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UNIVERSITÀ DEGLI STUDI DI MILANO

Centre for Metrological Traceability in Laboratory Medicine (CIRME)

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site: http://users.unimi.it/cirme

EUROMEDLAB ATHENS 2017

PREANALYTICAL AND ANALYTICAL ASPECTS AFFECTING CLINICAL RELIABILITY OF PLASMA GLUCOSE RESULTS

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Traceability in laboratory medicine: A matter of patient safety

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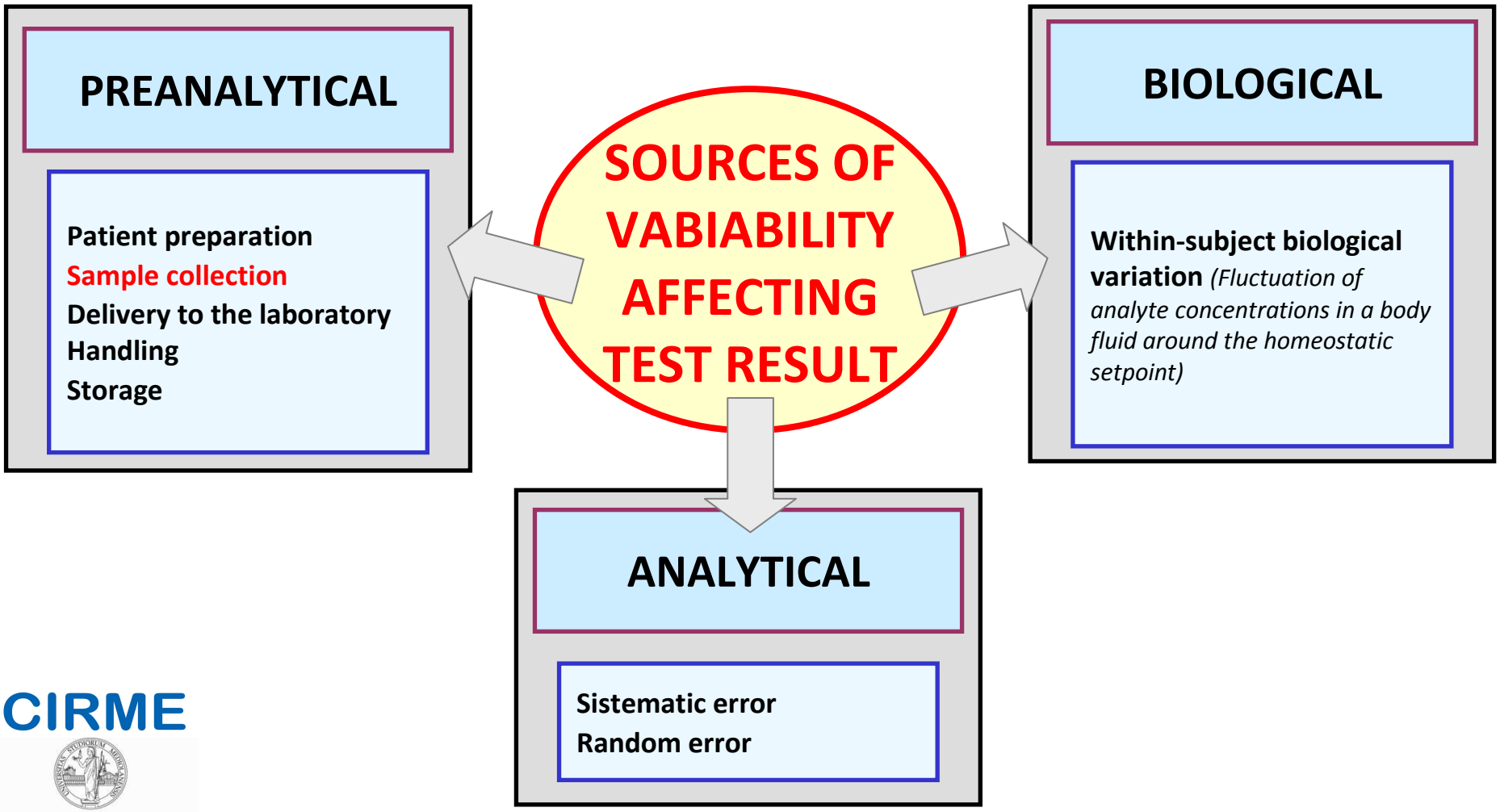
SYMPOSIUM

TOTAL VARIABILITY OF LABORATORY TEST RESULTS

$$V_{TOT} = (V_P^2 + V_A^2 + V_I^2)^{1/2}$$



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Pre-analitical sources of variation in glucose testing

$$V_{TOT} = (V_P^2 + V_A^2 + V_I^2)^{1/2}$$

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CRITICAL ISSUE:
TO PREVENT *in-vitro* GLYCOLYSIS

