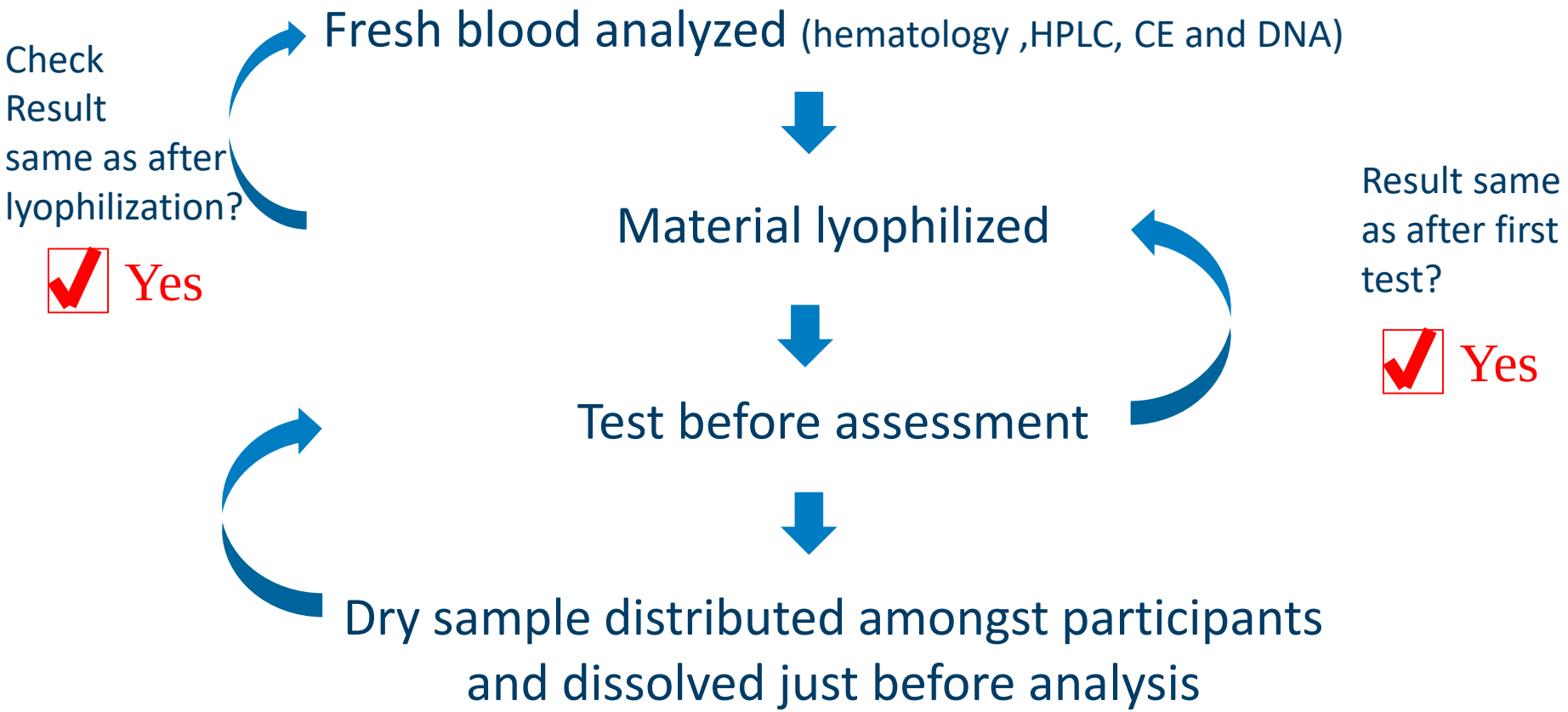


… feed-back of a Tosoh user:

"In daily practice we do much better ….
is the assessment material commutable?"

Lyophilized material

How are assessment samples prepared?

