

HbA1c

The last issues to be addressed

Cas Weykamp

IFCC HbA1c Network Coordinator

Queen Beatrix Hospital, Winterswijk, the Netherlands

IEQAS Annual Conference, Dublin, 4th October 2018

The amazing year 1969

The amazing year 1969



1969 – July 20

The amazing year 1969



1969 – July 20



1969 – February 9

The amazing year 1969



1969 – July 20



1969 – February 9

1969 – July 18

???

The amazing year 1969



1969 – July 20



1969 – February 9

1969 – July 18

Studies of an unusual hemoglobin in patients with diabetes mellitus

Samuel Rahbar ^{a, b, 1}, Olga Blumenfeld ^{a, b, 2}, Helen M. Ranney ^{a, b, 3}

^a Heredity Unit Department of Medicine and Biochemistry Albert Einstein College of Medicine New York, New York 10461 USA

^b Unit for Research in Aging Department of Medicine and Biochemistry Albert Einstein College of Medicine New York, New York 10461 USA

Received 18 July 1969, Available online 29 October 2004.

Discovery HbA1c



ELSEVIER



Studies of an unusual hemoglobin in patients with diabetes mellitus

Samuel Rahbar^{a, b, 1}, Olga Blumenfeld^{a, b, 2}, Helen M. Ranney^{a, b, 3}

^a Heredity Unit Department of Medicine and Biochemistry Albert Einstein College of Medicine New York, New York 10461 USA

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Abstract

The properties of an unusual hemoglobin found in patients with diabetes mellitus resembled those of hemoglobin A_{1c} prepared from normal subjects. A two-fold increase of hemoglobin A_{1c} was found in diabetic patients. Structural studies suggest the possibility that an amino sugar is bound to hemoglobin A_{1c} in diabetic patients.



The Last Issues of.....

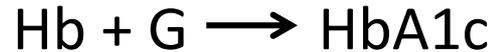
HbA1c

.....in Diabetes Care

HbA1c

HbA1c: an Elegant and Simple Concept

HbA1c: an Elegant and Simple Concept



$$(\text{HbA1c}) = (\text{Hb}) \times (\text{G}) \times \text{T} \times \text{C}_{\text{ELS}}$$

(Hb) and C_{ELS} are constant

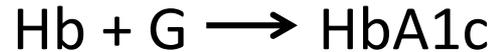
(Hb) = Hemoglobin concentration

T = Time

G = Glucose concentration

C_{ELS} = Erythrocyte Life Span

HbA1c: an Elegant and Simple Concept



$$(\text{HbA1c}) = (\text{Hb}) \times (\text{G}) \times \text{T} \times \text{C}_{\text{ELS}}$$

(Hb) and C_{ELS} are constant

$$(\text{HbA1c}) = (\text{G}) \times \text{T}$$

(HbA1c) = Averaged Glucose = Longterm Diabetic Control

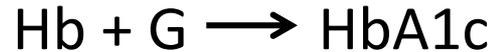
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Challenge Variants

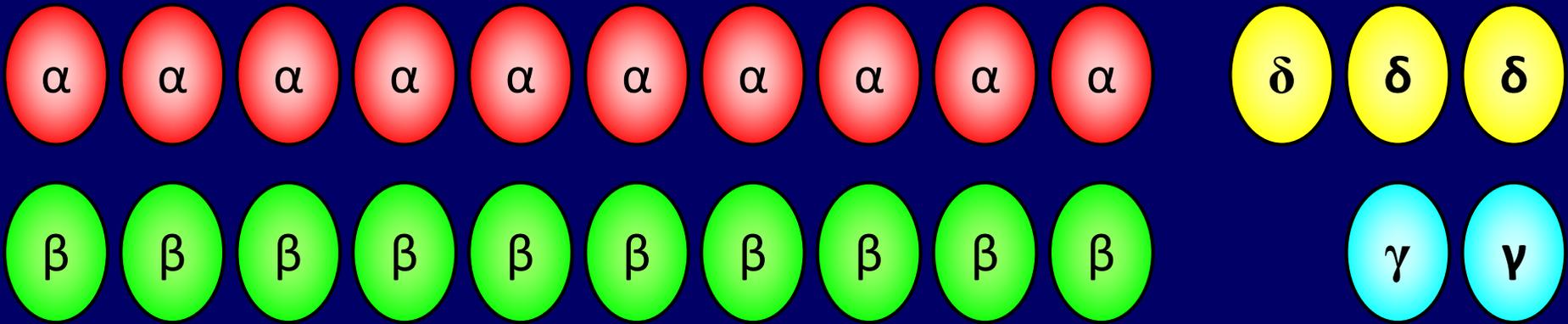
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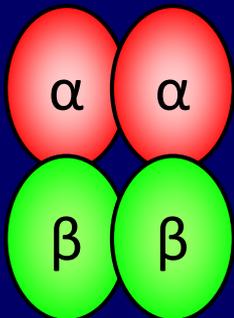
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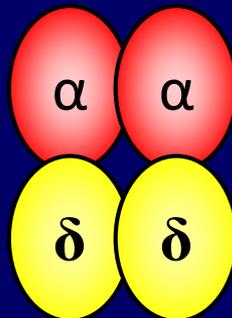
Normal



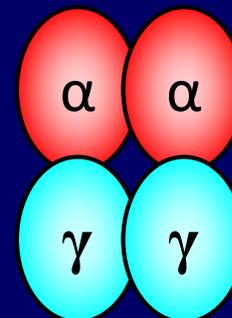
- * Equal Amounts of α - and β -chains synthesized
- * Only small amounts of γ - and δ -chains
- * Thus nearly all hemoglobins assembled are $\alpha_2\beta_2$: HbA



HbA
97%

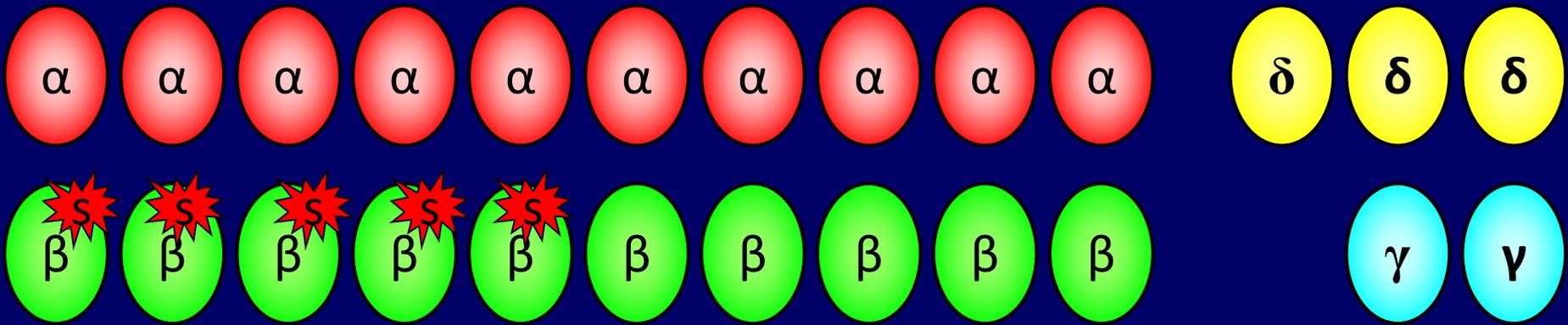


HbA2
2.5%

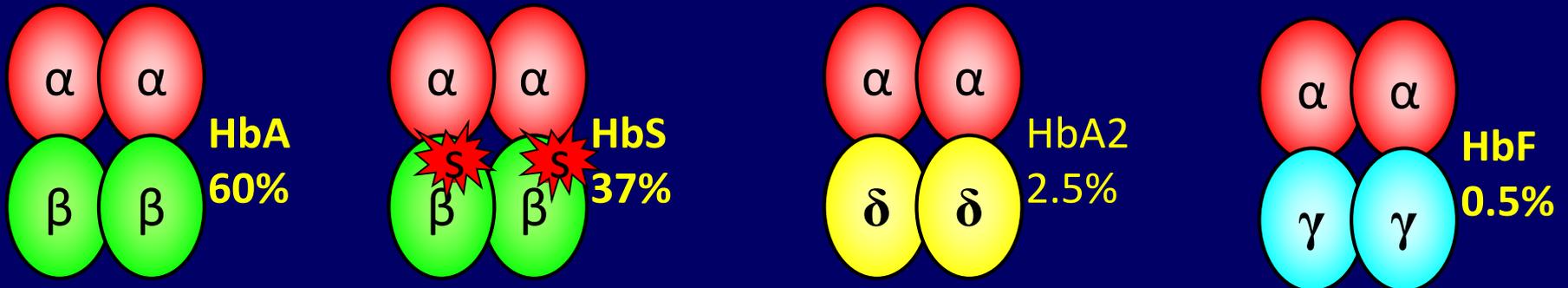


HbF
0.5%

Heterozygous Variant S



Half of the β -chains contain the mutation S



Facts Variants!

- * There are about 900 Variants
- * Big four: S,C,E and D ~ 99%
- * Highly dependent on ethnicity
 - S and C: African Americans/Middle East
 - E: Southeast Asia
 - D: Any population; higher Iran/India
- *Prevalence: 7% of the world population

Variants and HbA1c methods

Variants are seen

- HPLC methods
- Capillary Electrophoresis

Variants are not seen

- Immunochemical methods
- Enzymatic methods
- Affinity chromatographic methods

Impact Variants HbA1c

Analytical

Interference Measurement

But solved for most modern methods

Ask your manufacturer

Biological

Presence of a variant might indicate clinical conditions associated with change in erythrocyte life and thus with interpretation of HbA1c results

Variant Controversy

“Do you want to see Variants in your HbA1c test?”

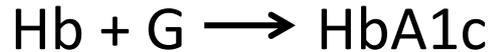
No!

HbA1c is meant to diagnose or monitor diabetes and therefore I do not want information on Variants

Yes!

Visibility of variants may indicate the presence of clinical conditions associated with altered erythrocyte lifespan and thus prevent wrong interpretation of the result. In addition, knowledge is of use for genetic counseling

HbA1c: an Elegant and Simple Concept



$$(\text{HbA1c}) = (\text{Hb}) \times (\text{G}) \times \text{T} \times \text{C}_{\text{ELS}}$$



Challenge

**Diagnosis &
Erythrocyte
Lifespan**

(Hb) = Hemoglobin concentration

T = Time

G = Glucose concentration

C_{ELS} = Erythrocyte Life Span

HbA1c for Diagnosis?

Many Reasons to say “Yes”

Biology

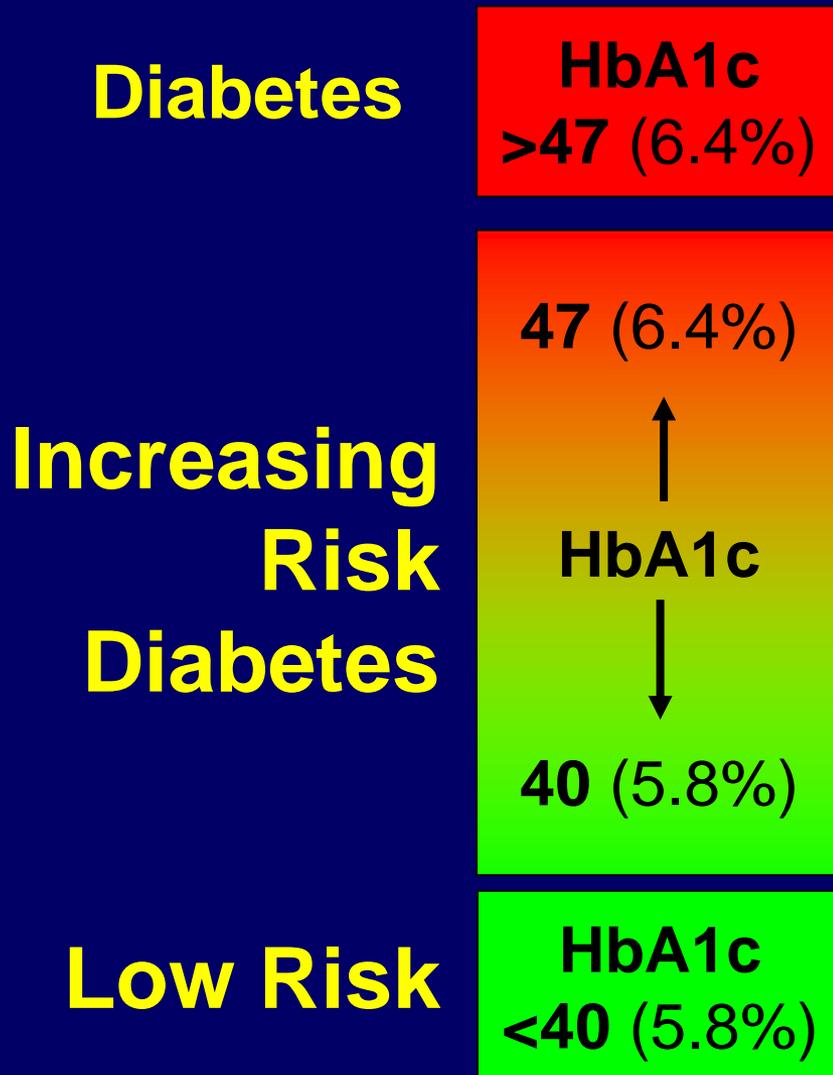
- Reflects chronic glycemia (2-3 months)
- Independent acute factors (e.g. stress; exercise)
- Very low intra-individual variability (CV ~1%)

Analysis

- Blood collected any time of the day
- Sample is stable
- Fasting not necessary
- “Well standardized” assay

HbA1c for Diagnosis; Why doubt?