GLUCOSE @ physiological concentrations in sample stored at room temperature IS LOST through an average rate of 5-7% per hour

Clin Chem 1989;35:315-7

GOLD STANDARD FOR SAMPLE COLLECTION

- **NATIONAL ACADEMY OF CLINICAL BIOCHEMISTRY (NACB) GUIDELINES FOR LABORATORY ANALYSIS IN DIABETES**
- **WORD HEALTH ORGANIZATION**

1- SEPARATE plasma from blood cells IMMEDIATELY after sample collection

OR

2- PLACE the sample tube immediately in an ICE-WATER SLURRY and SEPARATE plasma from the cells WITHIN 30 MIN

OR

3 - USE OF AN *EFFECTIVE GLUCOSE STABILIZER*

✓ **Tubes with only *enolase inhibitors, such as FLUORIDE, should not be relied on to prevent glycolysis***

✓ **Tube containing a *rapidly effective glycolysis inhibitor, such as CITRATE BUFFER, should be used***

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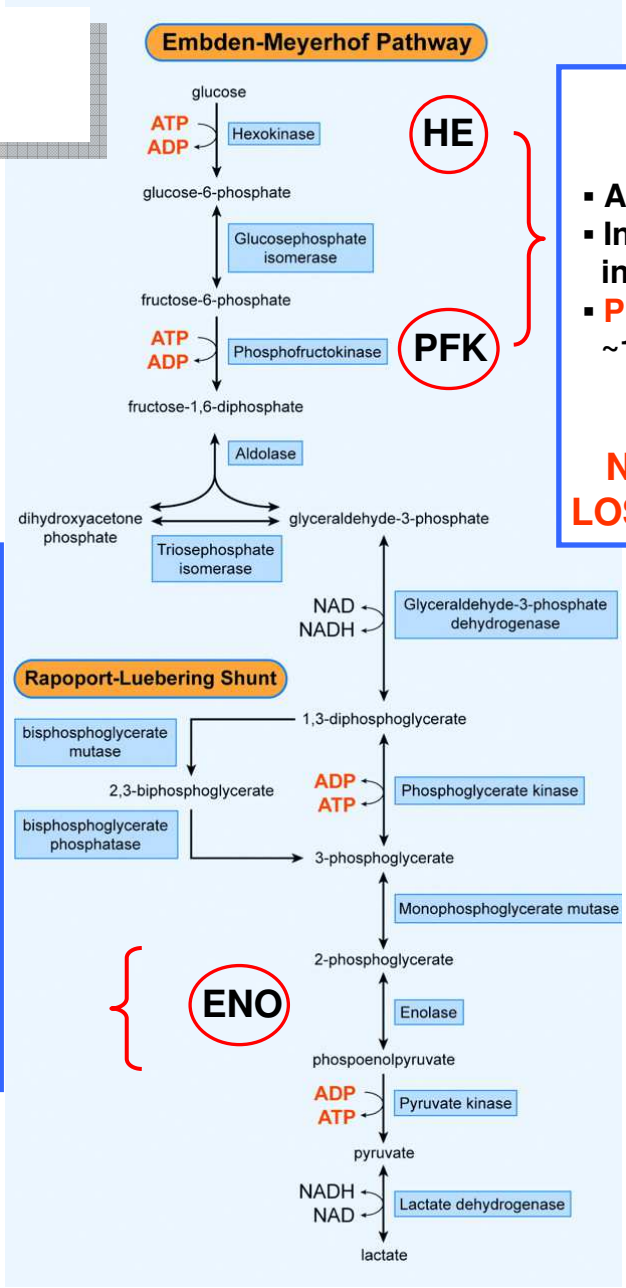
in-vitro GLYCOLYSIS STABILIZERS



CITRATE BUFFER

- Acidification to pH 5.3-5.8
- Inhibition of HE and PFK which act earlier in the glycolytic pathways
- Prompt stabilizing effect, guaranteed for ~10 h at room temperature

NO LOSS OF GLUCOSE AFTER 2h
LOSS OF GLUCOSE ~1.2% AFTER 24h

FLUORIDE
(and oxalate mixture)

- It forms a complex with enolase in the presence of P and Mg
- Inhibition of ENO which acts downstream in the glycolytic pathway
- Complete stabilizing effect achieved after 4 h from withdrawal

LOSS OF GLUCOSE DURING THE FIRST HOURS

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Effectiveness and Reliability of citric/citrate to prevent in-vitro glycolysis

Table 1. Effect of collection tube type and additives on stability of glucose.