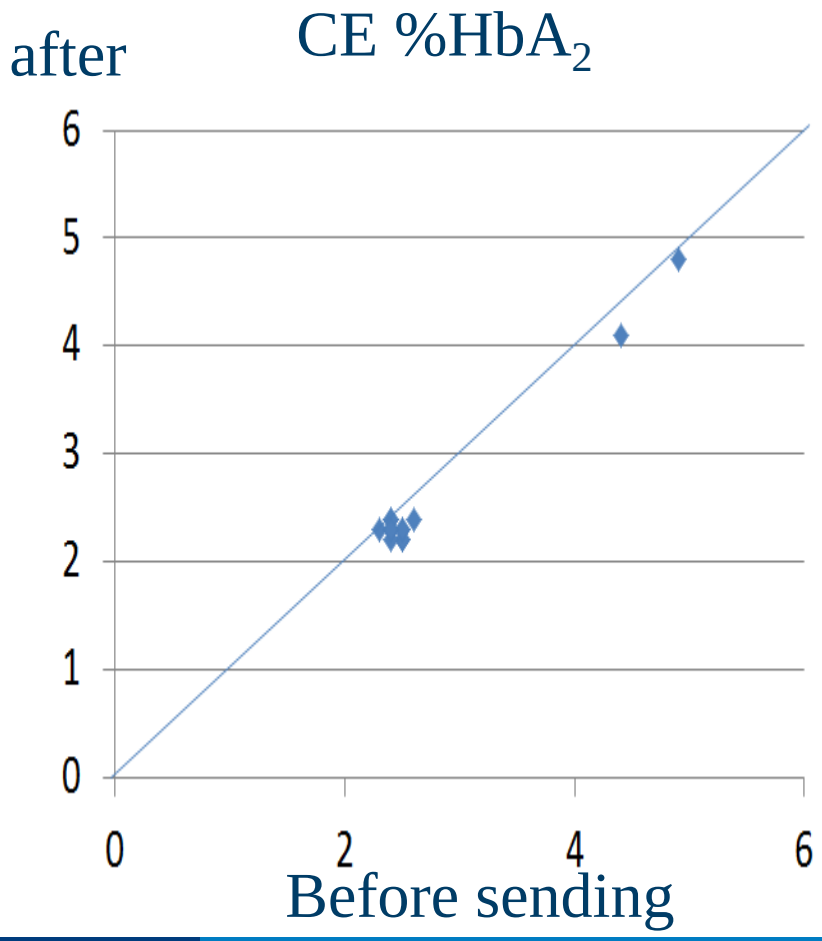


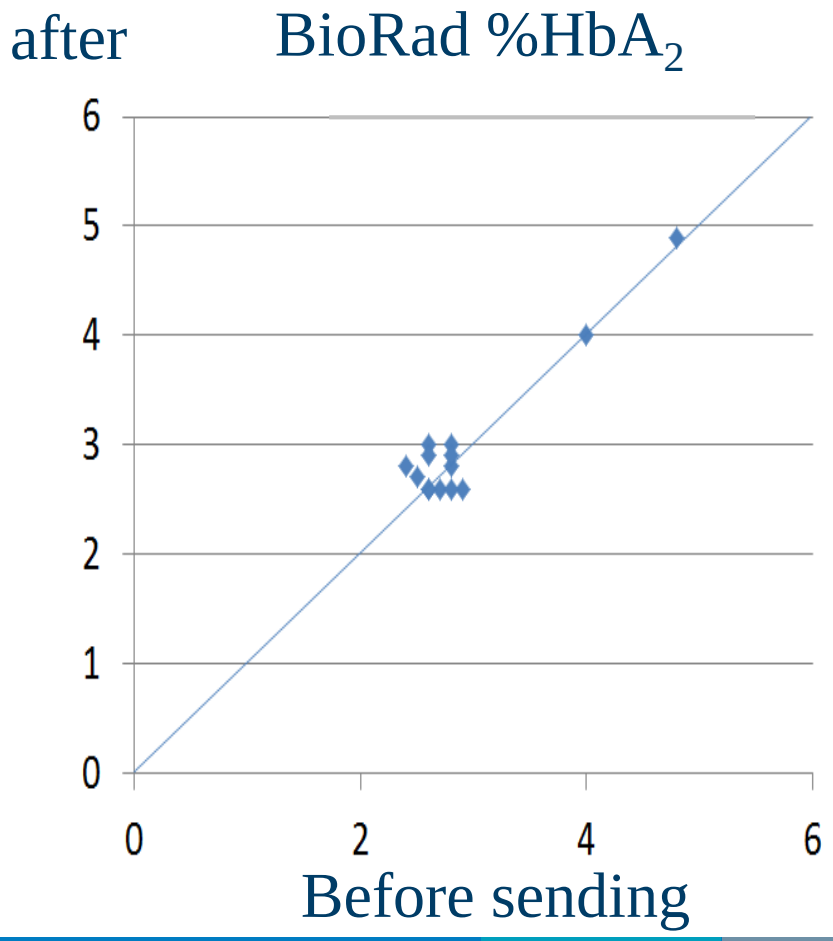
Result still the same?

CE (Sebia Capillarys) en HPLC (BioRad Variant II) HbA_2 %:

1st measurement 13 samples = before sending

2nd measurement 13 samples = after sending





Is lyophilized material unsuitable for Tosoh users?

Exchange of fresh blood:

Tosoh G7

	HbA0	HbA2	HbF
Anoniem 1	81.2	2.5	0.6
Anoniem 2	80.6	2.6	0.5
Anoniem 3	79.1	2.8	0.7
Anoniem 4	81.7	2.9	0.7
Anoniem 5	82.2	3.2*	0.8

BioRad VariantII

	HbA	HbA2	HbF
Anoniem 1	86.7	2.6	0.5
Anoniem 2	86.2	2.5	0.2
Anoniem 3	84.6	2.7	0.4
Anoniem 4	86.4	2.8	0.3
Anoniem 5	87	2.8	0.5

Capillarys (Sebia)