HbA1c for Diagnosis; Why doubt?



HbA1c for Diagnosis; Why doubt?

Diabetes

HbA1c
>47 (6.4%)

Window

“Normal” and “Diabetic”

Narrow

Increasing
Risk
Diabetes

47 (6.4%)



HbA1c



40 (5.8%)

Low Risk

HbA1c
<40 (5.8%)

HbA1c for Diagnosis; Why doubt?

Diabetes

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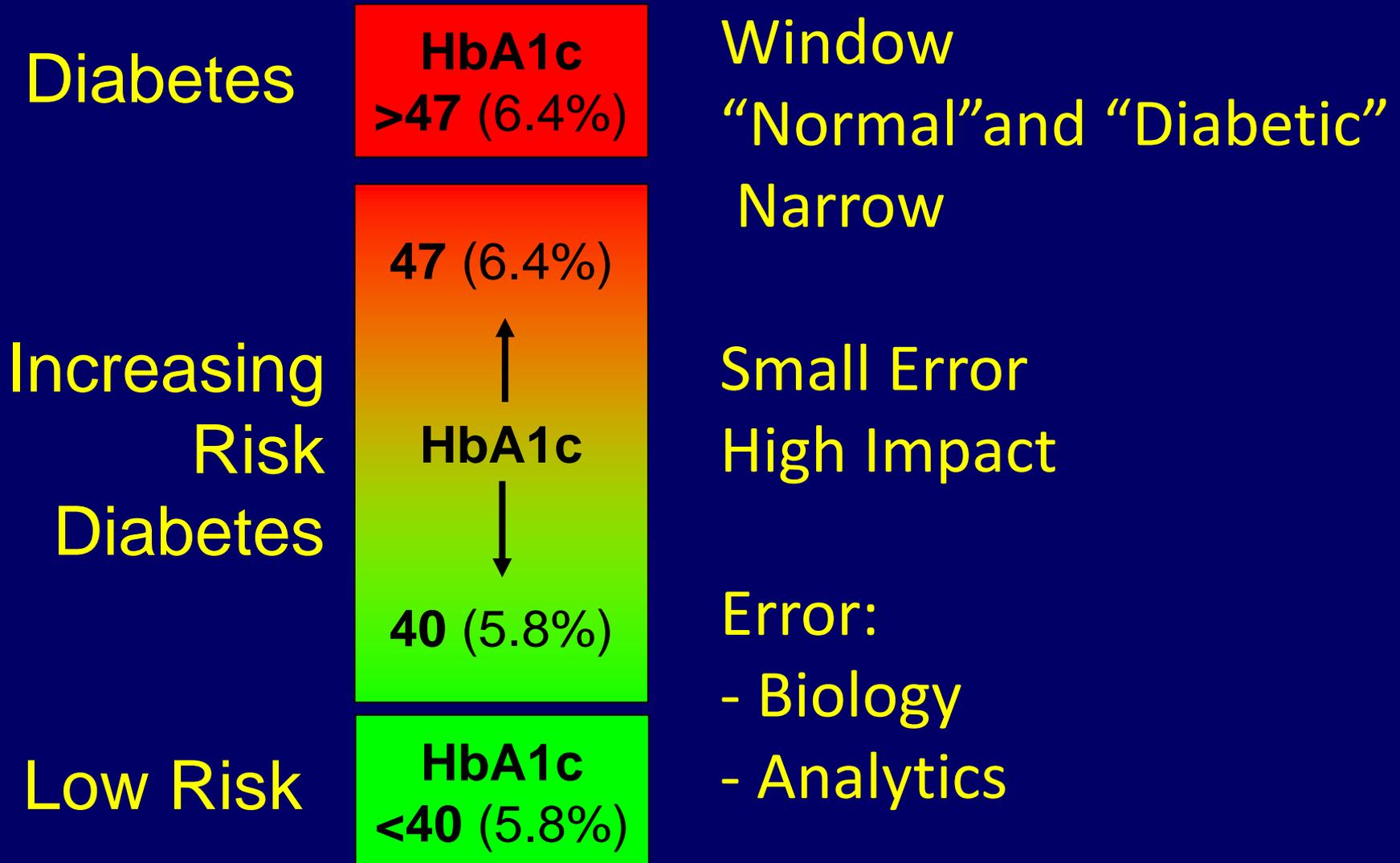
Small Error

High Impact

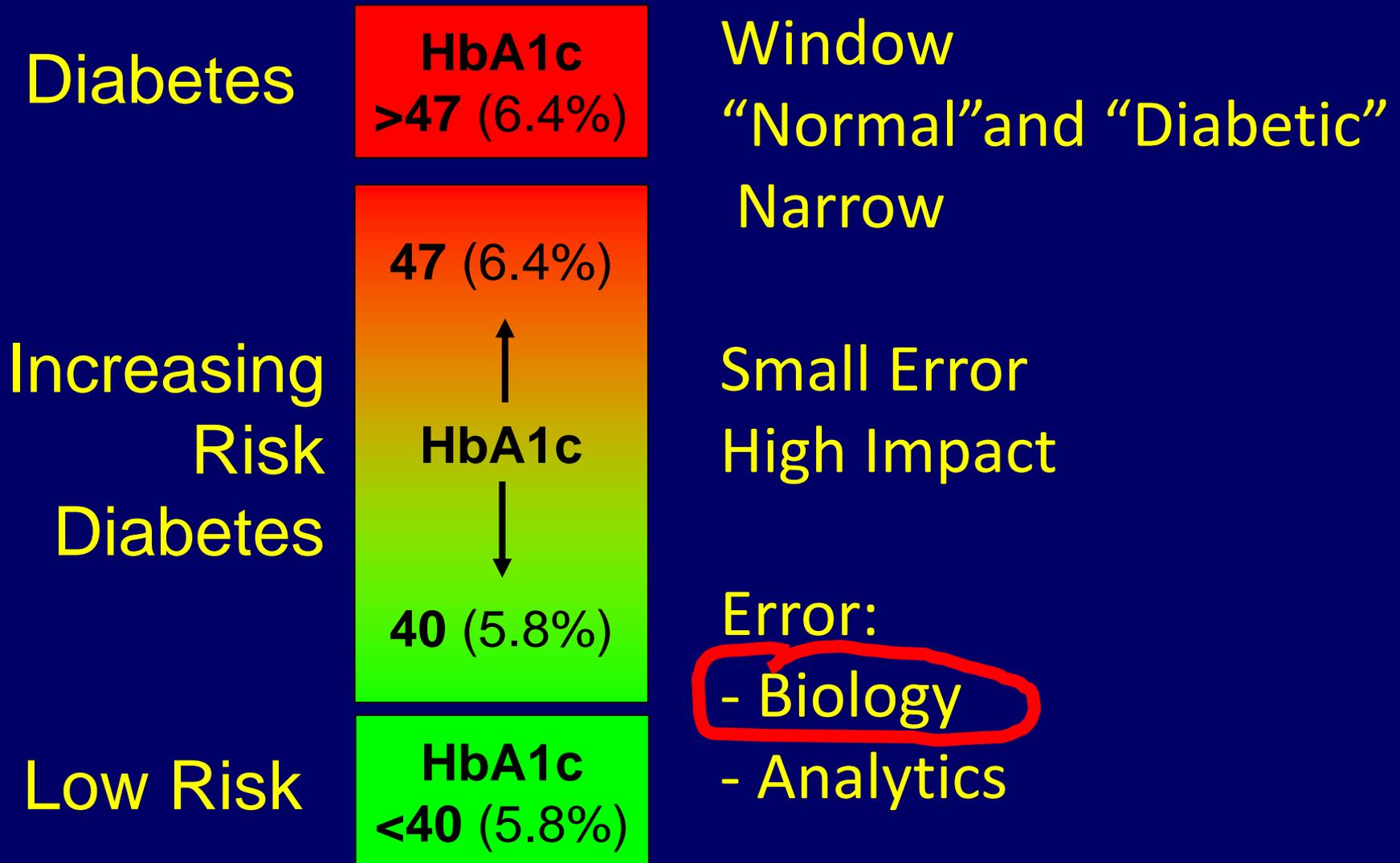
Low Risk

HbA1c
<40 (5.8%)

HbA1c for Diagnosis; Why doubt?



HbA1c for Diagnosis; Why doubt?



Biology: Erythrocyte Lifespan

Assumption: 120 days; equal for all

But

Physiological

Intra-individual Variation: CV 1%

Inter-individual Variation: CV 5% ?

We do not know

Pathological

Systematically lower: hemolytic anemia; acute blood loss

Systematically higher: iron deficiency (iron supplementation)

Biology: Erythrocyte Lifespan

Assumption: 120 days; equal for all

But

Physiological

Intra-individual Variation: CV 1%

Inter-individual Variation: CV 5% ?

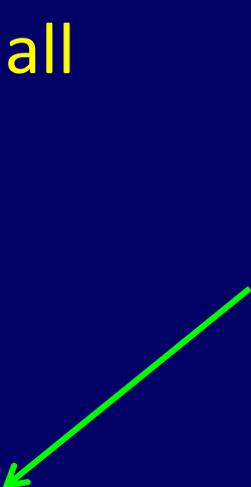
We do not know

Pathological

Systematically lower: hemolytic anemia; acute blood loss

Systematically higher: iron deficiency (iron supplementation)

Monitoring



Diagnosis



Interpretation HbA1c: Impact Erythrocyte Lifespan

Biology "Error"

HbA1c
>47 (6.4%)

Longer



47 (6.4%)