Sample type, postdraw storage	Comparator, postdraw storage	Mean delta, mmol/L[a]		
		Delta (%)	95% CI	*P* (n)[b]
Citric acid plasma, 2 h at 37 °C	Heparin plasma, 30 min at 0 °C	6.393 − 6.414 = −0.021 (0.3)	−0.07–0.02	0.33 (30)
Citric acid plasma, 24 h at 37 °C	Heparin plasma, 30 min at 0 °C	6.393 − 6.316 = 0.077 (1.2)	−0.002–0.06	0.05 (30)
Fluoride plasma, 2 h at 37 °C	Heparin plasma, 30 min at 0 °C	6.393 − 6.099 = 0.294 (4.6)	0.23–0.35	<0.001 (30)
Fluoride plasma, 24 h at 37 °C	Heparin plasma, 30 min at 0 °C	6.393 − 5.943 = 0.450 (7.0)	0.37–0.53	<0.001 (30)
Plasma, 30 min, ambient	Serum, 30 min, ambient	5.589 − 5.638 = −0.049 (0.9)	0.021–0.077	<0.001 (90)
Barrier serum, 24 h at 37 °C	Barrier serum, 30 min, ambient	5.826 − 5.819 = 0.007 (0.1)	−0.011–0.025	0.45 (66)

NACB Reference

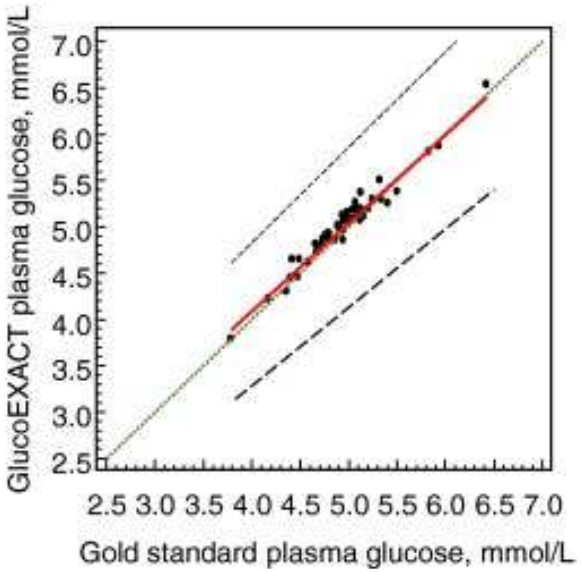
Gambino R et al, Clin Chem 2009;55:1019-21

Postdraw storage

T 20-24 °C
4 h

Mean Delta %, 0.95%

(95% CI, 0.44–1.46)



Bonetti G et al, Prim Care Diabetes 2016;10:227-32

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citric/citrate buffer vs fluoride

	AUTHORS	GLUCOSE mmol/L	MEAN DIFFERENCE
VENOSAFE GRANULAR	**Del Pino IG et al** *Clin Chem Lab Med* 2013;51:1943-9	**Mean 6.43 *vs* 5.98**	**+ 7.0%**
	Szőke D et al *Clin Chem Lab Med* 2014;52:e87-9	**Range 4.5 to 11.1 *vs* 4.1 to 10.7**	**+6.7%**
	Van den Berg SA et al *Sci Rep* 5 2015 n. 8875	**Mean 5.8 *vs* 5.5**	**+5.5%**
	Bonetti G et al *Biochemia Medica* 2016;26:68-76	**Median (range) 5.60 (5.47- 5.73) *vs* 5.21 (5.05 - 5.32)**	**+6.8%**
	Carta M et al Ann Clin Biochem 2016;53:715-6	**Median (95% CI) 4.4 (5.1-5.7) *vs* 5.1 (4.8-5.3)**	**+5.9%**
FC-MIX DRY	**Dimeski et al** *Clin Chem Lab Med* 2016	**Mean 5.35 vs. 5.05**	**+5.9%**
GLUCOMEDICS LIQUID	**Dimeski et al** *Ann Clin Biochem* 2014;52:270-5	**Mean 5.7 vs. 5.3**	**+7.5%**
	Juricic G et al *Clin Chem Lab Med* 2016;54:363-71	**Mean (±SD) 6.2 (±1.1) vs 5.6 (±1.0)**	**+10.7%**
	Juricic G et al *Clin Chem Lab Med* 2016;54:411-8	**Mean (±SD) 6.0 (±0.8) vs 5.5 (±0.8)**	**+8.5%**
	Carta M et al *Ann Clin Biochem* 2016;53:715-6	**Median (95%CI) 5.6 (5.5-5.9) vs 5.1 (4.8-5.3)**	**+8.9%**

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The difference between LIQUID vs. GRANULAR citric/citrate buffer

AUTHORS	GLUCOSE mmol/L	MEAN DIFFERENCE
Bakliza A et al *Clin Chem Lab Med* 2015;53:eA226-P46	Mean (±SD) 5.8 (0.8) vs. 5.6 (0.7)	+3.2%
Pasqualetti S et al *Clin Chem Lab Med* 2016;54:e281-3	Rap 	+3.8%
Cart 	an (95% CI) 5.6 (5.5-5.9) vs. 5.4 (5.1-5.7)	+3.7%
c G et al *Clin Biochem* 2016;49:1402-5	Mean (±SD) 6.0 (1.0) vs. 5.8 (0.9)	+3.4%

NOT AL CITRATE TUBES ARE CREATED EQUAL !!

