Towards a Certified Reference Material for HbA_2

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Stimulating innovation
Supporting legislation

What are Reference Materials (RMs)?

RMs are essential tools to achieve traceability and ensure long-term comparability of results.

CRM Requirements

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Traceability of values

- To ensure the continuity of measurement results from assays calibrated against successive reference materials

Commutability: resemble patient samples

Homogeneity

- The difference between the vials must be sufficiently small

Stability

- The material must be stable over many years

Correct concentration range:

- The relevant decision interval should be covered 2.0 – 8.1 %

Low turbidity (for turbidimetry, nephelometry)

Traceability

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Being traceable **to a** common standard **or stated reference should ensure that** independently obtained measurement results will overlap **within their stated uncertainties and at a certain level of confidence with the true value and consequently with each other**

- provided that measurement procedures applied in the traceability chain determine the **same measurand**
- if the comparison measurements do not introduce **unrecognised bias** (e.g. matrix effects, differential extraction etc.)
- if **all relevant uncertainty components** are included in the estimate of the combined uncertainties

Traceability chain

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Definition of S.I.

AAA, refractive index, dry mass determinations

"Pure" protein

reference procedure, immunoassay

i.e., ERM-DA470k
(matrix material)

value transfer procedure 1, immunoassay

Manufacture working calibrator
(matrix material)

value transfer procedure 2, immunoassay

Manufacture's product calibrator
(matrix material)

Immunoassay
(Routine method)

Routine sample result

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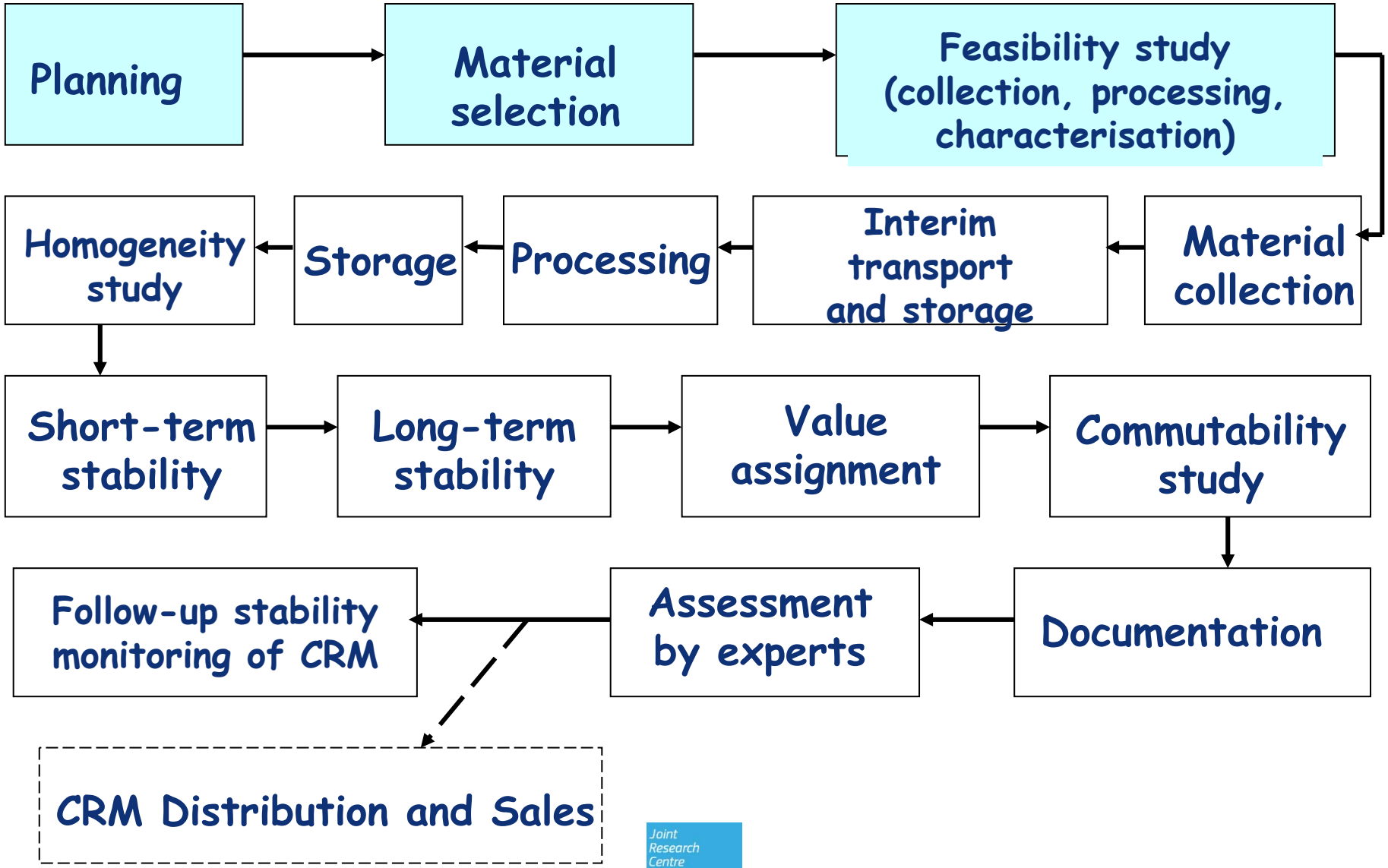
Production design



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Value is assigned together with uncertainty