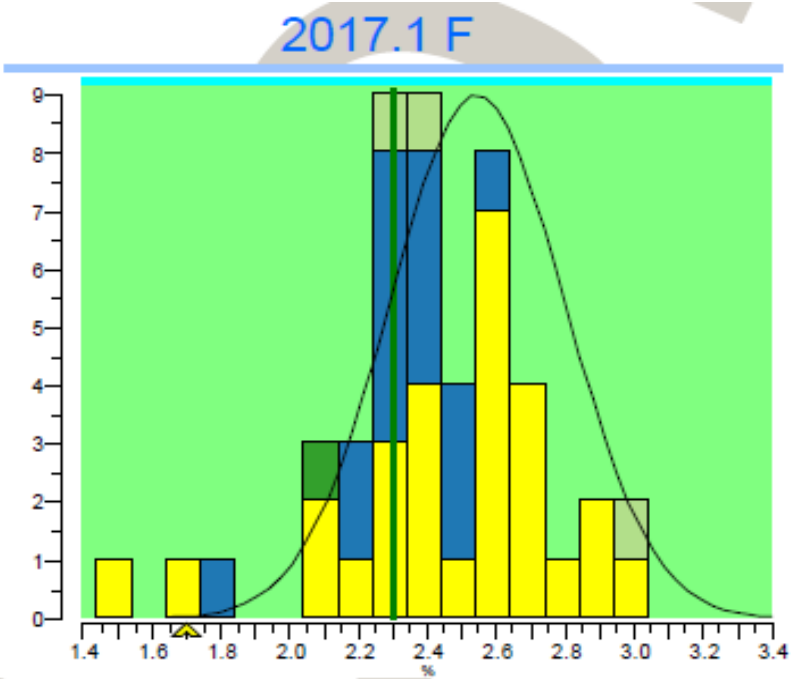
	HbA	HbA2	HbF
Anoniem 1	97.5	2.5	-
Anoniem 2	97.6	2.4	-
Anoniem 3	96.5	2.7	0.8*
Anoniem 4	97.5	2.5	-
Anoniem 5	97.4	2.6	-

Seems to correlate well…

Could it be the lyophilized material?

2017 assessment normal sample 2017.1F

Frozen blood distributed instead of lyophilized material



	cons.	meth.	exp.	lab
gem.	2.5	2.5	2.3	1.7
SD	0.3	0.3		
n	28	28		
nu	2	2		

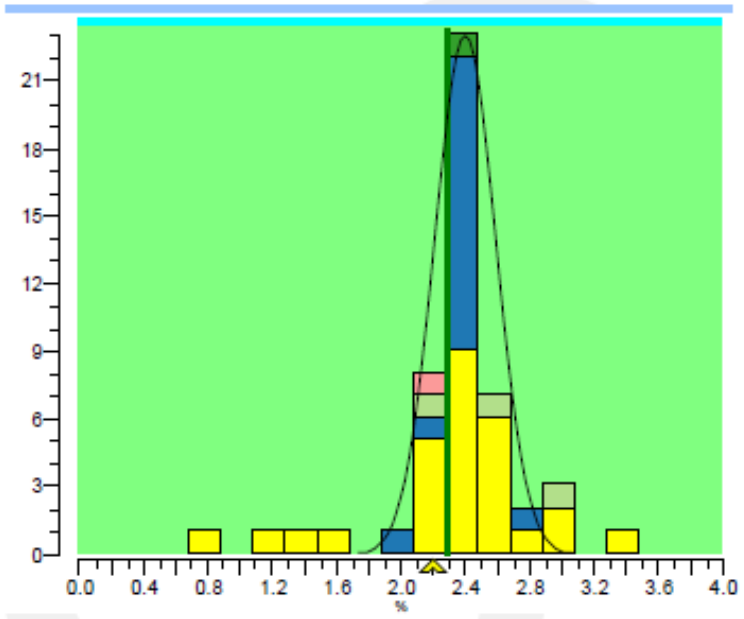
Legenda

HPLC Hb modus | CE Hb modus | HPLC HbA1c modus | CE HbA1c modus

2017 assessment normal sample 2017.2F

Frozen blood distributed instead of lyophilized material

2017.2 F



	cons.	meth.	exp.	lab
gem.	2.4	2.4	2.3	2.2
SD	0.2	0.2		
n	28	28		
nu	5	5		

Legenda

HPLC Hb modus | CE Hb modus | HPLC HbA1c modus | CE HbA1c modus

CONCLUSION

- In general the correct diagnosis can be made with all dedicated devices
- frozen blood samples seem to work better than lyophilized material but not for all participants
- Diagnosis of HbP carriers needs to be done using hematological data and Hb separation. DNA analysis for confirmation
- Participants are not only examined for accuracy of equipment, but also for their knowledge about HbP
- Participants play a major role in the assessment (but it is difficult to please everyone)

Leids Universitair Medisch Centrum

Acknowledgements

HbP lab/LDGA, LUMC, Leiden:

Jeanet ter Huurne

Maaike Verschuren

Sharda Bisoen

Sandra Arkesteijn

Rianne Schaap

Linda Vijfhuizen

Hakima el Idrissi

Frank Baas

SKML:

Warry van Gelder (coordinator Red Blood Cell Assessment)

Ron Meijer (SKML)

Cas Weijkamp (Beatrix Hospital, Winterswijk NL)

Marc Theelen (director SKML)

.....and all participants