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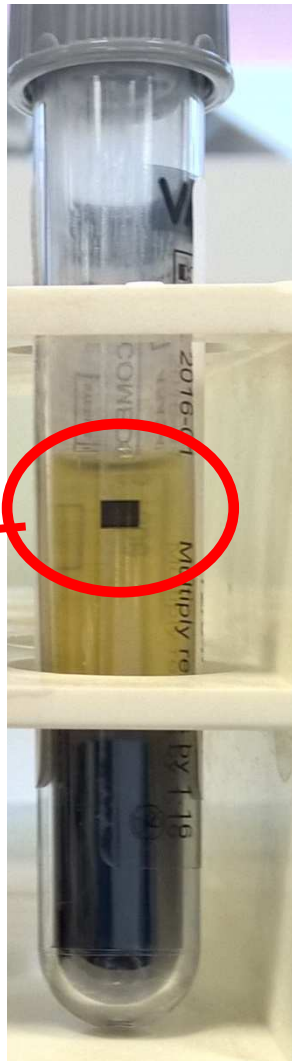
The difference between LIQUID vs. GRANULAR citric/citrate buffer: why?

1 INCORRECT DILUTION CORRETION FACTOR

Carta M et al Ann Clin Biochem 2016;53:715-6

GRANULAR	LIQUID (Diluition Factor, 1.16)	LIQUID (Diluition Factor, *1.10)
MEDIAN	MEDIAN	
5.4 mmol/L	5.6 mmol/L	5.4 mmol/L

**experimental DF suggested by Dimeski et al Ann Clin Biochem 2014;52:270-5*



2 IMPRECISE VACUUM ACTION

Perfect correction factor may become incorrect when tubes are not exacty filled as intended

….our experience

- well trained phlebotomists,
- tubes underfilled considered indicative of human error

….we speculated some problems in tubes manufacturing

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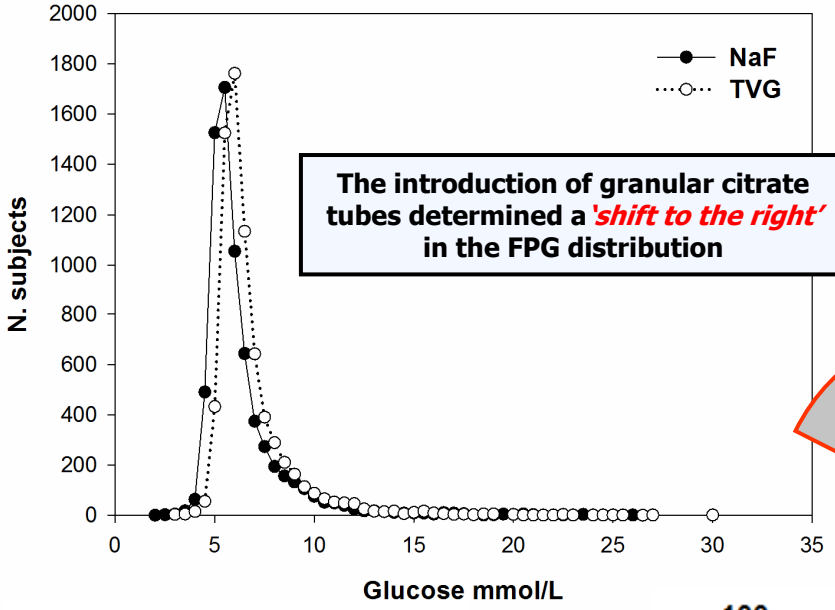
FASTING PLASMA GLUCOSE DISTRIBUTION



