Biologic variability of HbA_2 and related parameters

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NEW BIOLOGIC AND ANALYTIC ISSUES ON HEMOGLOBIN A_2 AND OTHER MINOR HEMOGLOBINS
November 27th, 2012
Milano
Aula Magna - Settore Didattico Colombo

agenda

- What about analytical goals?
- Goals for HbA_2
  - Experimental approach
  - Clinical needs/outcomes
  - Opinion of experts
- Conclusions

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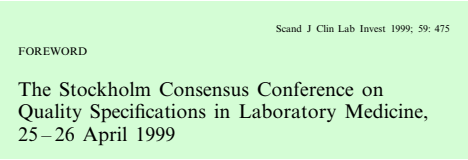
What about analytical goals? IFCC-IUPAC conference, Stockolm 1999

1. Evaluation of the effect of analytical performance on clinical outcomes in specific (general) clinical settings
   - data based on components of biological variation
   - data based on analysis of clinicians' opinions
2. Published professional recommendations
3. Performance goals set by regulatory bodies or EQAS organizers
4. Goals based on the current state of the art



Scand J Clin Lab Invest 1999; 59: 475

FOREWORD

The Stockholm Consensus Conference on Quality Specifications in Laboratory Medicine, 25–26 April 1999

Kenny et al, Scand J Clin Lab Invest 1999;59:585

Defining the analytical goals

- Biological variation
- Clinical needs
- Opinion of experts

Analytical goals clinical outcomes

- Few examples in Laboratory Medicine
- For HbA_{1c}: simulation based on the DCCT study (CLSI C54-P, 2007)
  - Poor glycemic control: HbA_{1c} >8%
  - Good glycemic control: HbA_{1c}<7%

Allowable total error: to correctly classify a subject who has a true HbA_{1c} value of 7.5%, measurement error must not exceed 0.5% in absolute terms

±0.5 % (absolute)
±6.7 % (relative)

Clin Chem Lab Med 2010;48(5):623–626 © 2010 by Walter de Gruyter • Berlin • New York. DOI 10.1515/CCLM.2010.140

Recommendations for the implementation of international standardization of glycated hemoglobin in Italy[1)]

Analytical goals derived from biological variation

IMPRECISION

- $CV_A \leq 0{,}25\ CV_I$ (Optimal)
- $CV_A \leq 0{,}5\ CV_I$ (Desirable)
- $CV_A \leq 0{,}75\ CV_I$ (Minimal)

TRUENESS

- $BA < 0.125\ (CV_I^2 + CV_G^2)^{0.5}$ (Optimal)
- $BA < 0.25\ (CV_I^2 + CV_G^2)^{0.5}$ (Desirable)
- $BA < 0.375\ (CV_I^2 + CV_G^2)^{0.5}$ (Minimal)

TOTAL ERROR

- $TEa\ \% = 1.65\ (0{,}25\ CV_I) + 0{,}125\ (CV_I^2 + CV_G^2)^{1/2}$ (Optimal)
- $TEa\ \% = 1.65\ (0{,}5\ CV_I) + 0{,}25\ (CV_I^2 + CV_G^2)^{1/2}$ (Desirable)
- $TEa\ \% = 1.65\ (0{,}75\ CV_I) + 0{,}375\ (CV_I^2 + CV_G^2)^{1/2}$ (Minimal)

Fraser et al, Ann Clin Biochem 1997;34:8-12

Desirable Specifications for Total Error, Imprecision, and Bias, Derived from Biologic Variation

This most recent and extensive listing of biologic goals has been provided by Ricos C, Alvarez V, Cava F, Garcia-Lario JV, Hernandez A, Jimenez CV, Minchinela J, Perich C, Simon M. "Current databases on biologic variation: pros, cons and progress." Scand J Clin Lab Invest 1999;59:491-500. *These data were updated with new data from 2008: see what was updated here.*

Annex I, Part I: Within-subject and between-subject CV values of analytes and *Desirable Analytical Quality Specifications for imprecision, bias and total error*

11-Desoxycortisol **through** *α-Fetoprotein*
Albumin **through** *CA 549 antigen*
Calcium **through** *Cystein*
Dehydroepiandrosterone sulfate **through** *Homocysteine*
Immunoglobulin A **through** *Lycopenene*
Magnesium **through** *Oxalate, output*
pCO_2 **through** *Rheumatoid factor*
SCC antigen **through** *Zinc*