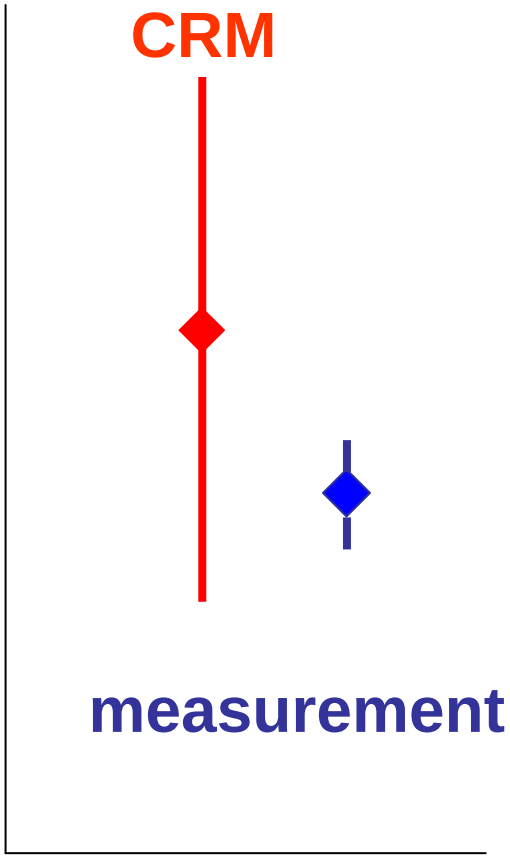
Certified value [mg/L]	U_{CRM} (k = 2) [mg/L]
xx.x	x.x

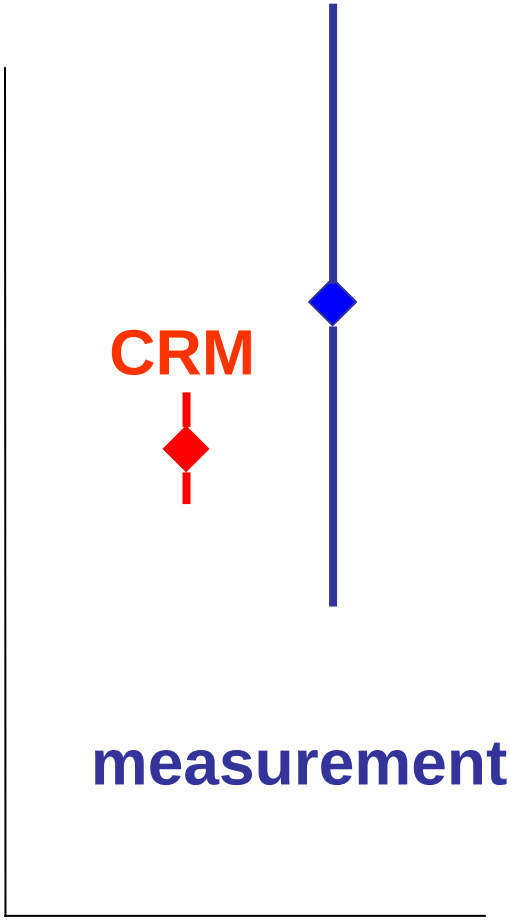
Main components of the uncertainty budget:

- ✓ characterisation
- ✓ calibrant purity
- ✓ homogeneity (bb)
- ✓ stability

$$U_{CRM} = k\sqrt{u_{char}^2 + u_{cal}^2 + u_{bb}^2 + u_{lts}^2}$$

Uncertainty





CRM unsuitable

Method suitable?

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HbA_2 marker for β-thalassemia

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Feasibility study

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- Characterisation

- Correlation studies:
  - do results on clinical samples form different methods correlate? If not, harmonisation is not possible by recalibration only

- Commutability studies:
  - can we select an process a material so that is has similar properties as clinical samples?

- Stability:
  - producing a material stable for 10-20 years

- Value assignment:
  - develop a strategy for assigning values to the reference material

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HbA2 – marker for β-thalassemia

First batch

- human hemolysate with normal HbA_2 concentration
- lyophilized form
- 100 vials

Preparation of stabilized hemolysate
Milan (Nov. 2007)

↓ shipment in dry ice

Lyophilization
IRMM (Apr. 2008)