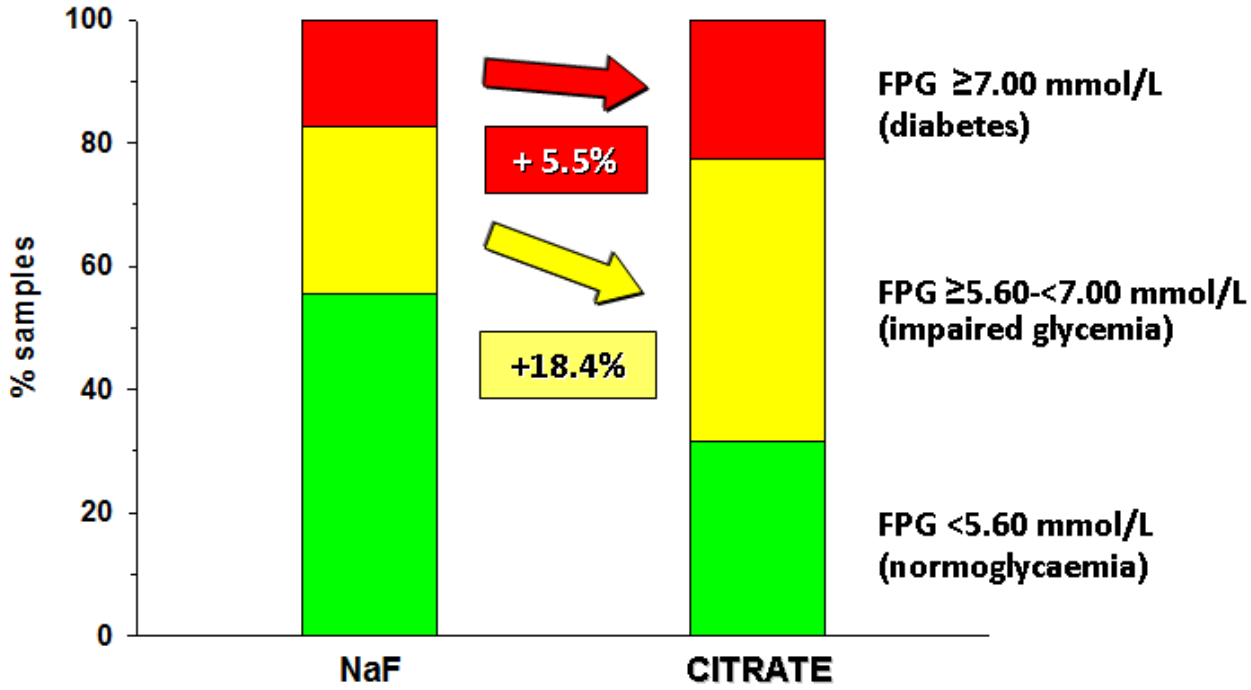
The introduction of granular citrate tubes determined a *'shift to the right'* in the FPG distribution

Szőke D et al., Biochim Clin 2015;39:76

	n	Range	Median FPG	25-75th
Fluoride (NaF) Apr-Sept 2013	7120	2.05-25.7 mmol/L	5.44 mmol/L	4.94-6.33 mmol/L
Granular citrate Apr-Sept 2014	7192	2.61-29.8 mmol/L	5.94 mmol/L	5.44-6.88 mmol/L

CLINICAL IMPACT WITH THE INTRODUCTION OF GRANULAR CITRATE

CLINICAL CLASSIFICATION OF SUBJECTS UNDERGONE FASTING PLASMA GLUCOSE TEST



Pasqualetti S et al., Clin Chem Lab Med 2015;53:S104-T067

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CLINICAL CLASSIFICATION OF SUBJECTS UNDERGONE GESTATIONAL DIABETES MELLITUS (GDM) TEST AFTER THE IMPLEMENTATION OF ADA RECOMMENDATION ON PREANALYTICAL FOR GLUCOSE

Table 1. Comparison of mean glucose concentrations between research and usual conditions for each test.[a]

Glucose	Research conditions	Usual conditions	P[b]
Fasting			<0.0001
mg/dL	90.0 (12.6)	81.0 (12.6)	
mmol/L	5.0 (0.7)	4.5 (0.7)	
1-h			<0.0001
mg/dL	140.4 (43.2)	133.2 (41.4)	
mmol/L	7.8 (2.4)	7.4 (2.3)	
2-h			<0.0001
mg/dL	102.6 (32.4)	99.0 (32.4)	
mmol/L	5.7 (1.8)	5.5 (1.8)	

[a] Data are mean (SD).
[b] Paired Student *t* test.

IADPSG, International Association of the Diabetes and Pregnancy Study Groups, diagnostic criteria*

Table 2. Comparison of the incidence of GDM between research and usual conditions for each test.[a]

Glucose	*75 g OGTT GDM	Research conditions	Usual conditions	P[b]
Fasting	>5.1 mmo/L	51 (32.9)	10 (6.5)	<0.0001
1-h	>10.0 mmo/L	20 (13.3)	17 (11.0)	NS
2-h	>8.5 mmol/L	4 (2.6)	4 (2.6)	NS
Total[c]		59 (38.1)	22 (14.2)	<0.0001

[a] Data are n (%). NS, not significant.
[b] McNemar test of correlated proportions.
[c] Some overlap of cases (see Fig. 2).

GDM
+27%
+5%

Screened subjects, 155

****According to the HAPO study performed under well controlled preanalytical conditions for glucose testing***

HAPO Study Cooperative Research Group. Clin Trials 2006;3:397-407

IADPSG GDM criteria:
- implementation of ADA/NACB & WHO protocols
- or tube types that yields compatible results

- To rightfull classificate subjects as diabetics
- To receive the needed treatments that will deprived from in presence of preanalytical invalid conditions.

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Daly N et al, Clin Chem 2016;62:387-91
Daly N et al, Am J Obstet Gynecol 2015;213:84:e1-5

The introduction of citrate in clinical practice: which caveat?