- See The Reference List
- See The References
- See The Guest Essay

	Analyte	Biological Variation		Desirable specification		
		CVw	CVg	I(%)	B(%)	TE(%)
S-	11-Desoxycortisol	21.3	31.5	10.7	9.5	27.1
S-	17-Hydroxyprogesterone	19.6	52.4	9.8	14.0	30.2
S-	5'Nucleotidase	11.3	12.6	5.7	4.2	13.6
U-	5'-Hidroxiindolacetate, concentration, 24 h	20.3	33.2	10.2	9.7	26.5
S-	α1-Acid Glycoprotein	11.3	24.9	5.7	6.8	16.2
S-	α1-Antichymotrypsin	13.5	18.3	6.8	5.7	16.8
S-	α1-Antitrypsin	5.9	16.3	3.0	4.3	9.2
S-	α1-Globulins	11.4	22.6	5.7	6.3	15.7

Pre-analytical variation: general sources

- Sex
- Age
- Race
- Food and drugs
- Seasonal variations
- Sample collection and storage

agenda

- Goals for HbA_2
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Th ideal protocol for the determination of the biological variability of an analyte

- Apparently healthy subjects
- No drugs or alcohol, usual life styles
- Phlebotomy by the same person at the same time of the day
- Optimal protocol for sample transport, processing and storage at -80 °C
- Analysis of all samples in a single run, in duplicate