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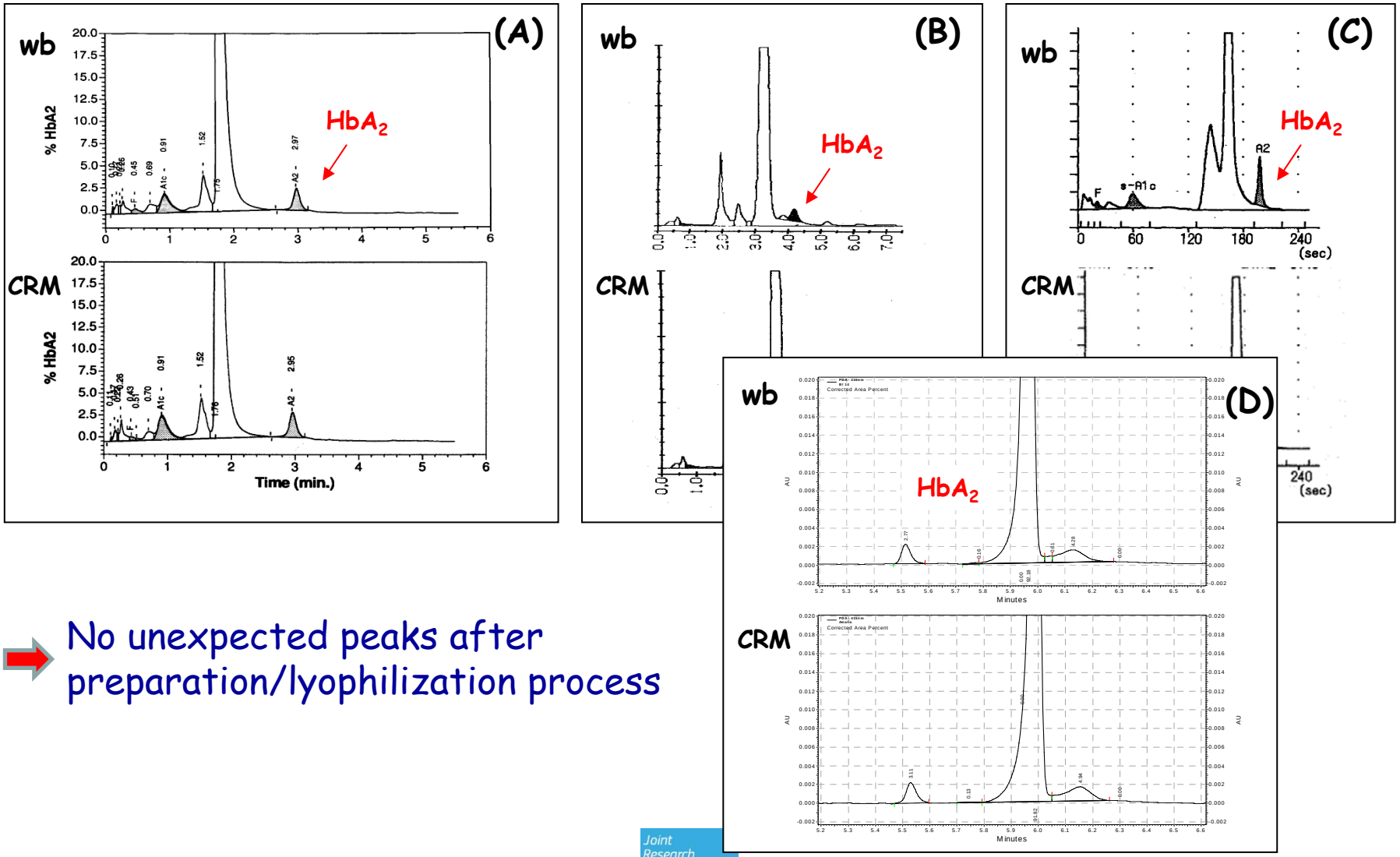
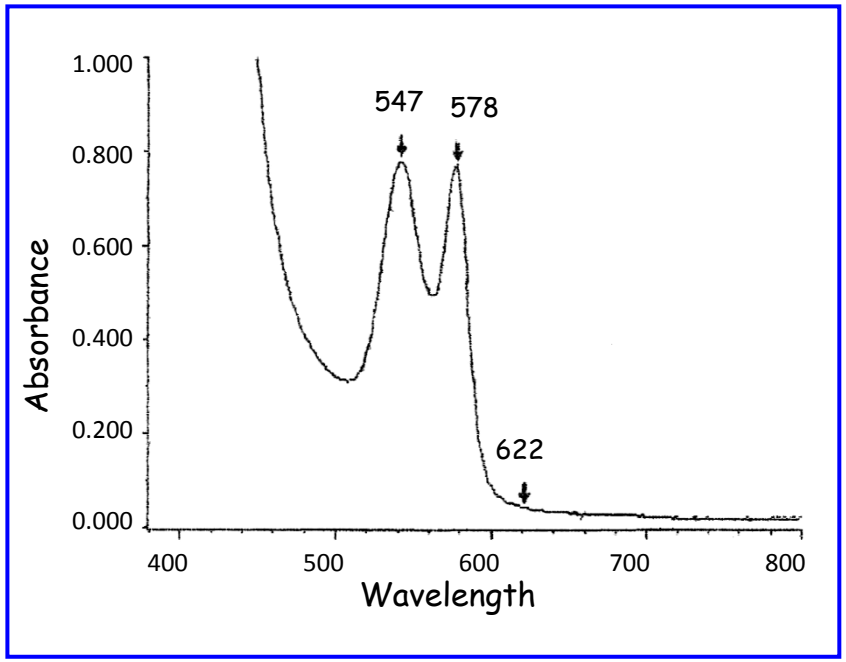
Characterization



No unexpected peaks after preparation/lyophilization process

Characterization



Hemoglobin absorption spectrum

- Typical of oxygenated Hb, no oxidation

	Total Hb g / L	Met Hb %
Mean	129	0.8
SD	1	0.2
n	9	4

Total hemoglobin and derivatives

- Concentrations similar to that of whole blood

Commutability test

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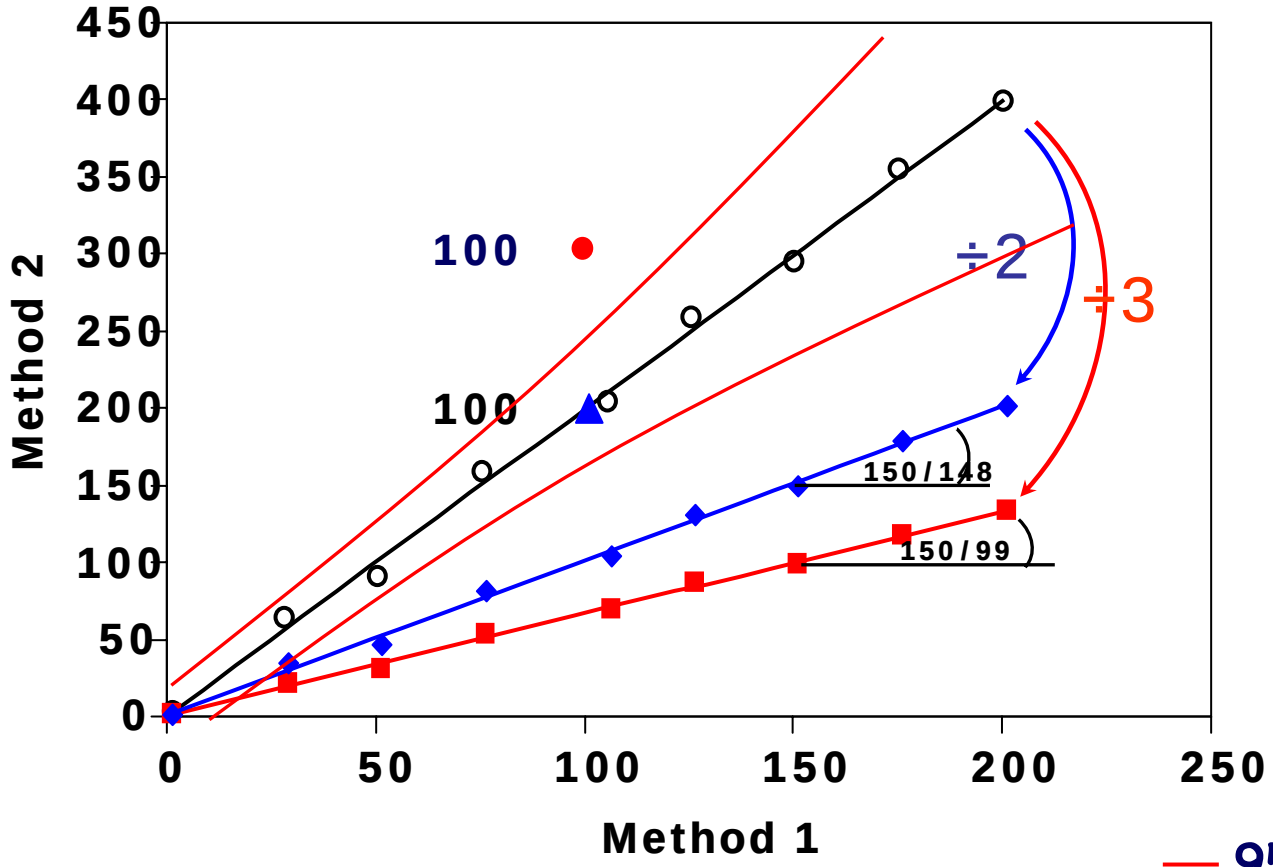
Comparing the response of Reference Materials (RMs) with the response of patient samples