Evidence 1 **- data about the performance of different "citrate tubes" are confused**

Caveat 1 – **selection of tubes containing citrate requires caution**

Evidence 2 **- reliable tubes that promptly inhibit *in vitro* glycolysis may lead to a different clinical classification of subjects**

Caveat 2 – **which decision limits should be applied to plasma glucose?**

- **should these be redefined when tubes are used that promptly inhibit in vitro glycolysis**

or

- **should they be maintained, so that more subjects at increased risk for diabetes will be identified earlier?**

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Pasqualetti S, Panteghini M. Ann Clin Biochem 2017;54:302-3

DE GRUYTER Clin Chem Lab Med 2016; 54(10): e281–e283

Letter to the Editor

Sara Pasqualetti*, Dominika Szőke, Sarah Birindelli, Alberto Dolci and Mauro Panteghini

Optimal collection tubes for plasma glucose determination: confusion reigns supreme

FROM EU MARKET

(Terumo Venosafe™ Glycaemia – citrate buffer/NaF/Na_2EDTA - GRANULAR FORM)

✓ Grainer Bio-one GLUCOMEDICS – NaF/EDTA & citrate – LIQUID FORM

✓ Sarstedt GlucoEXACT - NaF/citrate – LIQUID FORM

✓ Grainer Bio-one Vacuette® FC Mix tube – citrate buffer/NaF/Na_2EDTA - DRY FORM

….. A MESSY STATE OF AFFAIRS

Need for a well-designed clinical study comparing the suitable options using blood acidification offered by the market

….. IN THE MEANTIME

Staying (*returning*) to tubes containing sodium fluoride only as these have been used in the majority of studies generating the current glucose cut-points for diabetes diagnosis

Plasma Glucose and its Biological Variation

$$V_{TOT} = (V_P^2 + V_A^2 + V_I^2)^{1/2}$$

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The concentrations of *measurands* in body fluids are physiologically *variable* as they *fluctuate around the individual homeostatic set point*

- of each individual ***Within-subject*** ***(CV_i)***
- random fluctuation of setting points among individuals ***Between-subject*** ***(CV_g)***

Application of Biological Variation Data

"Result interpretation"

INDEX OF INDIVIDUALITY

To select the right criteria for results interpretation (reference interval, longitudinal variation)

REFERENCE CHANGE VALUE (RCV)

Clinically significative change in two consecutive results

SPECIMENS NEEDED TO ESTABILISH *INDIVIDUAL'S* HOMEOSTATIC SET POINT

BIOLOGICAL VARIATION

ANALITYCAL PERFORMANCE SPECIFICATIONS

"Reliability of test results"

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Problems with Biological Variation Data

- Published data are of varying quality and quite heterogeneous
- Safe application requires prior critical appraisal
- Need for standards (i.e. a minimum set of attributes to enable the data to be effectively transmitted and applied)

Braga F, Panteghini M. Crit Rev Clin Lab Sci 2016;53:313-25

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Glucose CV_i and CV_g in literature

PLASMA

First Author	Year of Publication	CV_i	CV_g
Cummings	1988	4.9	6.1
Godsland	1985	4.6	
Davie	1993	13.1	3.2
Rohlfing	2002	5.7	5.8
Lacher	2005	8.3	12.5
Lacher	2010	7,5	11.7
Bailey	2013	11.4	9.1
Loh	2014	12.2	

SERUM

First Author	Year of Publication	CV_i	CV_g	Age	Sex
Harris	1970	5.6	7.8		
Young	1971	6.6	2.7		
Williams	1978	11.5, 6.1, 6.3, 6.6, 7.8, 7.8, 6.9	12.9, 5.6, 6.7,8.3, 6.8, 10, 8		
Costangs	1985	13.3; 7.9; 12			
Fraser	1989	4.7	5.4		
Ricos	1989	10.8			
Eckfeldt	1994	4.2	10.8		
Carlsen	2011	5.4	5.6		
Pineda-Tenor	2013	5.5	8.2	>80	♂
Pineda-Tenor	2013	3.7	8.8	19-42	♂
Pineda-Tenor	2013	6.8	7.3	>80	♀
Pineda-Tenor	2013	4.5	7.5	19-42	♀
Loch	2015	8.5; 10.4	16.2; 16.8		

Issues with (Glucose) BV data

- ✓ ***Heterogeneity of protocols*** **for derive biological variation data**
- ✓ **CV_i and CV_g values possibly dependent from *different biological MATRICES***
- ✓ **CV_i and CV_g values different for healthy and diseased individuals**

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Quantifying Biological Variation

How do you do the experiment?

✓ Subjects	How many?
✓ Collect specimens	Number? Frequency?
✓ Analyse specimens	Minimise analytical variation?
✓ Analyse data	Outliers? Statistics?