Normal

Erythrocyte

Lifespan

HbA1c



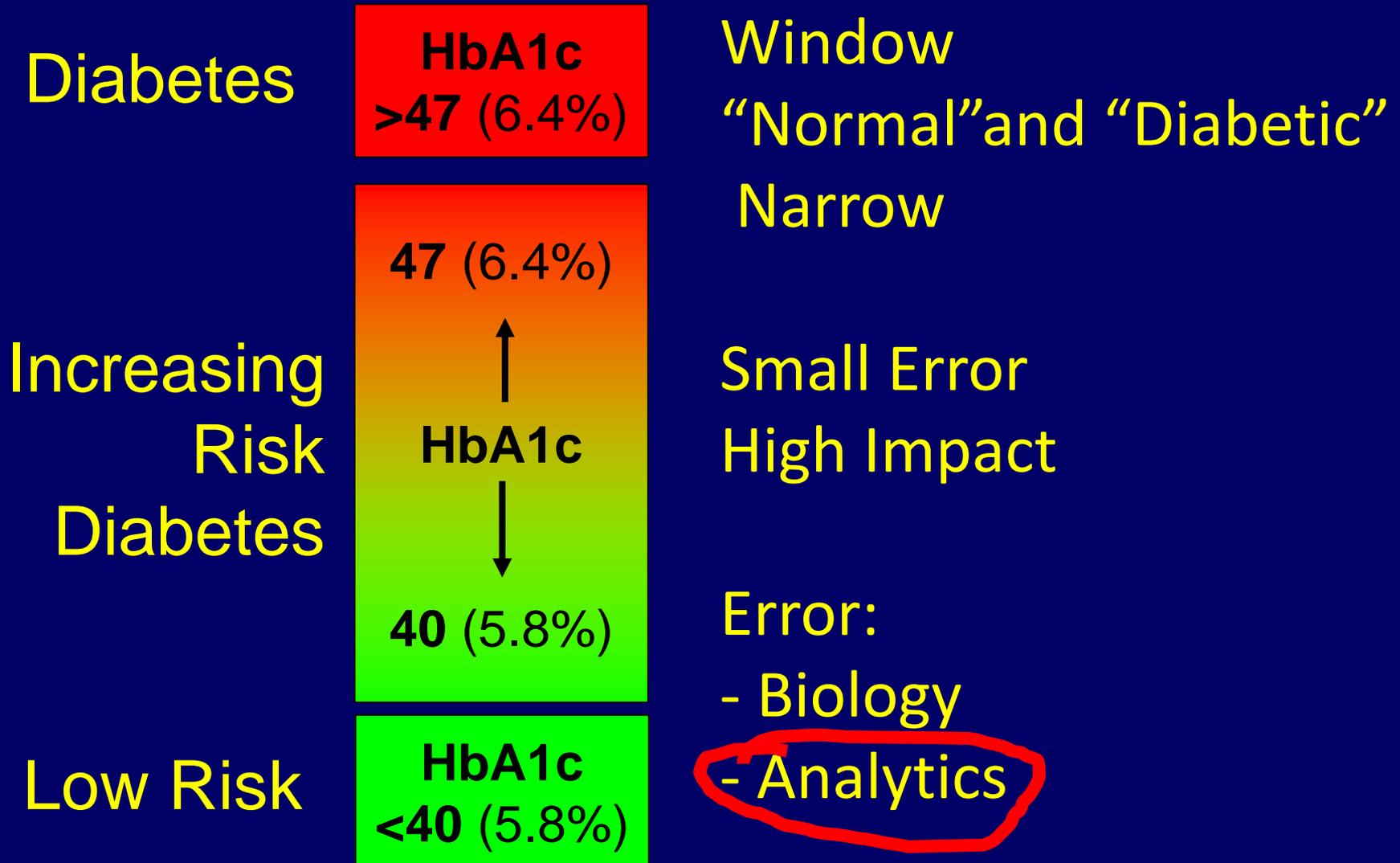
Shorter



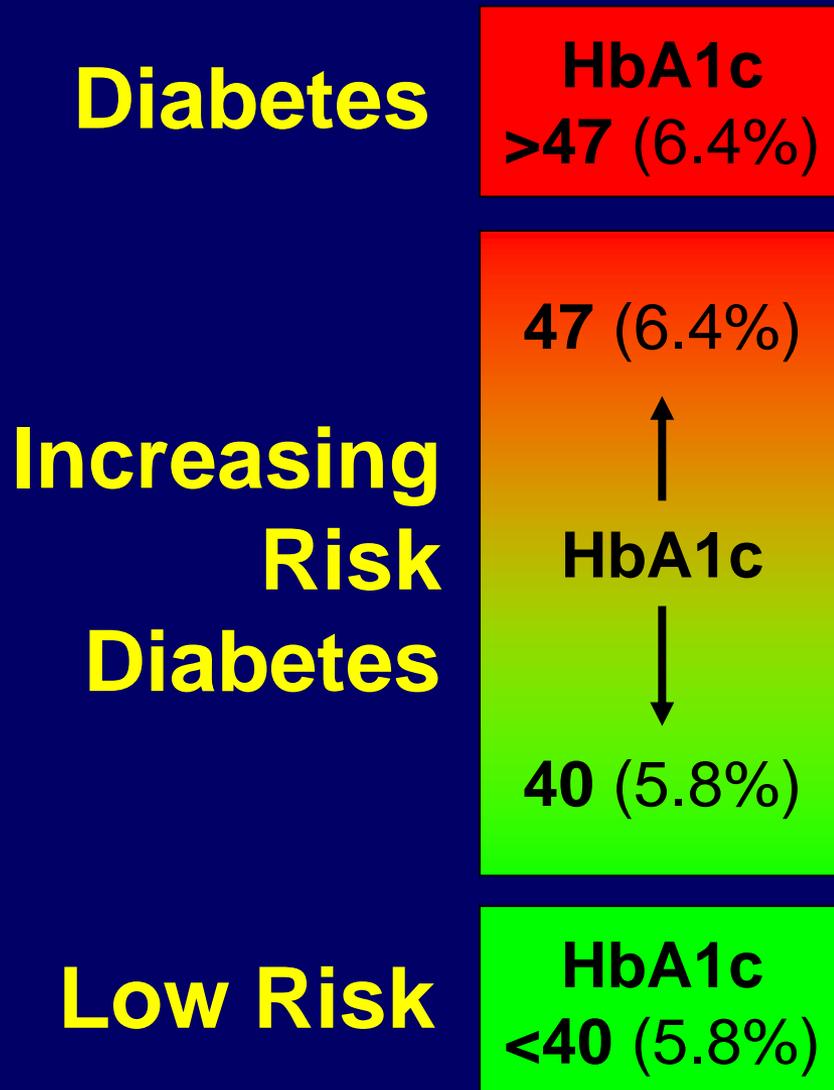
40 (5.8%)

HbA1c
<40 (5.8%)

HbA1c for Diagnosis; Why doubt?



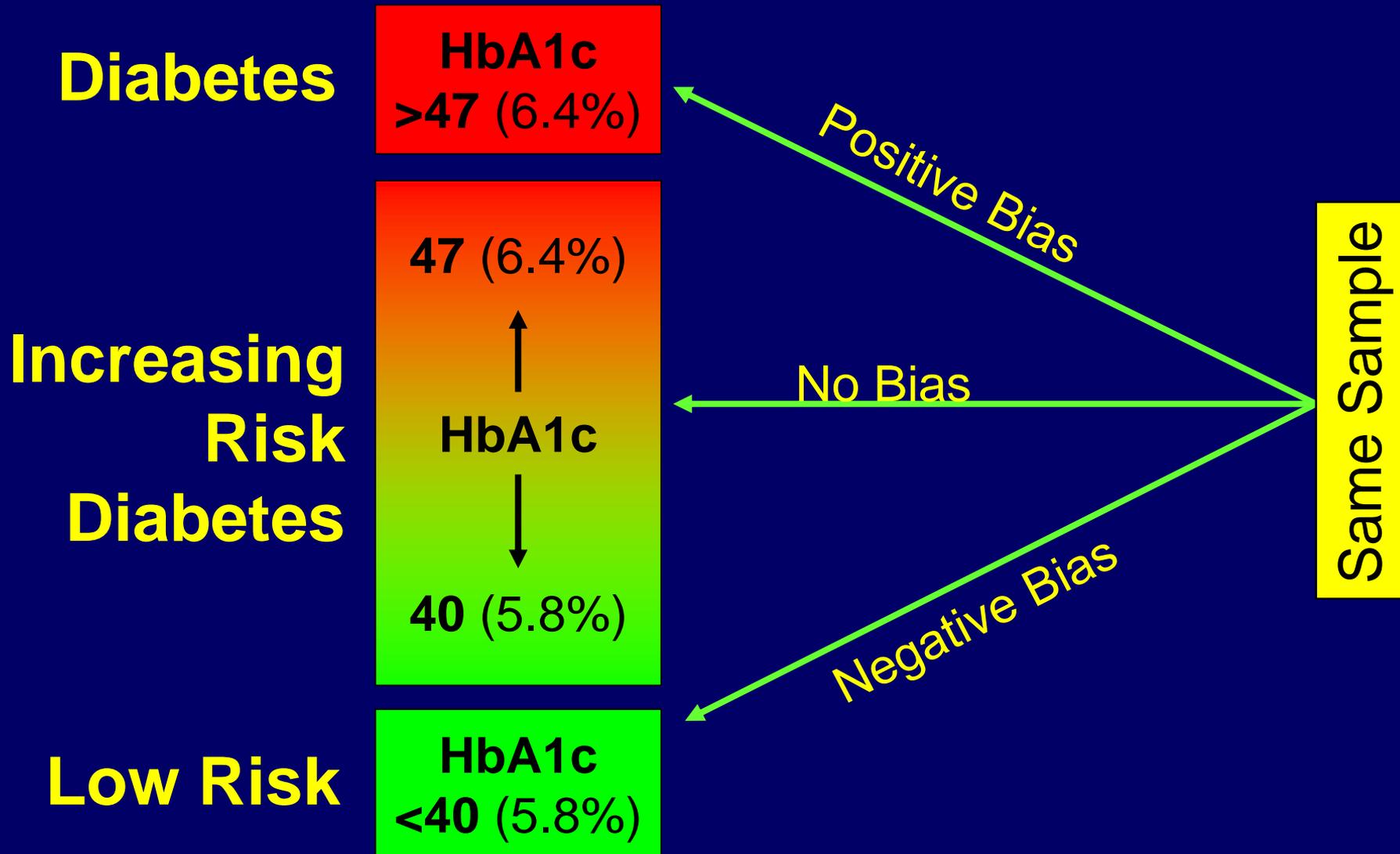
Discussion: Small Differences



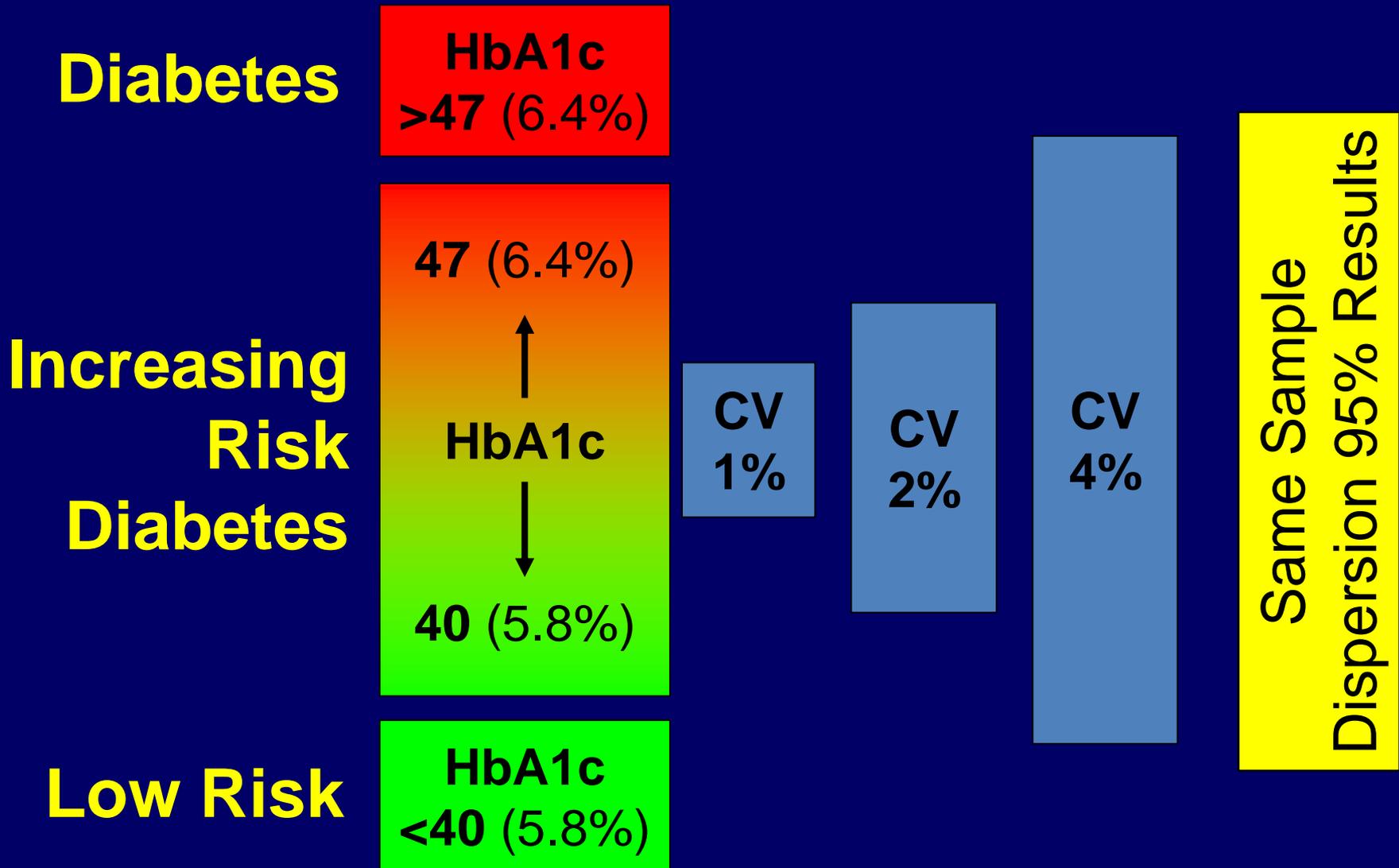
Error:

- Bias
- Imprecision

Impact Bias on Interpretation



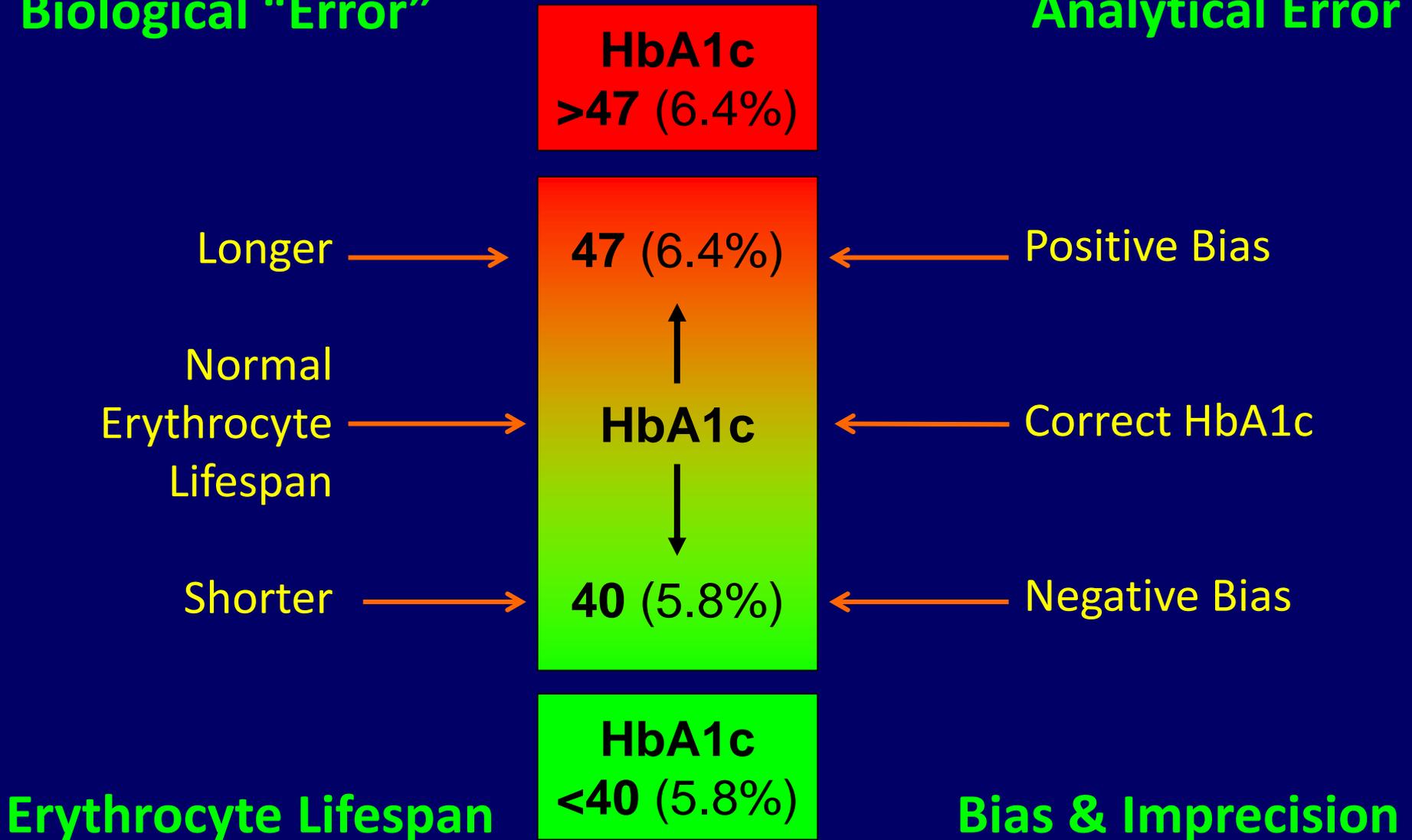
Impact Imprecision on Interpretation



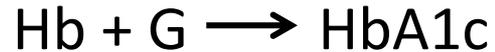
Interpretation HbA1c: Impact Error

Biological "Error"

Analytical Error



HbA1c: an Elegant and Simple Concept



$$(\text{HbA1c}) = (\text{Hb}) \times (\text{G}) \times \text{T} \times \text{C}_{\text{ELS}}$$



Challenge Quality

(Hb) = Hemoglobin concentration

T = Time

G = Glucose concentration

C_{ELS} = Erythrocyte Life Span

Quality of HbA1c Measurement

1. What is Quality?
2. How good is good enough?
3. How can I know my performance?
4. In case of Poor Performance:
 - Blame Lab or Manufacturer?
 - How do I know?

Quality of HbA1c Measurement

1. What is Quality?

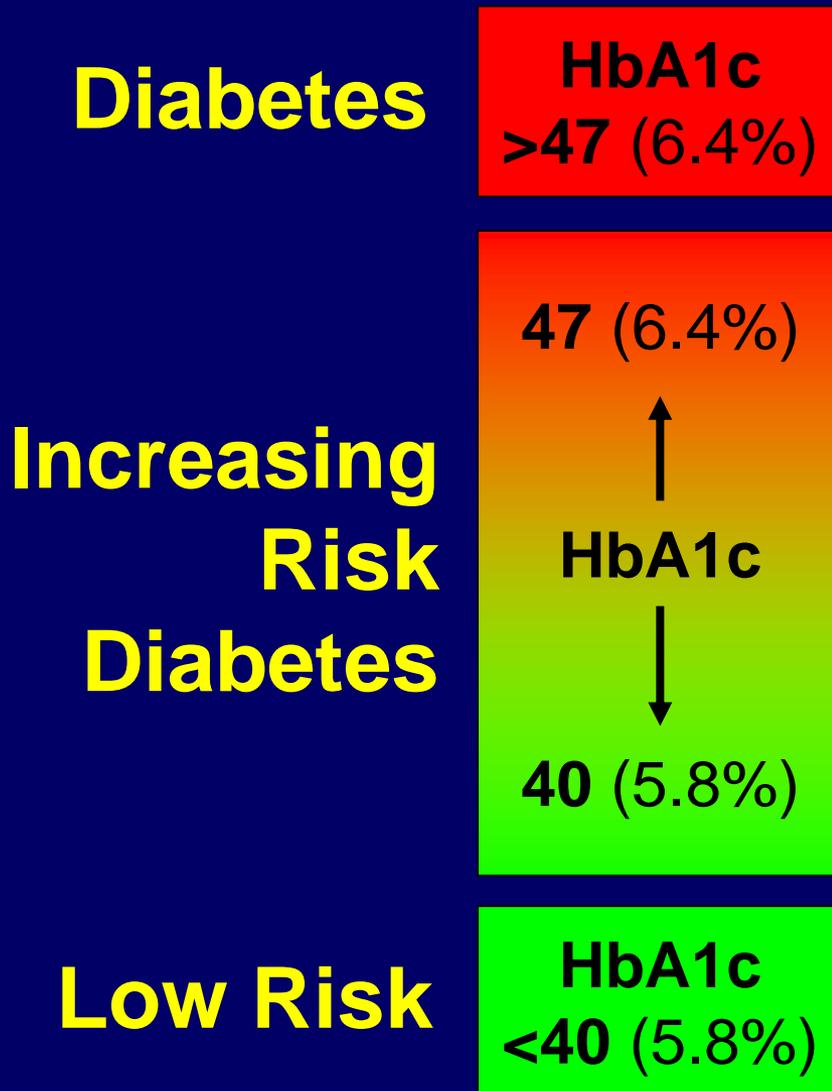
2. How good is good enough?

3. How can I know my performance?

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Remember: Sources of Error



A small error has high impact on interpretation

Error:

- Bias
- Imprecision

Quality of HbA1c Measurement

1. What is Quality?

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Clinical Chemistry

CLCH

Clin
Chem
2015

International Journal of Laboratory Medicine and Molecular Diagnostics

Investigation of 2 Models to Set and Evaluate Quality Targets for Hb A_{1c}: Biological Variation and Sigma-Metrics

Cas Weykamp,^{1,2*} Garry John,³ Philippe Gillery,⁴ Emma English,⁵ Linong Ji,⁶ Erna Lenters-Westra,^{7,8}
Randie R. Little,⁹ Gojka Roglic,¹⁰ David B. Sacks,¹¹ and Izumi Takei,¹² on behalf of the IFCC Task Force
on Implementation of HbA_{1c} Standardization

Essence Model

1. Model includes Sources of Analytical Error
Bias and Imprecision combined = Total Error

2. Define Allowable Total Error
“Results should not differ more than
5 mmol/mol from the true value”

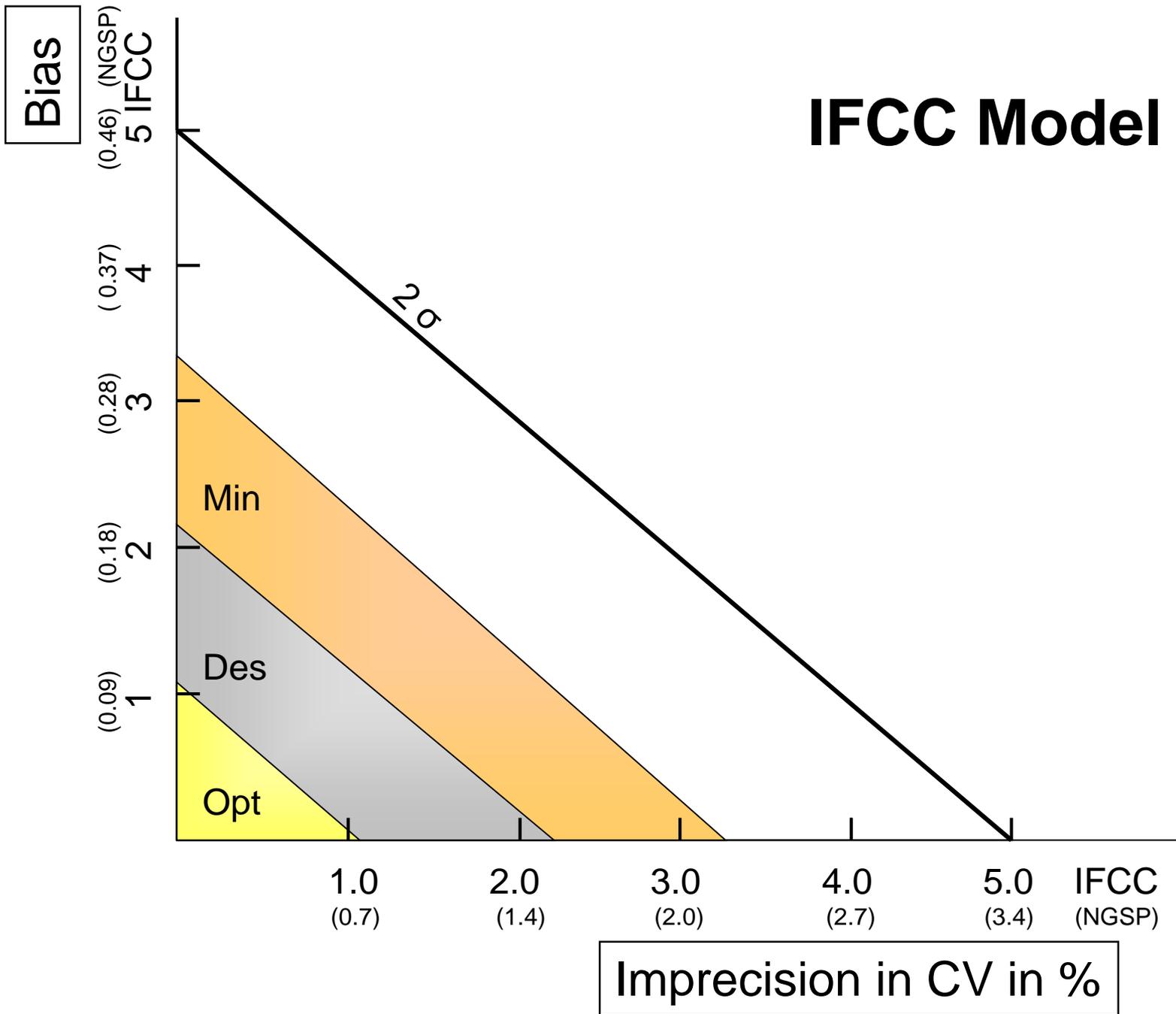
3. Define Risk of not Meeting the Criterion
“It is acceptable that 1 out of 20 results
will not meet the defined criterion”