- Patient samples
- Non-commutable RM, assigned value 100
- Calibration with non-commutable RM
- Commutable RM, assigned value 100
- Calibration with commutable RM
- 95 % prediction interval

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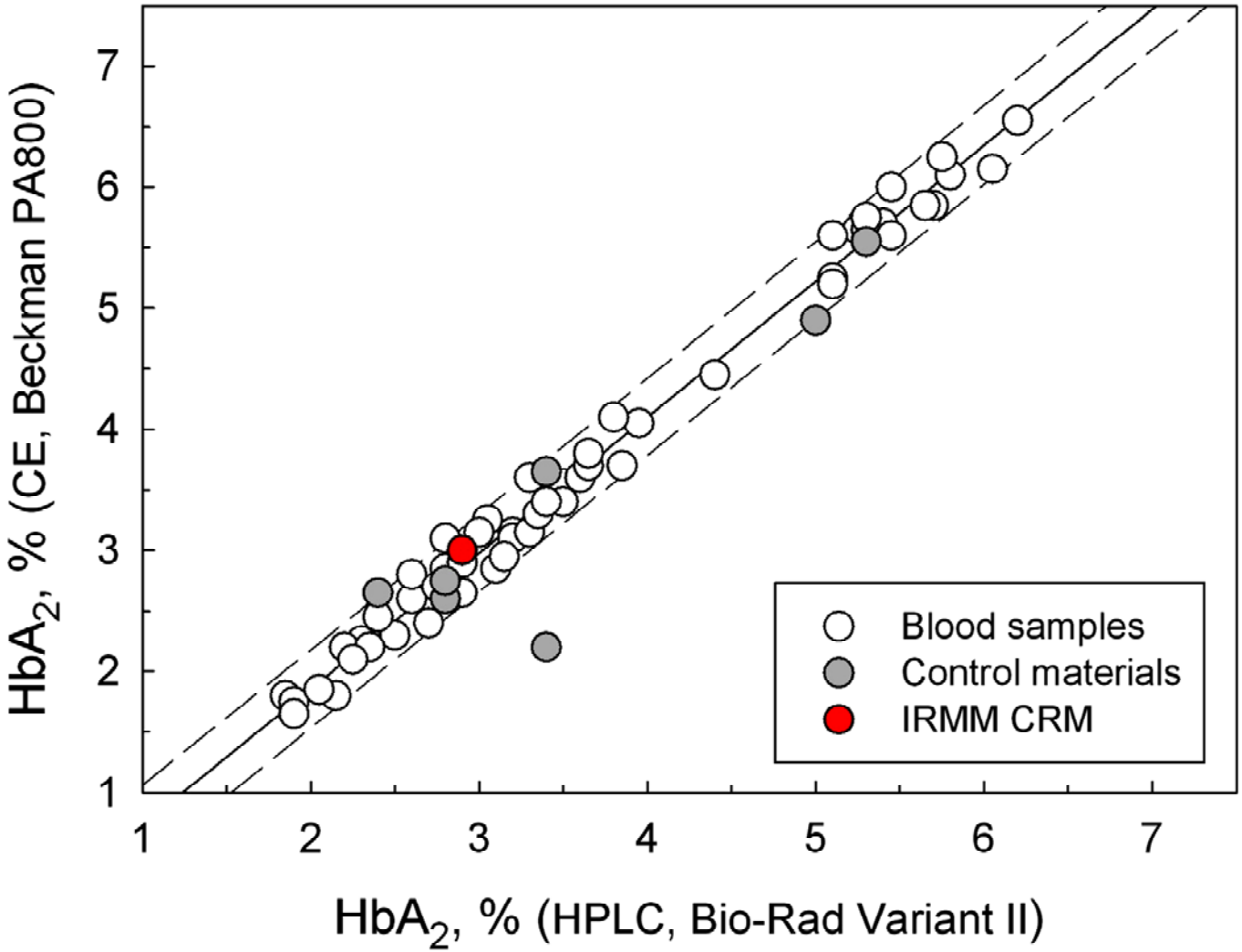
Commutability test



Sort Term Stability

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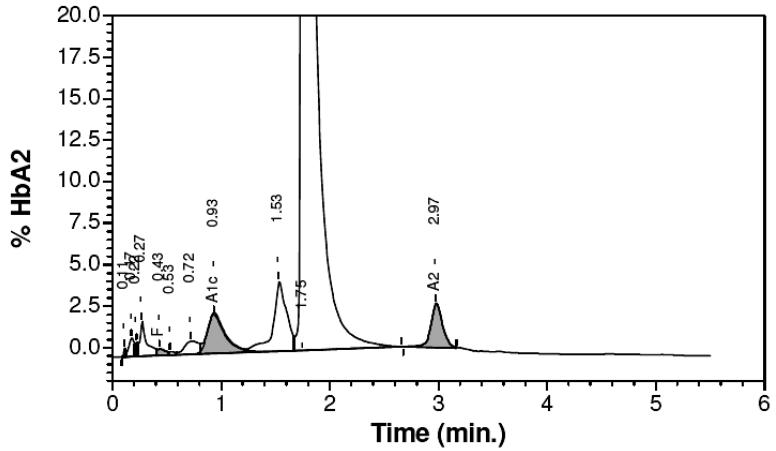
F Concentration = 0.7 %
A1c Concentration = 5.8 %
A2 Concentration = 2.7 %