Braga et al, Clin Chim Acta 2010;411:1606-1610

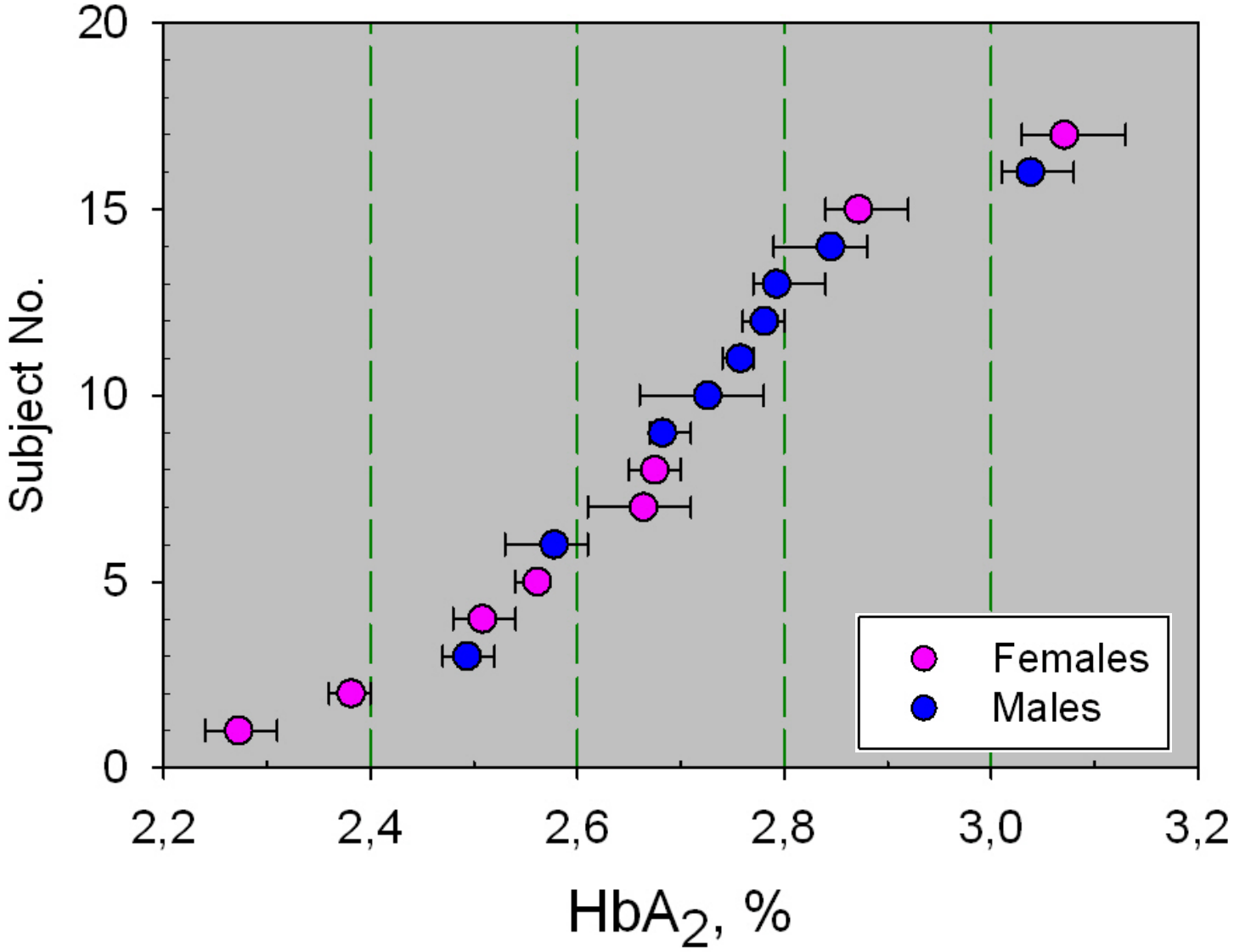
Experimental protocol

- N = 18 healthy subjects
  - N = 9 Men
  - N = 9 Women
  - Age: 26 – 52 y
- Five blood samples (every 2 weeks for 2 months)
- Parameters:
  - HbA_{1c}, glycated albumin, fructosamine, HbA_2
  - RBC, WBC, PLT, Hb, MCH, MCHC, MCV, RDW
- Measurements on fresh blood samples (whole blood cell count) and storage at -80 °C until analysis (minor hemoglobins, glycated albumin and fructosamine)
- Analysis of HbA_2 by HPLC

Data analysis

$$\sigma^2_{total} = \sigma^2_{anal} + \sigma^2_{I} + \sigma^2_{G}$$

- Analytical variation: from the duplicate results for each specimen or from internal QC (whole blood cell count)
- Intra-individual variation: from the serial results for each subject
- Inter-individual variation: from the total variance of data, minus the analytical and intra-individual components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Subject No.
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3,2
HbA2, %
Females
Males

Biologic variation of HbA_2

Group	HbA_2 %	CV_I %	CV_G %
Males	2.74	0.8	5.4
Females	2.63	0.6	9.2
All	2.69	0.7	7.7

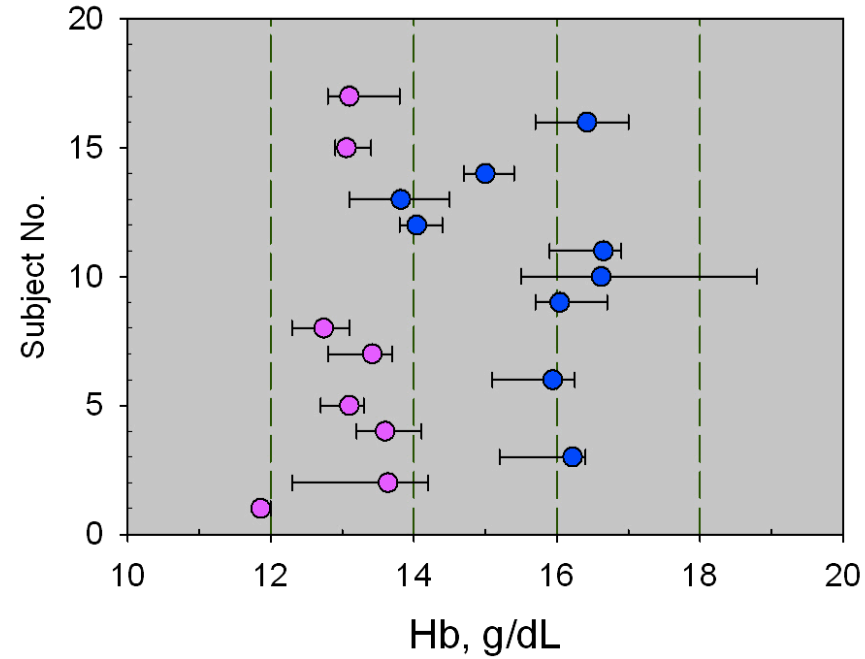
No difference in HbA_2 mean values between genders (p=0.265)

HbA_2, analytical goals (1)