No Mathematics

Just Graphs

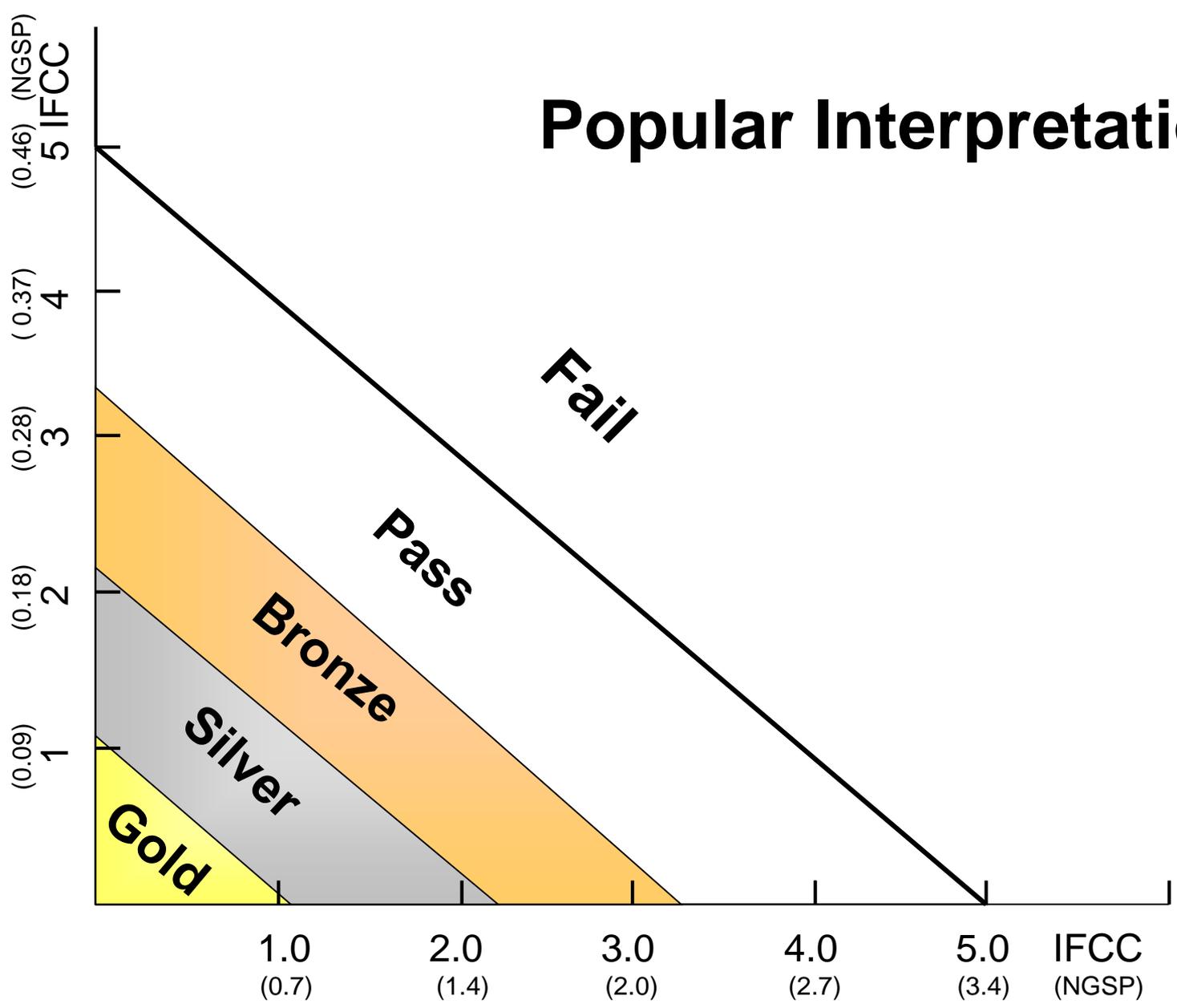
Few Examples

IFCC Model



Bias

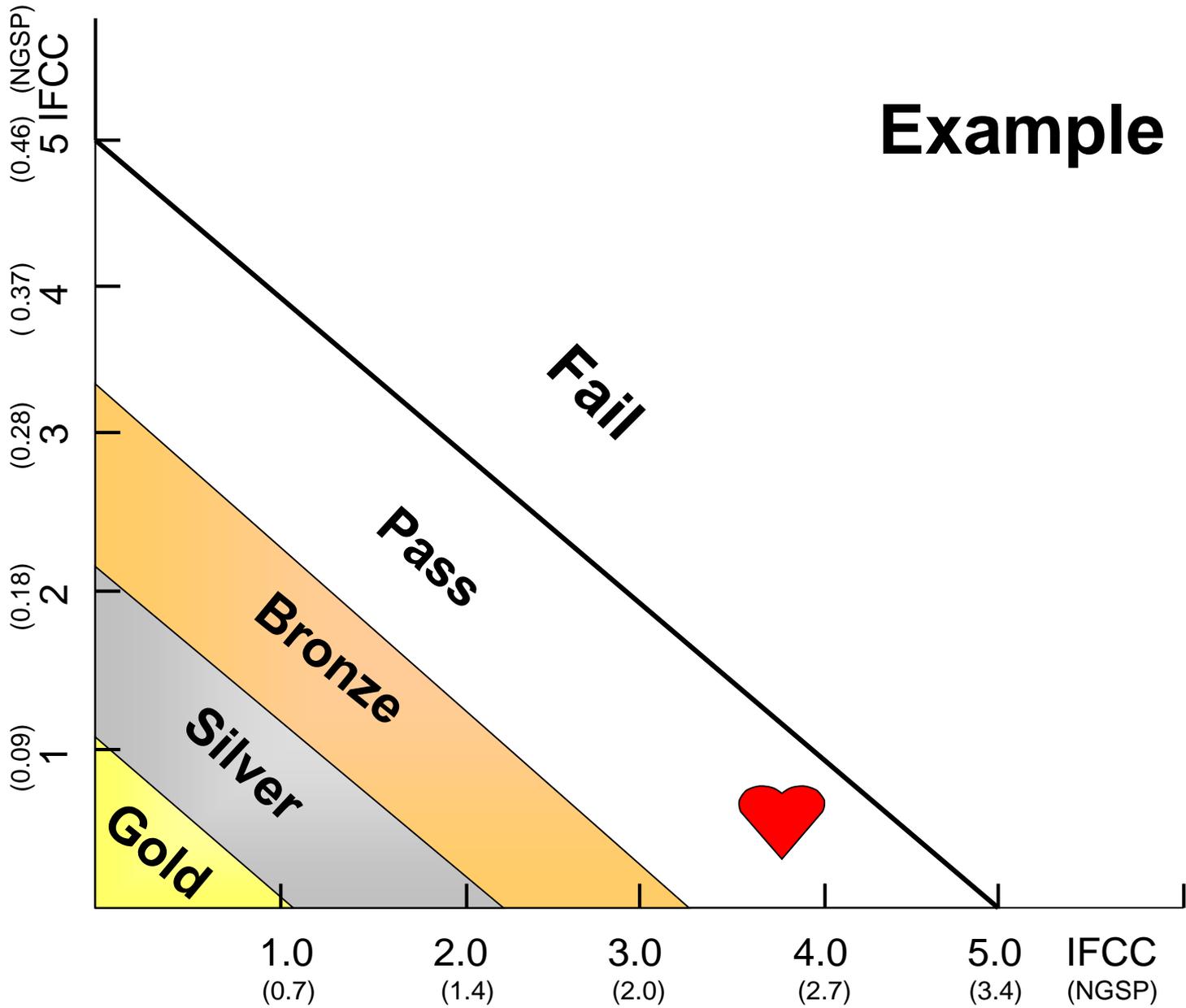
Popular Interpretation



Imprecision in CV in %

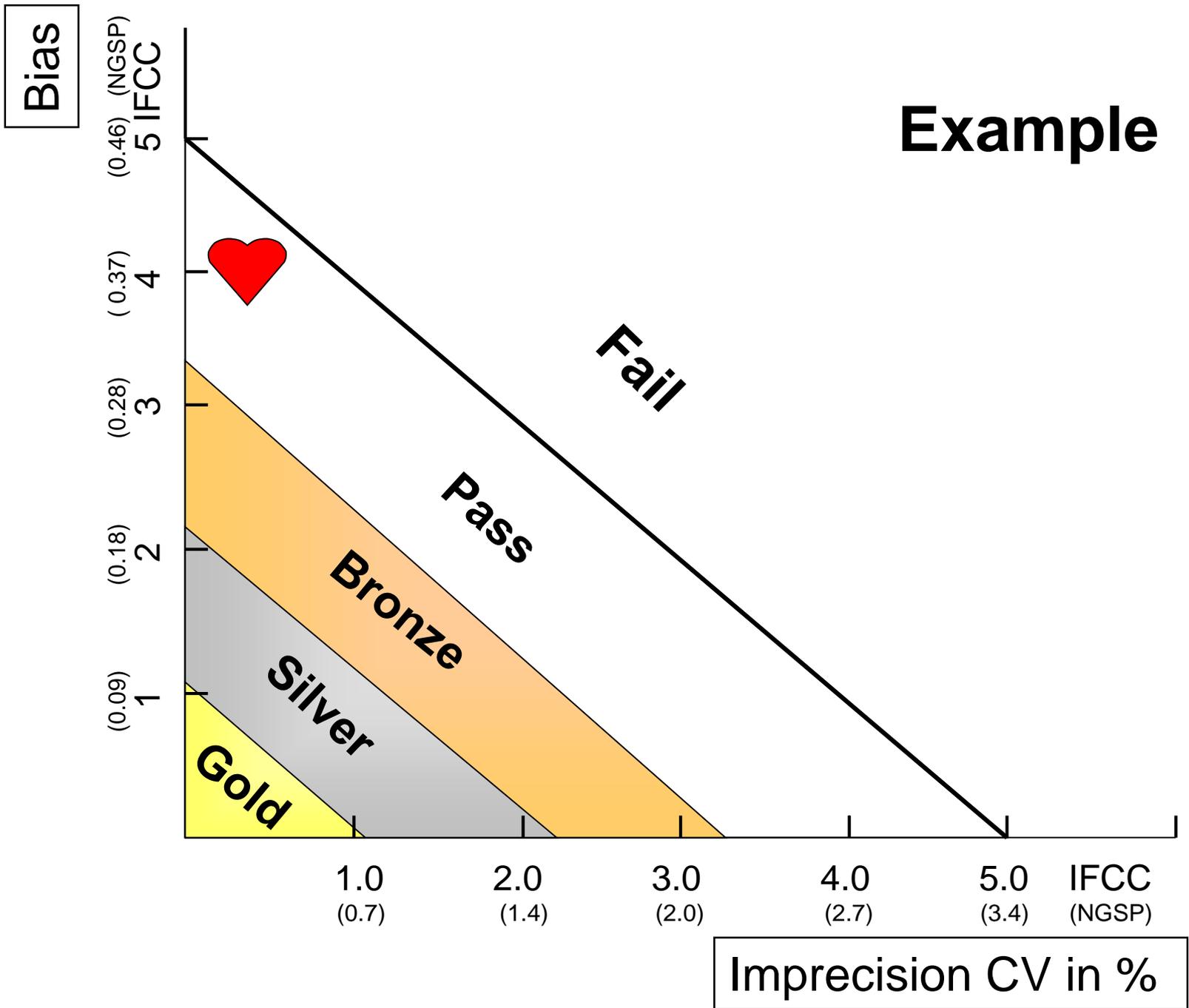
Example

Bias



Imprecision CV in %

Example



Quality of HbA1c Measurement

1. What is Quality?

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- How do I know?

How can I know my performance: IFCC Model

Option 1

Fill Imprecision and Bias Internal QC

Option 2

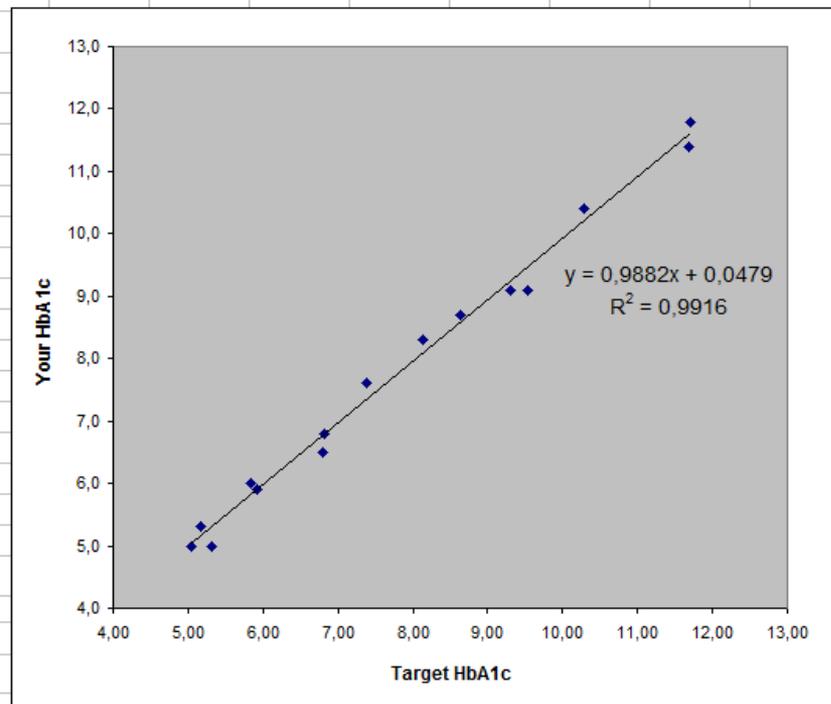
Fill: Imprecision Internal QC Bias IEQAS

Option 3

Fill Bias and Imprecision from IEQAS

Option 3: Fill data IEQAS

Calculation Imprecision							
x (target)	y (your)	x'	y'	(x') ²	(y') ²	(x'y')	
6,79	6,5	-1,05	-1,29	1,10	1,66	1,35	
10,28	10,4	2,44	2,61	5,95	6,81	6,37	
6,82	6,8	-1,02	-0,99	1,04	0,98	1,01	
8,63	8,7	0,79	0,91	0,62	0,83	0,72	
5,32	5,0	-2,52	-2,79	6,35	7,78	7,03	
5,84	6,0	-2,00	-1,79	4,00	3,20	3,58	
11,71	11,8	3,87	4,01	14,98	16,08	15,52	
9,53	9,1	1,69	1,31	2,86	1,72	2,21	
5,04	5,0	-2,80	-2,79	7,84	7,78	7,81	
7,38	7,6	-0,46	-0,19	0,21	0,04	0,09	
11,69	11,4	3,85	3,61	14,82	13,03	13,90	
5,93	5,9	-1,91	-1,89	3,65	3,57	3,61	
5,17	5,3	-2,67	-2,49	7,13	6,20	6,65	
8,14	8,3	0,30	0,51	0,09	0,26	0,15	
9,30	9,1	1,46	1,31	2,13	1,72	1,91	
Mean	7,84	7,79		Sum	72,77	71,66	71,91