Braga F, Panteghini M. Crit Rev Clin Lab Sci 2016;53:313-25

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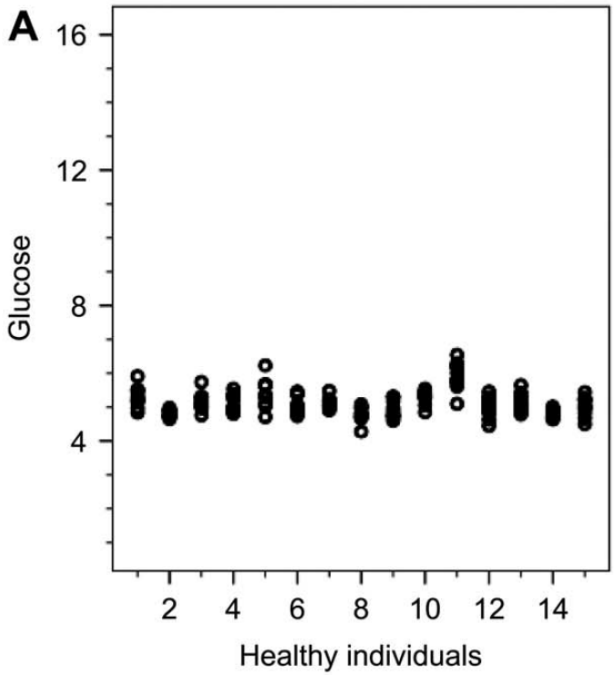
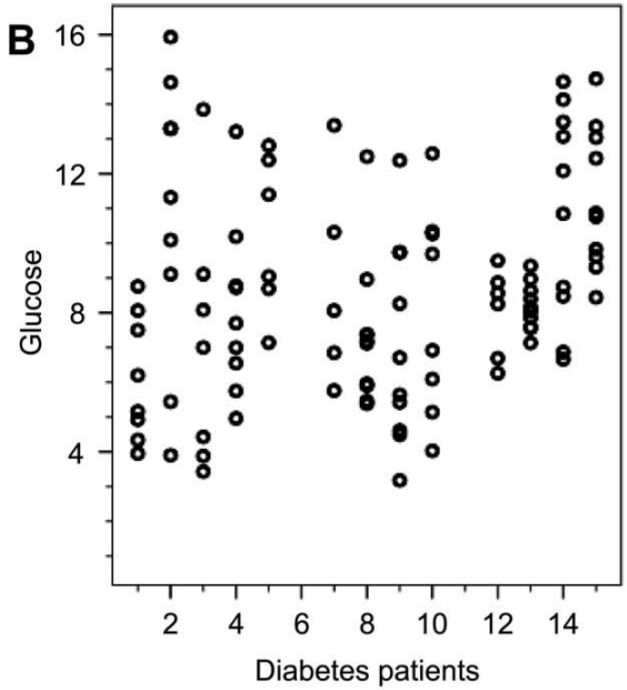
Biological variation from patients Should they be used?





Inherent biological variability

Inherent biological variability

+

disease (and treatment) related variability

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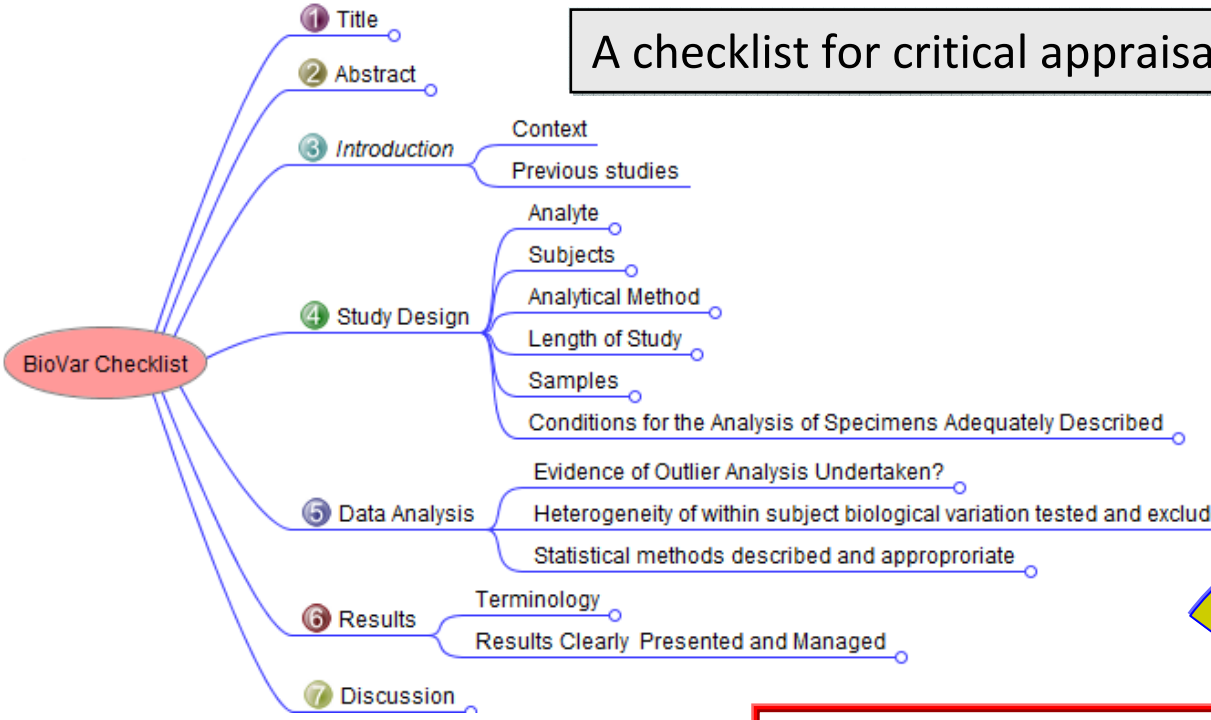
Carlsen S et al, Clin Chem Lab Med 2011;49:1501-7