- Biologic variation -

HbA_2

Analytical goal	Quality level		
	Minimal	Desirable	Optimal
Imprecision, %	0.5	0.3	0.2
Bias, %	2.9	1.9	1.0
Total error, %	4.5	3.0	1.5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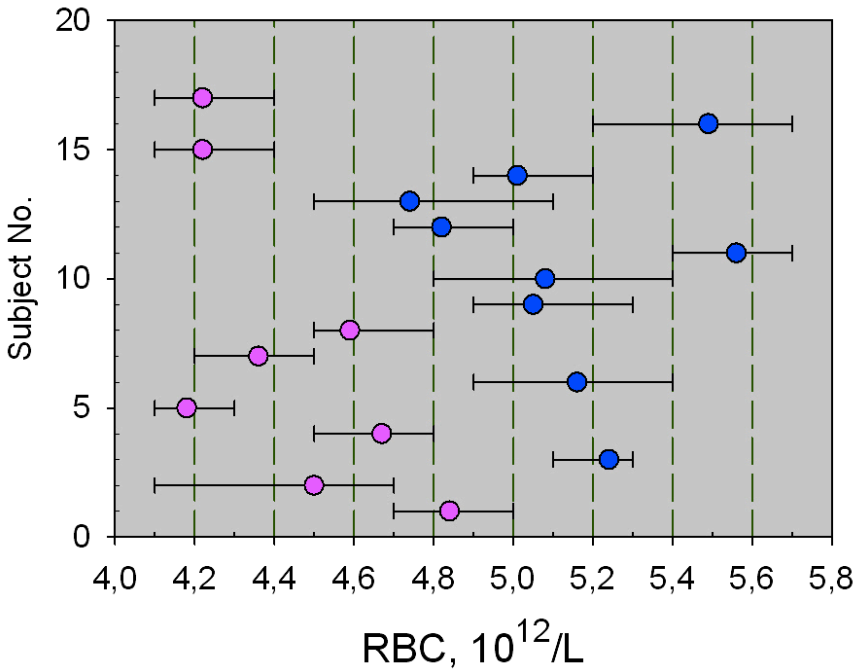
Hb, g/dL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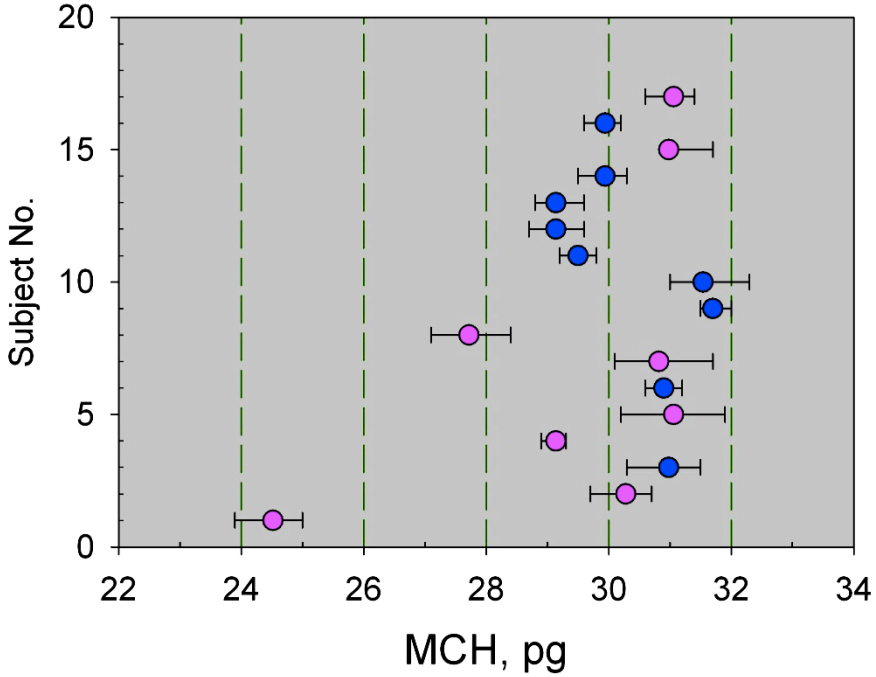
RBC, 10^12/L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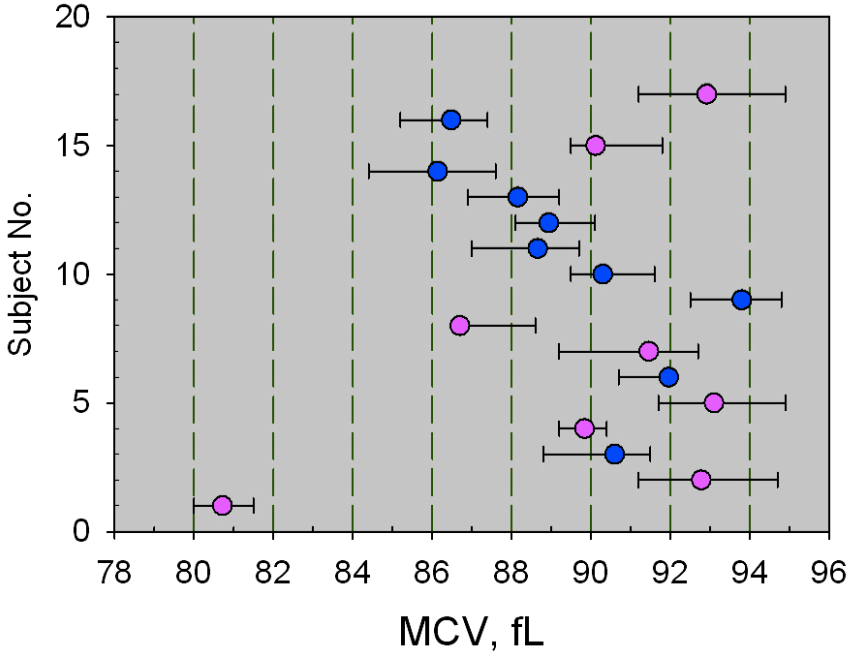
MCH, pg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MCV, fL