Analysis comments:

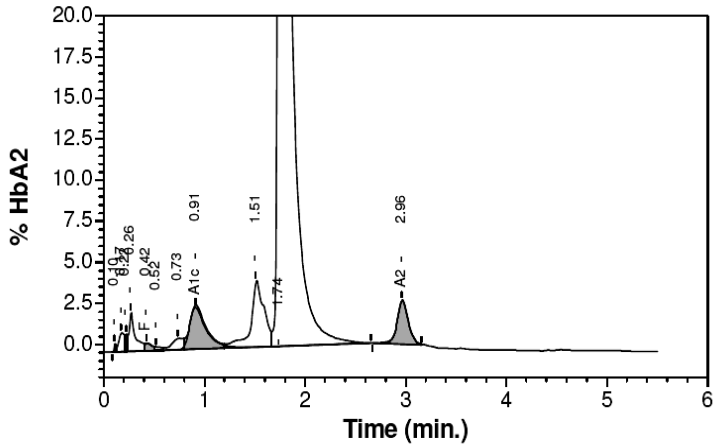


Just after the reconstitution

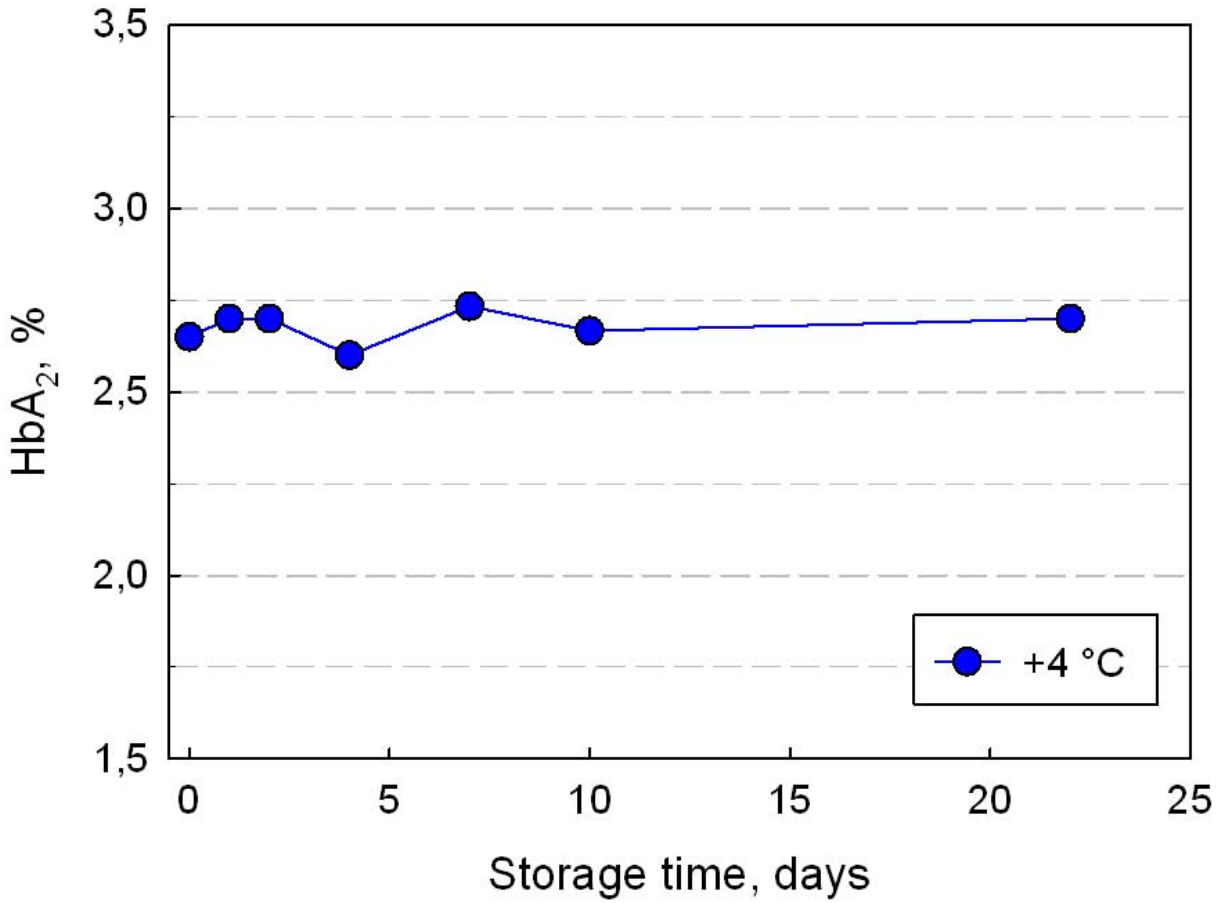
After 22 days of storage at +4 °C

F Concentration = 0.7 %
A1c Concentration = 5.9 %
A2 Concentration = 2.7 %

Analysis comments:



Sort Term Stability



Long Term Stability

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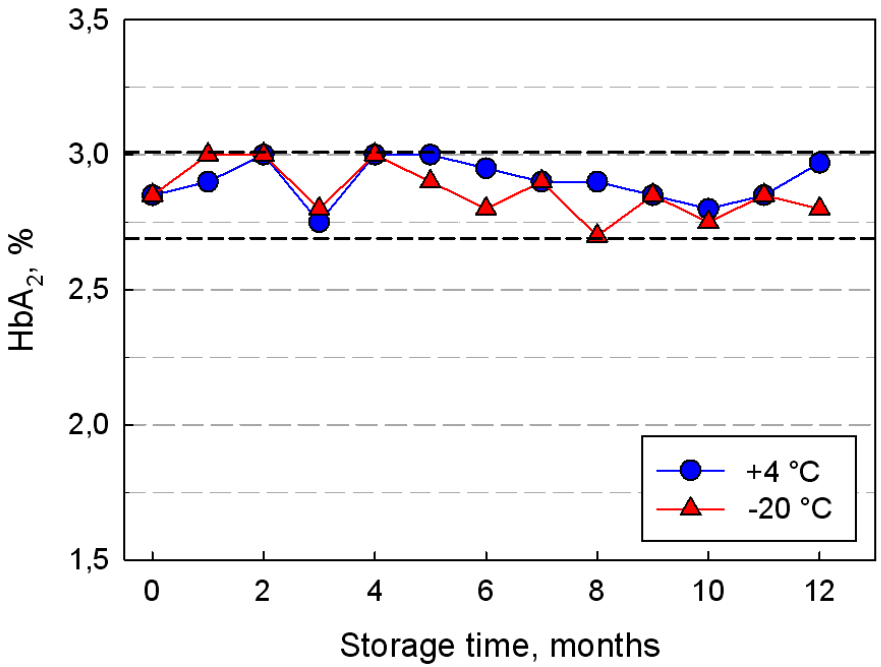
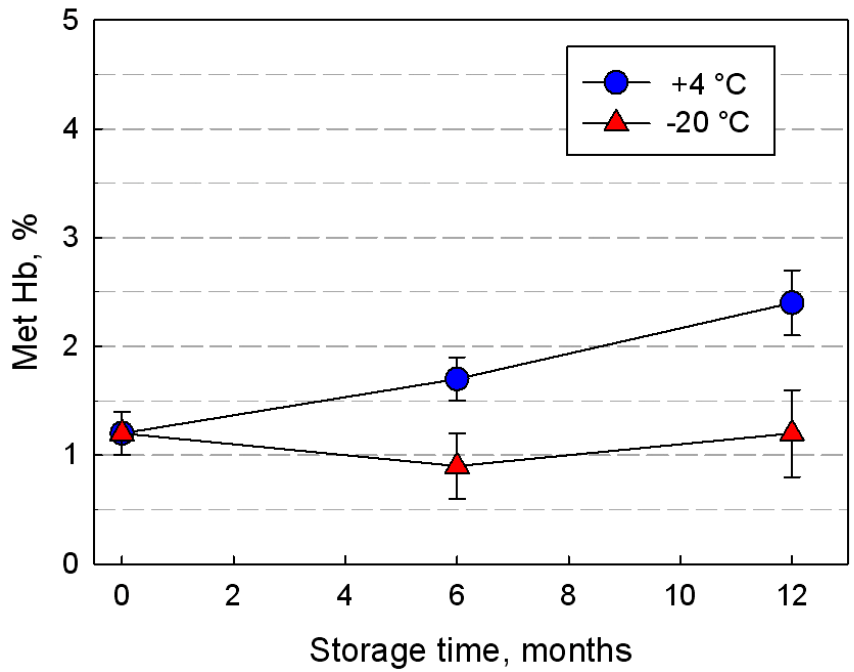
lyophilized material

- June 2009 -





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Long Term Stability

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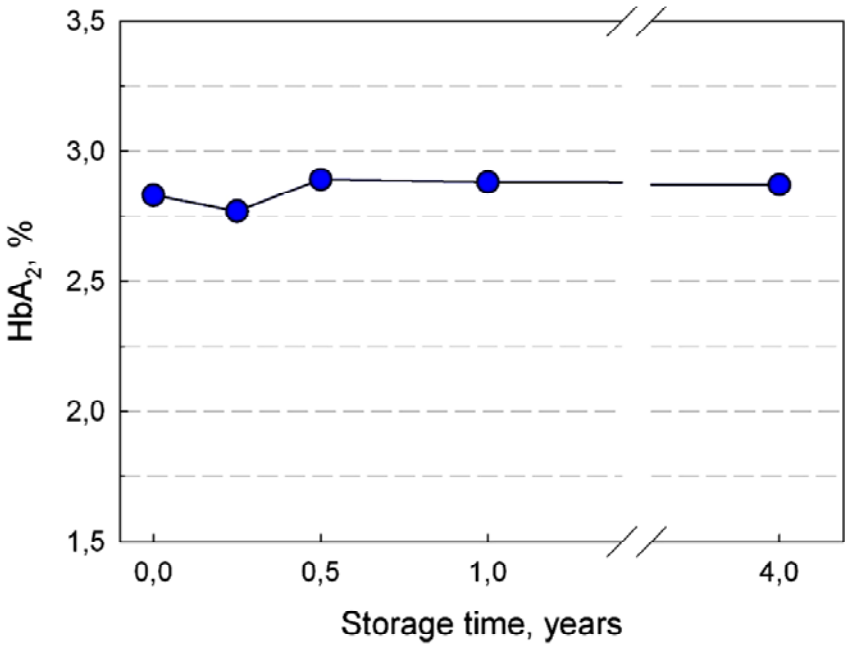
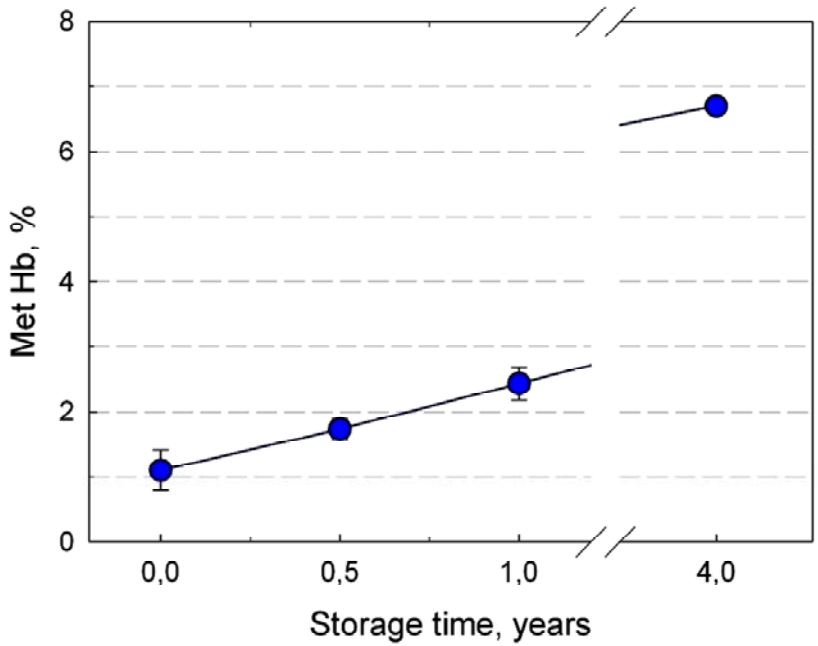
storage temp. +4 °C