Imprecision 2,8
Bias -0,05

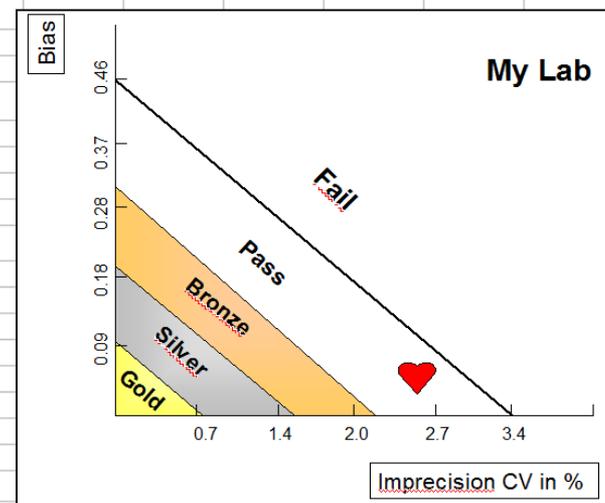
$$x' = (x_i - \bar{x})$$

$$y' = (y_i - \bar{y})$$

$$r = \sqrt{\frac{(\sum x'y')^2}{\sum (x')^2 \cdot \sum (y')^2}} \quad r^2 = \frac{(\sum x'y')^2}{\sum (x')^2 \cdot \sum (y')^2}$$

$$\text{Imprecision} = \sqrt{\frac{\sum (y')^2 \cdot (1-r^2)}{(n-2)}} \times 100\%$$

$$\text{Bias} = y - x$$



Quality of HbA1c Measurement

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European Summit on HbA1c



A Project of
IFCC C-EUBD
Education in the Use of
Biomarkers in Diabetes
&
15 National
EQA organisers



★ EurA1c ★

Garry John
Emma English
Rajiv Erasmus
David Sacks
Cas Weykamp

“EurA1c”

Concept

Once a year the respective European EQA/PT Organizers use the same 2 samples

“EurA1c”

Concept

Once a year the respective European EQA/PT Organizers use the same 2 samples

Information

- Overall performance in Europe
- Performance per country
- Performance per manufacturer
- Performance per country per manufacturer

Participants First “EurA1c” in October 2016



EQA Organisers

BE	WIV-ISP	Yolande Lenga
DE	INSTAND e.V.	Patricia Kaiser
GR	ESEAP	Alexander Haliassos, Kostas Makris, Otto Panagothinakis
IT	Centro di Ricerca Biomedica	Laura Sciacovelli
CZ	SEKK	Marek Budina, Marie Uhlířová
FR	Biologie Prospective	Jean-Pascal Siest
SA	Tygerberg Hospital	Rajiv Erasmus
UK	WEQAS	Annett Thomas, Samantha Jones
SE	EQUALIS	Gunnar Nordin, Carita Krook Persson
AT	ÖQUASTA	Christoph Buchta, Mathias M. Müller
ES	SEQC	Montserrat Ventura, Emma Ventura
PT	Inst. Nac. de Saude Dr. Jorge	Ana Paula Faria
IE	IEQAS	Hazel Graham
CH	Universitätsspital Zürich	Roman Fried
TR	Tubitak Ume	Diler Aslan, Fatma Akcadag, Muslum Akgoz
NL	SKML	Cas Weykamp
INT	ERL	Cas Weykamp

Samples “EurA1c”

Samples “EurA1c”

Fresh Whole Blood

Advantage: commutable and suitable all methods

Disadvantage: limited stability

Samples “EurA1c”

Fresh Whole Blood

Advantage: commutable and suitable all methods

Disadvantage: limited stability

Lyophilized Hemolysate

Advantage: Stable

Disadvantage: not commutable for all methods;
not suitable some POCT instruments

Samples “EurA1c”

Fresh Whole Blood

Advantage: commutable and suitable all methods

Disadvantage: limited stability

Lyophilized Hemolysate

Advantage: Stable

Disadvantage: not commutable for all methods;
not suitable some POCT instruments

Choice

National EQA organisers: Logistics in the Country

“EurA1c”: 15 countries - 2166 Labs

Country	Fresh Whole Blood	Lyophilized Lysate
Austria		107
Belgium	139	
Czech Republic		70
France	135	132
Germany	652	
Greece		73
Ireland	30	
Italy	84	48
Netherlands	136	54
Portugal		43
South Africa		2
Spain		76
Sweden	117	
Switzerland	29	
Turkey	48	45
United Kingdom	148	

Results

Fresh Whole Blood

“EurA1c”

Concept

Once a year the respective European EQA/PT Organizers use the same 2 samples

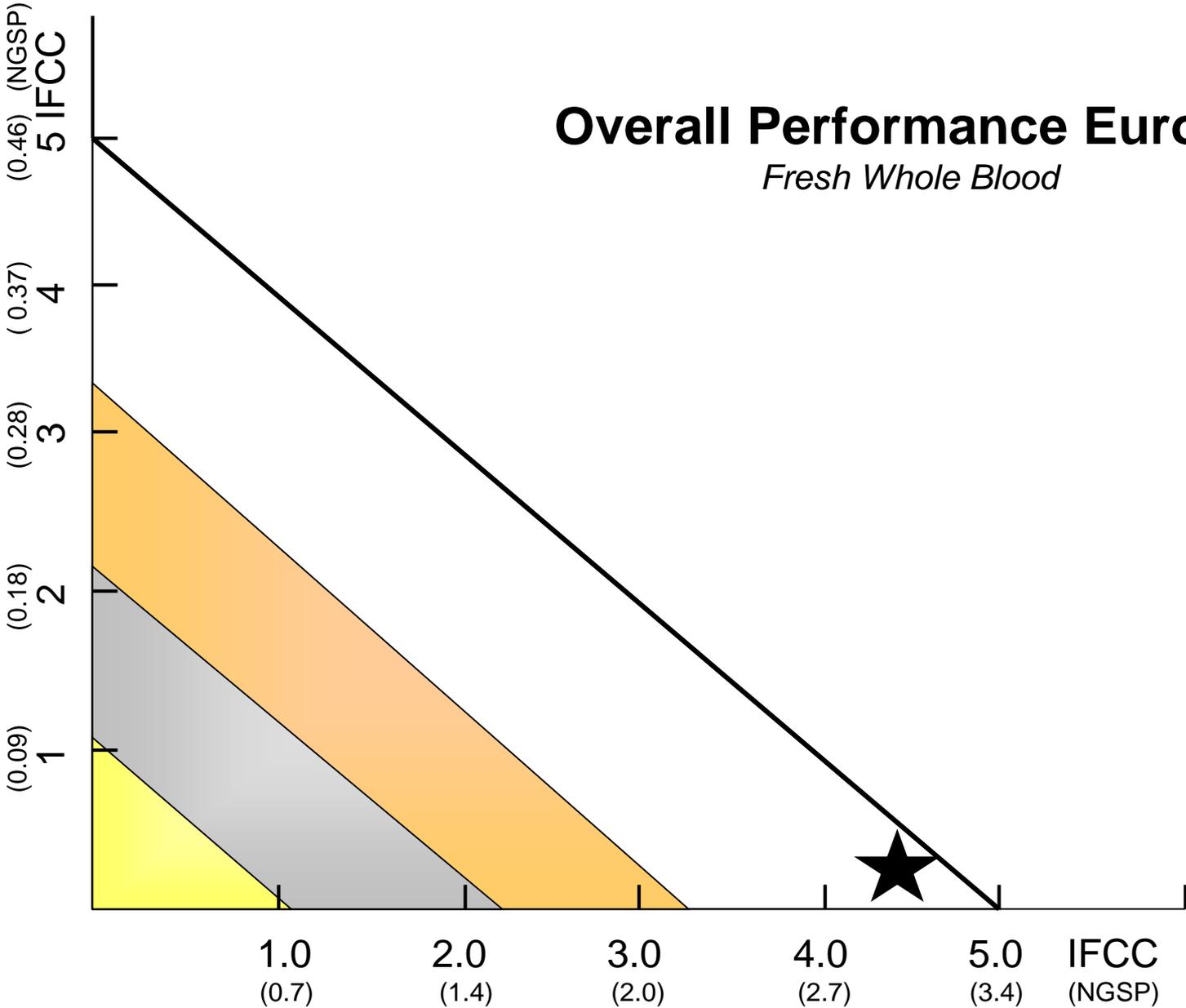
Information

- Overall performance in Europe
- Performance per country
- Performance per manufacturer
- Performance per country per manufacturer

Bias

Overall Performance Europe

Fresh Whole Blood



Imprecision in CV in %

“EurA1c”

Concept

Once a year the respective European EQA/PT Organizers use the same 2 samples

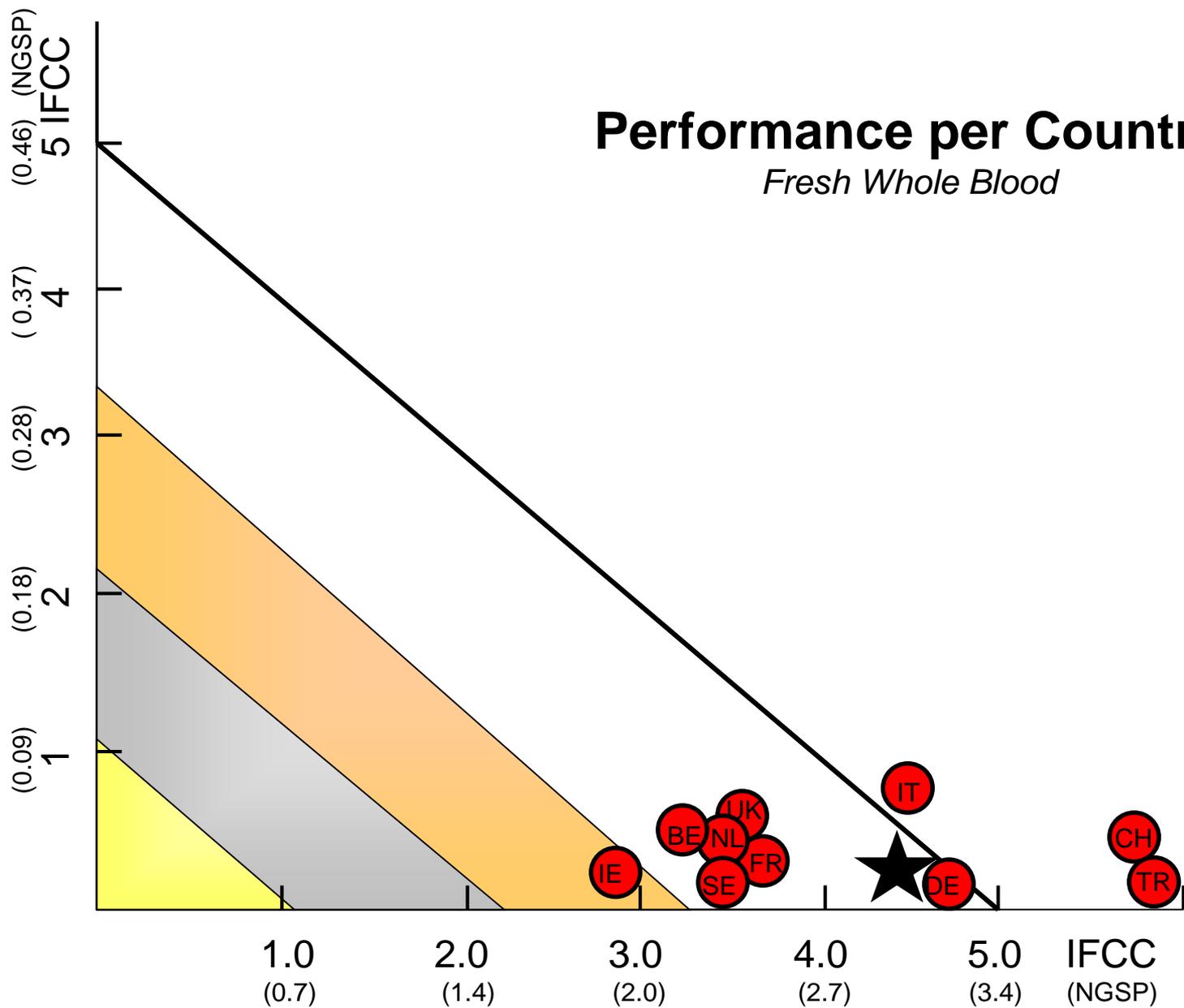
Information

- Overall performance in Europe
- **Performance per country**
- Performance per manufacturer
- Performance per country per manufacturer