Parameter		Value	CV_I %		CV_G %	
Hb, g/dL	M	15.5	2.6		6.0	
	F	13.1	3.2		3.9	
	All	14.4	2.8	2.8	10.2	6.6
MCH, pg	M	30.3	---		2.9	
	F	29.4	1.2		7.3	
	All	29.9	0.7	1.6	5.5	5.2
MCV, fL	M	89.3	1.0		2.4	
	F	89.8	1.1		4.3	
	All	89.5	1.0	1.3	3.4	4.8
RBC, 10^{12}/L	M	5.1	2.9		4.7	
	F	4.4	3.0		4.8	
	All	4.8	2.9	3.2	8.5	6.1

data from Westgard's database

Whole blood cell count

Parameter	Desirable analytical goal		
	Imprecision %	Bias %	TE %
Hb	1.4 1.4	2.6 1.8	7.3 4.1
MCH	0.4 0.8	1.4 1.4	2.5 2.7
MCV	0.5 0.7	0.9 1.2	2.6 2.3
RBC	1.5 1.6	2.3 1.7	7.1 4.4

data from Westgard's database

HbA_2, analytical goals (2)

- clinical needs -

HbA_2 = 3.3 % (upper normal)
HbA_2 = 3.8 % (low β-thal carrier)

HbA_2 = 3.55 % → ?

TE = 0.25/3.55 x 100 = 7.0 %

HbA_2, analytical goals (3a)

- opinion of the experts -

Case no.	Description/question
1	A pregnant woman is tested for β-thalassemia as part of her antenatal screening blood tests. The HbA_2 result is 3.5%. RBC, MCH, MCV are within the normal range. A repeat test is taken and this gives a result of 3.2%. Do you believe this new HbA_2 result to be significantly different from the previous value of 3.5%? Yes/No

Case no.	HbA_2, mean, %	HbA_2 change, %	TE, %	Team	No. answers	Significancy (percent of the answers)
1	3.35	0.3	9.0	A	11	No (73%)
				B	83	No (64%)

Mosca et al, Clin Chem Lab Med 2012, October (ahead of print)

HbA_2, analytical goals (3b)

- opinion of the experts -

2 A pregnant woman is tested for β-thalassemia as part of her antenatal screening blood tests. The HbA_2 result is 3.2%. Iron studies undertaken at the same time indicate that she has iron deficiency. She is given iron supplements for 3 months after which time still MCV, MCH and Hb are low and a repeat test is taken. This gives a result of 3.7%.
Do you believe this difference to be significant?
Yes/No

Case no.	HbA_2, mean, %	HbA_2 change, %	TE, %	Team	No. answers	Significancy (percent of the answers)
1	3.35	0.3	9.0	A	11	No (73%)
				B	83	No (64%)
2	3.45	0.5	14.5	A	12	Yes (75%)
				B	83	Yes (81%)

Mosca et al, Clin Chem Lab Med 2012, October (ahead of print)

HbA_2, analytical goals (4)

- Summary -

approach	total error, %
Biologic Variability	4.5
Clinical needs	7.0
Opinion of the experts	9.0÷15

Conclusions

- The biological variability of HbA_2 is very small
- CV_I is < than CV_G: limit to the use of reference intervals based on populations
- The analytical goal for CV_a is very stringent
- The analytical goals can be different depending on the criterium
- More time is needed to accomplish a complete reference system for HbA_2 → IQC and EQAS are essential in order to keep under strict control the HbA_2 methods

Aknowledgments

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(opinion of the experts)

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Looking forward to meeting you at

EUROMEDLAB Milano 2013

20th IFCC-EFCC European Congress of Clinical Chemistry and Laboratory Medicine

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