HbA_2



MetHb



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Long Term Stability

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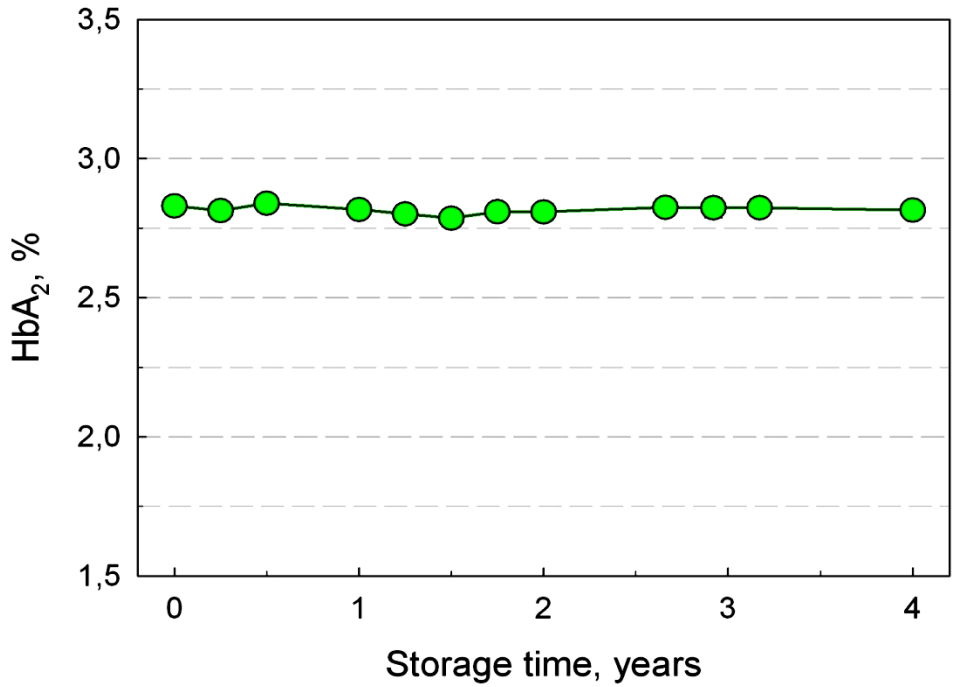
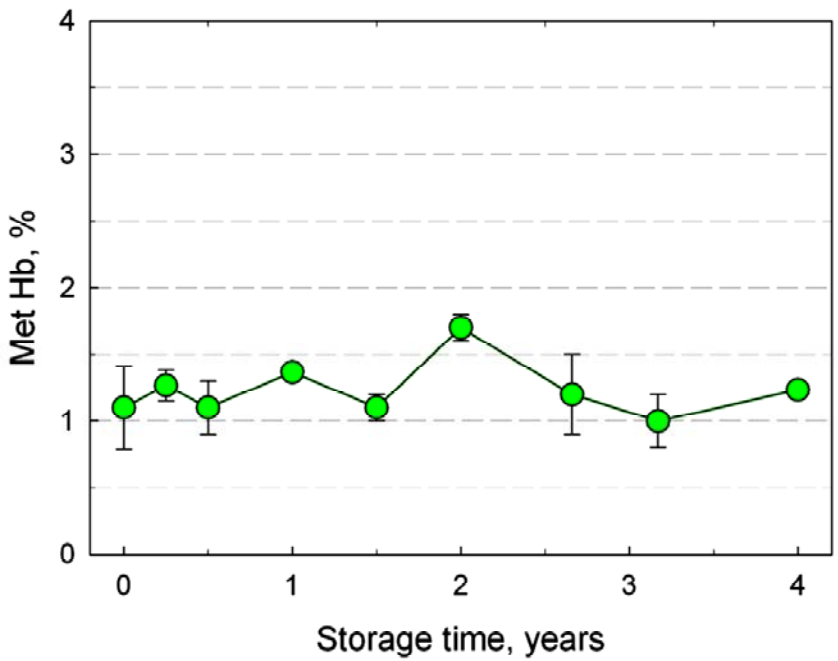
storage temp. -20 °C

- October 2012 -

HbA_2



MetHb



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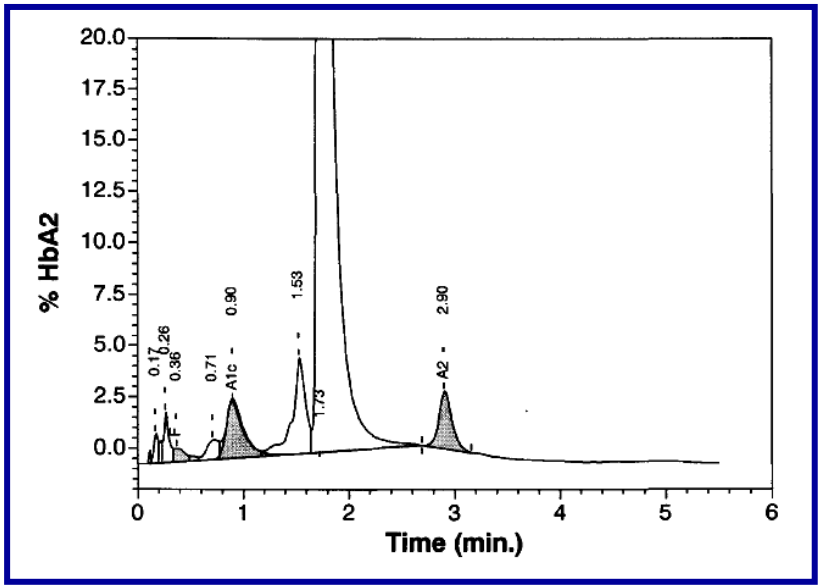
Long Term Stability