Bias

Performance per Country

Fresh Whole Blood



Imprecision in CV in %

“EurA1c”

Concept

Once a year the respective European EQA/PT Organizers use the same 2 samples

Information

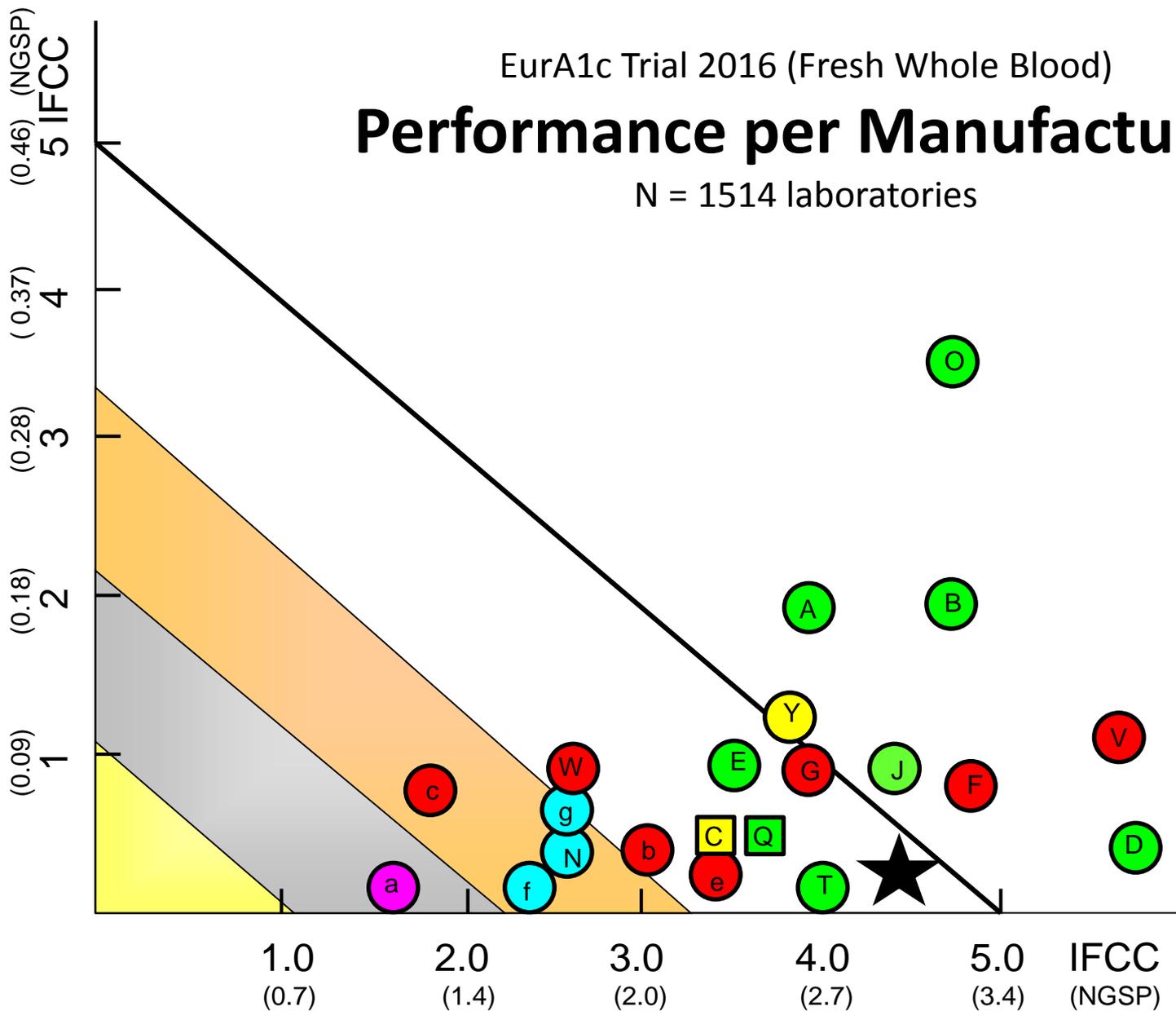
- Overall performance in Europe
- Performance per country
- **Performance per manufacturer**
- Performance per country per manufacturer

Bias

EurA1c Trial 2016 (Fresh Whole Blood)

Performance per Manufacturer

N = 1514 laboratories



Imprecision in CV in %

“EurA1c”

Concept

Once a year the respective European EQA/PT Organizers use the same 2 samples

Information

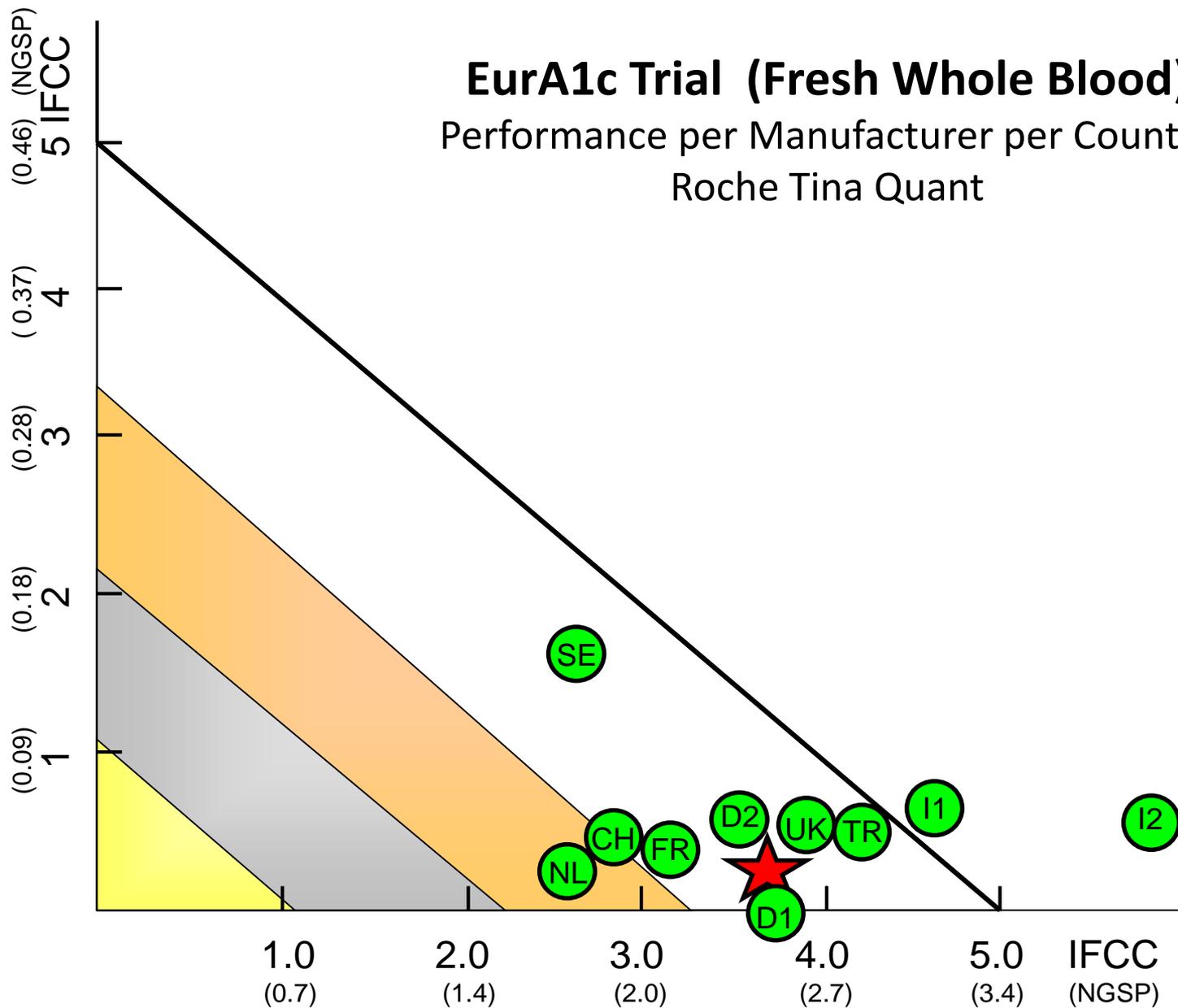
- Overall performance in Europe
- Performance per country
- Performance per manufacturer
- **Performance per country per manufacturer**

Bias

EurA1c Trial (Fresh Whole Blood)

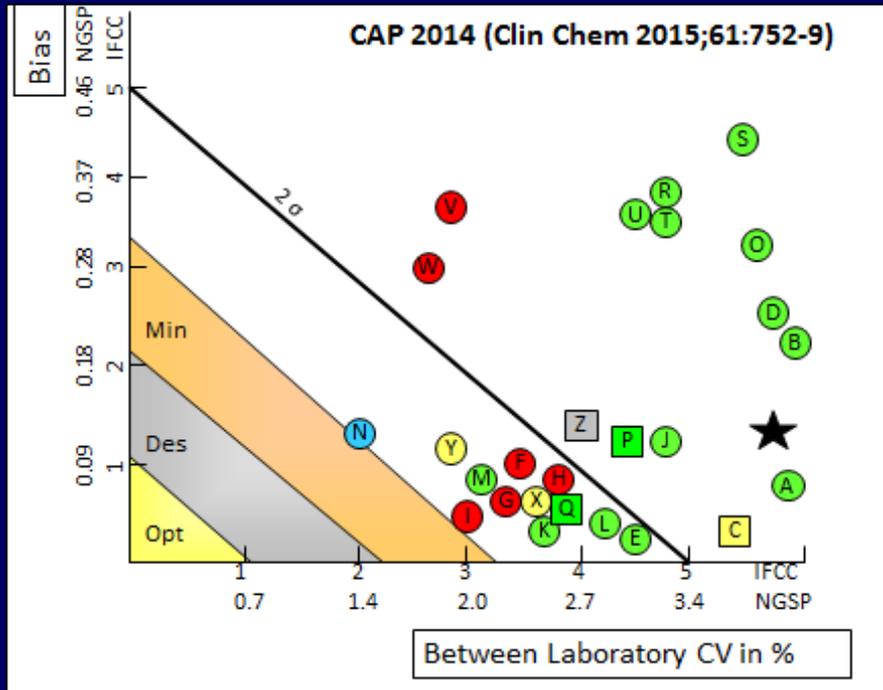
Performance per Manufacturer per Country

Roche Tina Quant

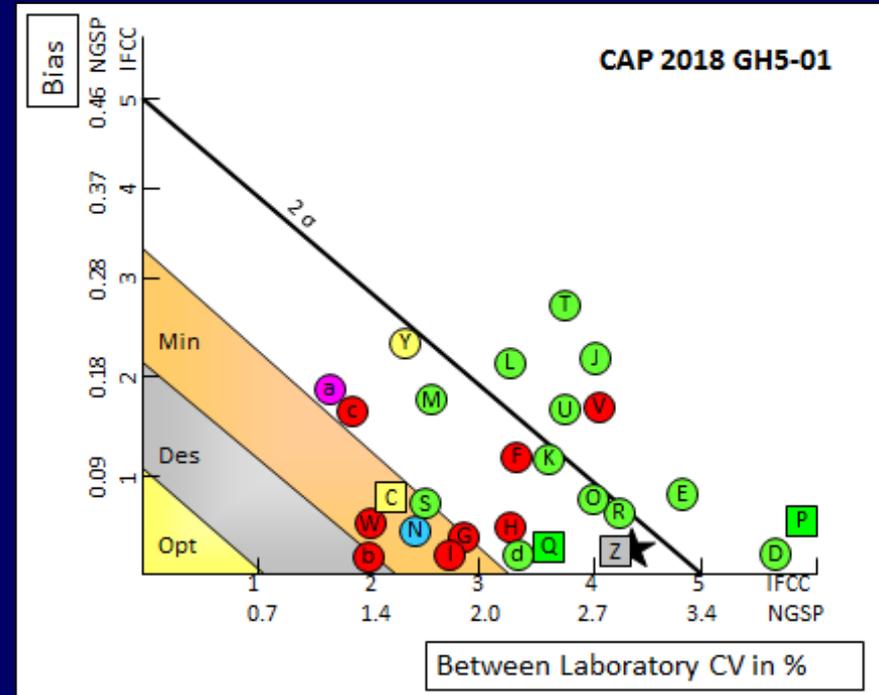


Imprecision in CV in %

Performance improves!