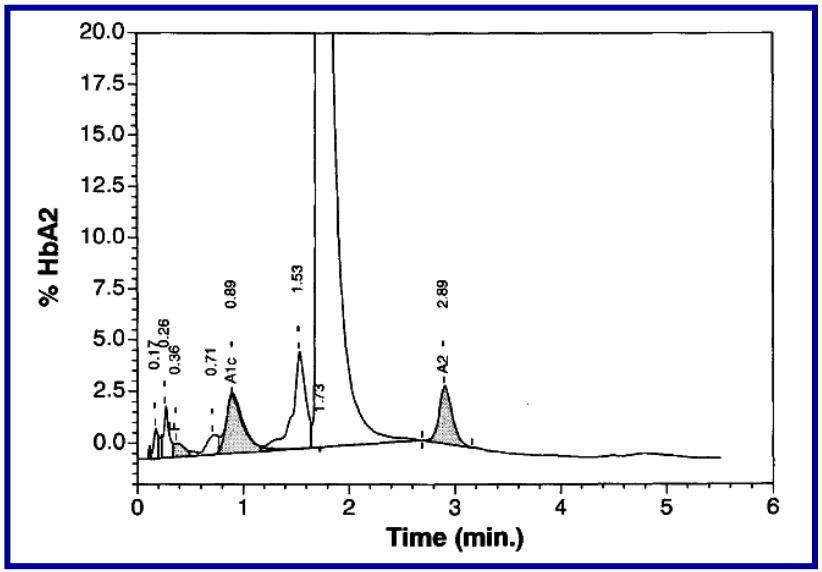


Just after the reconstitution

43 mmol/mol HbA1c (IFCC)
2.8% HbA2

After 4 years of storage at -20 °C

44 mmol/mol HbA1c (IFCC)
2.8% HbA2



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Second batch

human hemolysate with normal HbA_2 concentration

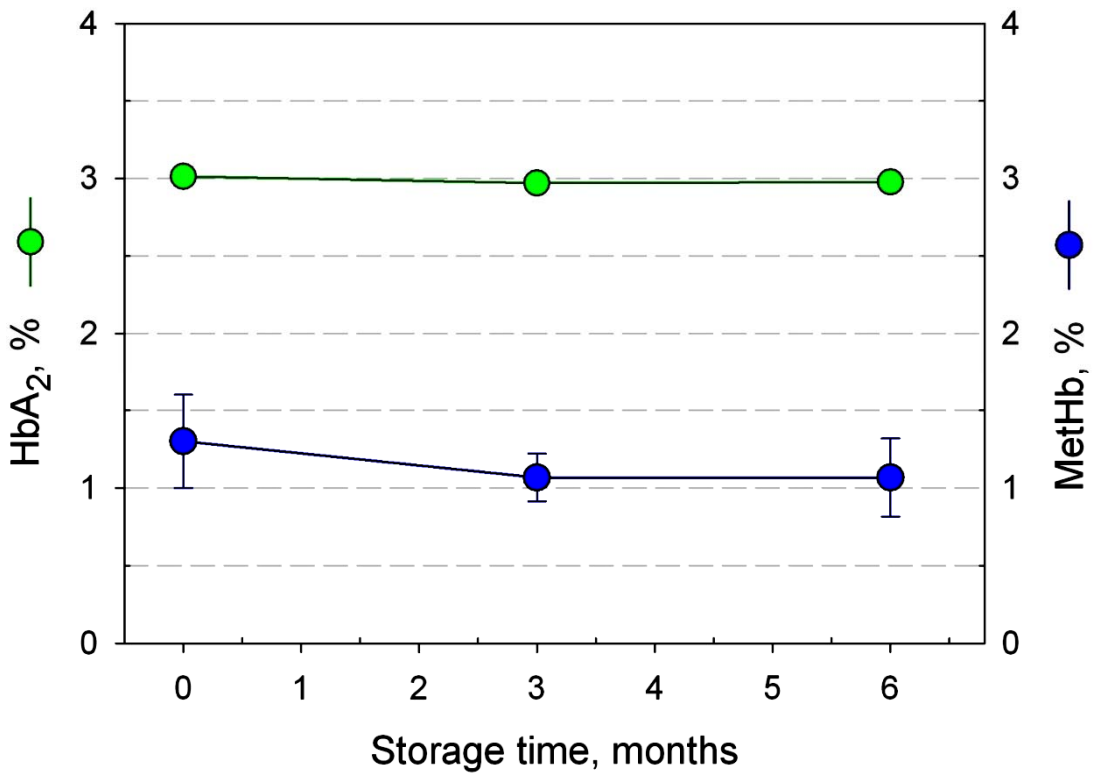
Preparation of stabilized hemolysate
Milan (Oc. 2010)

↓

Lyophilization under argon atmosphere
to prevent oxidation during storage
IRMM (Feb. 2011)

Long Term Stability

storage temp. +4 °C



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Lyophilised human hemolysate

- Characterisation by: UV, CE, electrophoresis ✓

- Commutable ✓
  - Correlation: further investigation on the differences in value assignment when using polyCATA, RP C4 globin and Sebia Hydrasys ✓

- STS : stable at 4 °C for 4 weeks ✓

- LTS : stable at: -20 °C ✓
  +4 °C under N_2 ✓

- Value assignment of purified calibrant: by LC/MS ✓

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