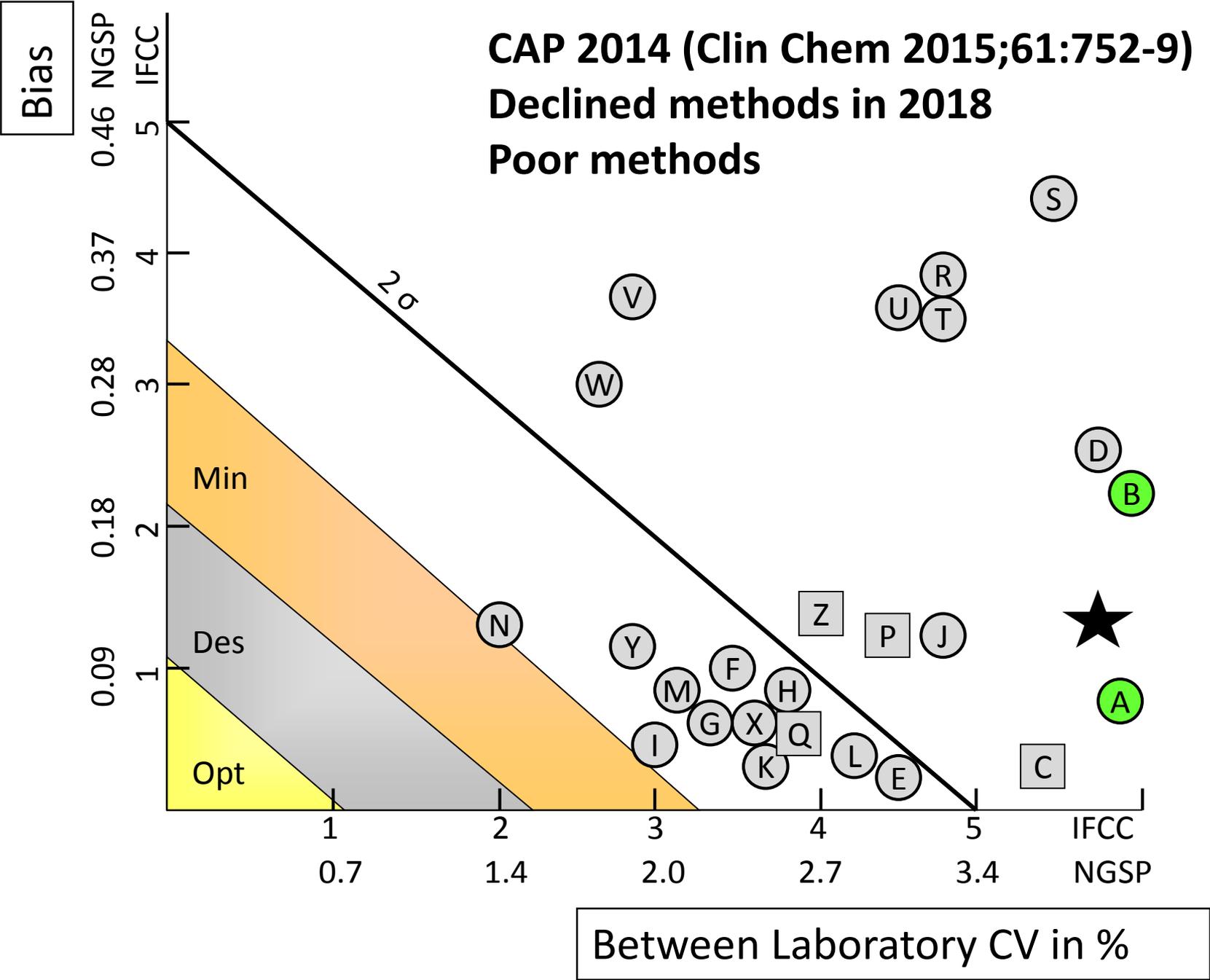


2014

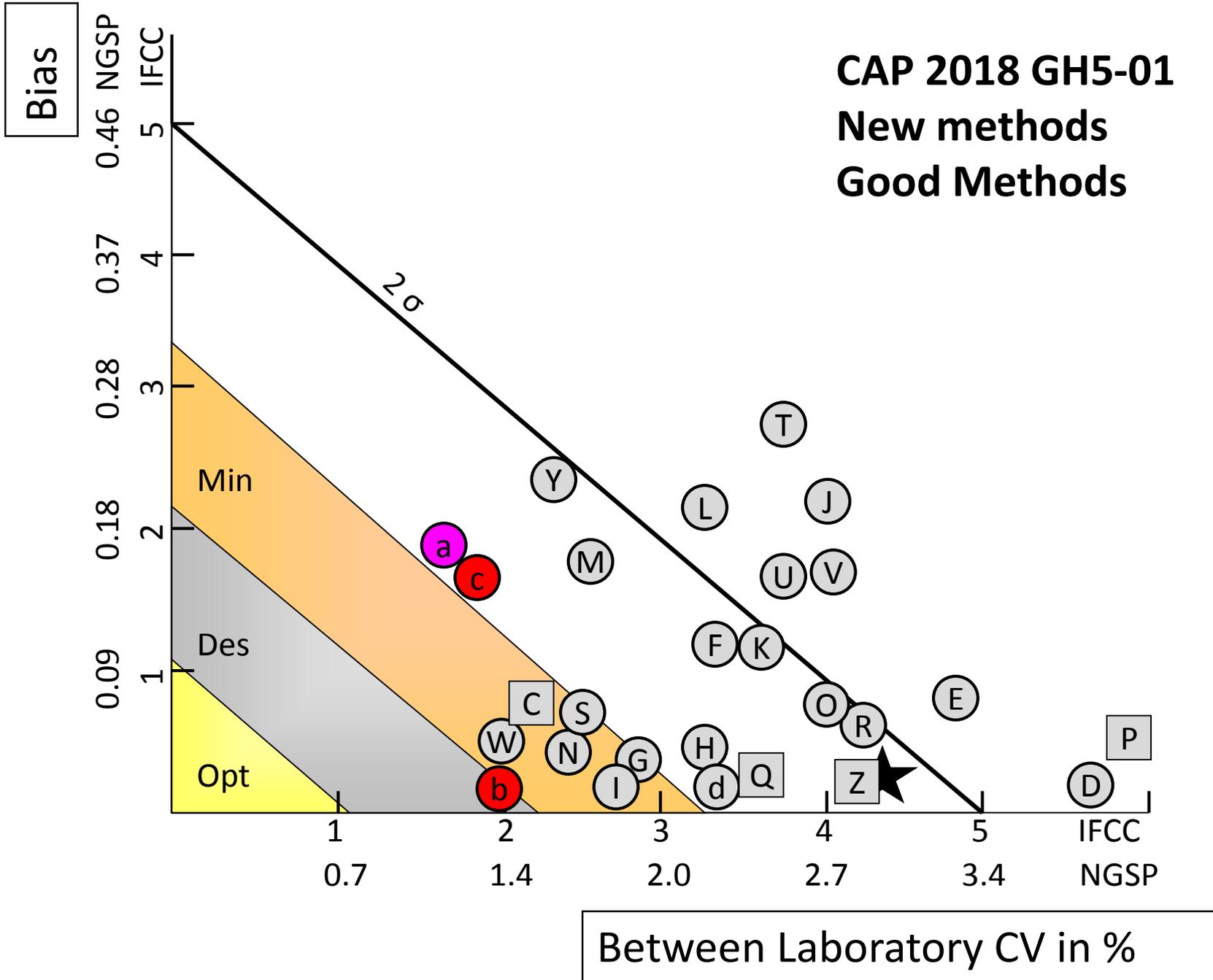


2018

CAP 2014 (Clin Chem 2015;61:752-9)
Declined methods in 2018
Poor methods



CAP 2018 GH5-01
New methods
Good Methods





EurA1c:

The European HbA1c Trial to Investigate the Performance of HbA1c Assays in 2166 Laboratories across 17 Countries and 24 Manufacturers by Use of the IFCC Model for Quality Targets

The EurA1c Trial Group

BACKGROUND: A major objective of the IFCC Committee on Education and Use of Biomarkers in Diabetes is to generate awareness and improvement of HbA1c assays through evaluation of the performance by countries and manufacturers.

With some limitations, fresh whole blood and well-defined lyophilized specimens are suitable for purpose.

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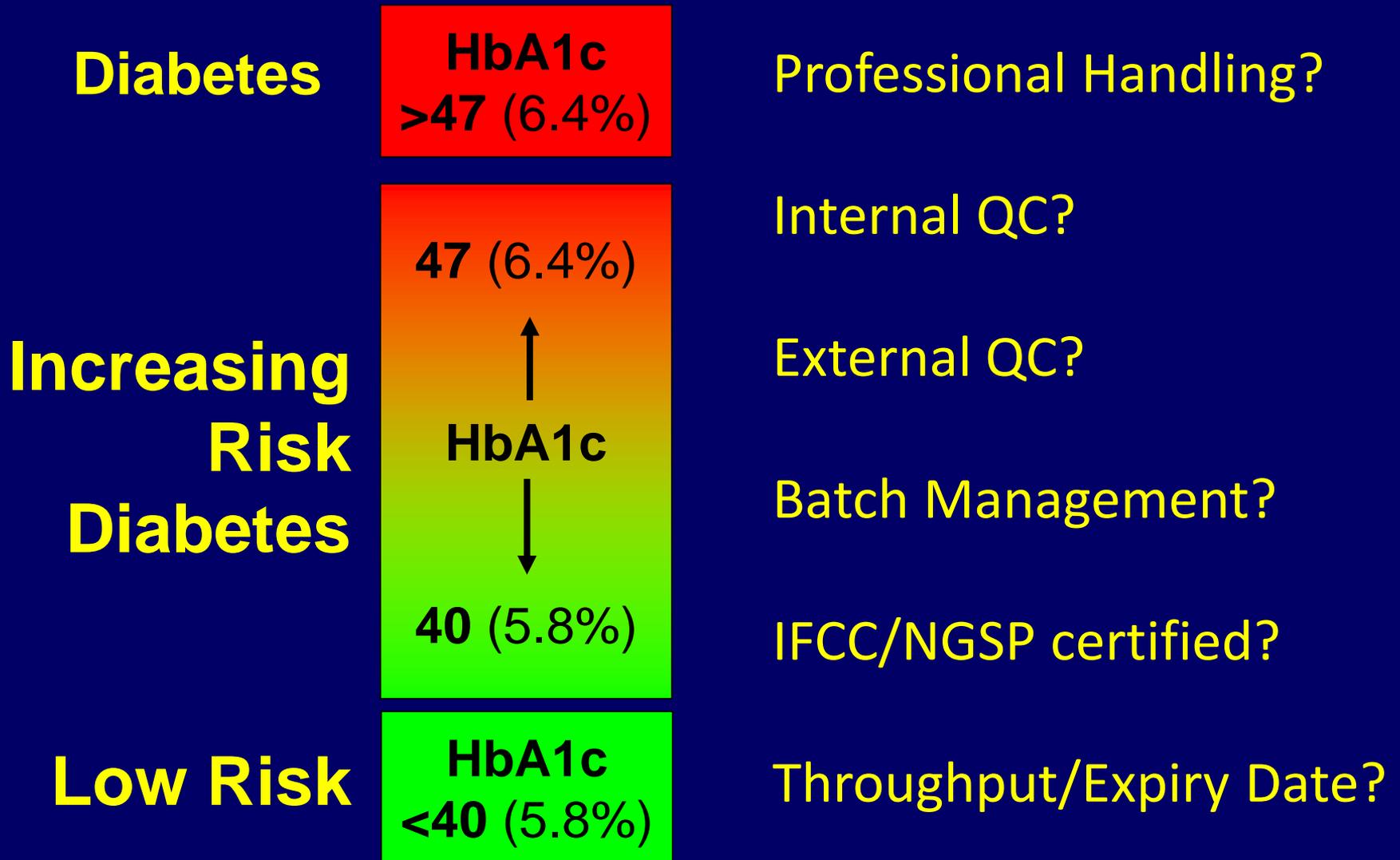
Clinical Chemistry 64:8
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Editorials

Toward a Global Overview of HbA1c Test Performance

Eric S. Kilpatrick^{1*}

POCT for Diagnosis?



POCT for Diagnosis?

Conditionally
Yes

Diabetes	HbA1c >47 (6.4%)	Professional Handling?
Increasing Risk Diabetes	47 (6.4%) ↑ HbA1c ↓ 40 (5.8%)	Internal QC?
		External QC?
		Batch Management?
Low Risk	HbA1c <40 (5.8%)	IFCC/NGSP certified?
		Throughput/Expiry Date?

Summary

1. HbA1c (nearly) perfect after 50 years

2. Variants: Interference: S-C-D-E 99%
Want to see?

3. Diagnosis Narrow Window normal/diabetic
Biological “error” (ery lifespan)
Analytical error (bias/imprecision)

4. IFCC Quality Targets Model

5. EurA1c Differences Manufacturers
IE best performing country 2016

6. POCT Diagnosis: Conditionally Yes

Once More: the amazing year 1969

One small step for the Laboratory
One giant leap for diabetes care...

HbA1c



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Thank you for your Attendance