A checklist for critical appraisal of studies of biological variation



Bartlett WA et al., Clin Chem Lab Med 2015;53:879

Biological Variation Working Group

EFLM
EUROPEAN FEDERATION OF CLINICAL CHEMISTRY AND LABORATORY MEDICINE

Clin Chem Lab Med 2011;49(9):1501–1507 © 2011 by Walter de Gruyter • Berlin • Boston. DOI 10.1515/CCLM.2011.233

Within-subject biological variation of glucose and HbA_{1c} in healthy persons and in type 1 diabetes patients

Siri Carlsen[1,2,*], Per Hyltoft Petersen[2], Svein Skeie[1,2], Øyvind Skadberg[1] and Sverre Sandberg[2]

[1] Department of Medicine, Stavanger University Hospital, Stavanger, Norway
[2] Norwegian Center for Quality Improvement of Primary Care Laboratories (NOKLUS), Section for General Practice, Department of Public Health and Primary Health Care, University of Bergen, Bergen, Norway

CV_i	CV_g
5.4%	5.6%

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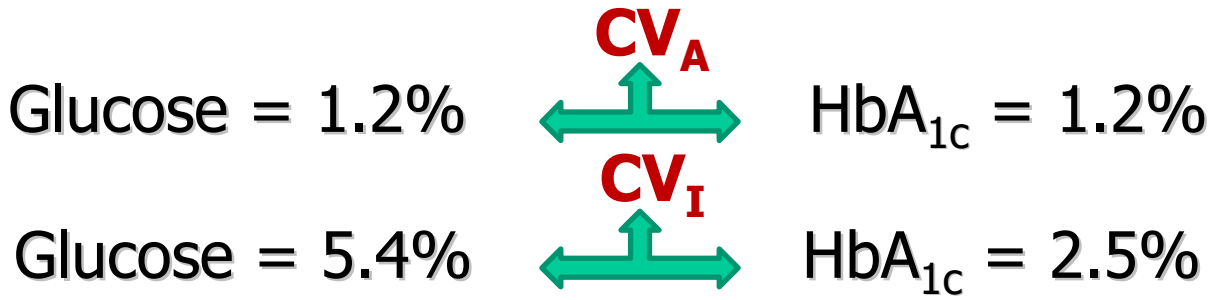
Assessing the number of specimens (**n**) required to estimate the individual's homeostatic setpoint of plasma glucose

$$n = 1.96^2 * (CV_A^2 + CV_i^2) / D^2$$

CV_A, Analytical coefficient of variation
CV_I, Within-subject biological coefficient of variation
D, desired percentage of closeness (usually, 95%)



Glucose n = 4.7

HbA_{1c} n = 1.2

?

Table 2.2—Criteria for the diagnosis of diabetes

FPG ≥126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 h.*

OR

2-h PG ≥200 mg/dL (11.1 mmol/L) during an OGTT. The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.*

OR

A1C ≥6.5% (48 mmol/mol). The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay.*

OR

In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dL (11.1 mmol/L).

*In the absence of unequivocal hyperglycemia, results should be confirmed by repeat testing.

Diabetes Care 2017;40suppl1:s1-135

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DE GRUYTER Clin Chem Lab Med 2015; aop

Sverre Sandberg*, Callum G. Fraser, Andrea Rita Horvath, Rob Jansen, Graham Jones, Wytze Oosterhuis, Per Hyltoft Petersen, Heinz Schimmel, Ken Sikaris and Mauro Panteghini

Defining analytical performance specifications: Consensus Statement from the 1st Strategic Conference of the European Federation of Clinical Chemistry and Laboratory Medicine

Model 1: Based on the effect of analytical performance on clinical outcomes

a. Done by direct outcome studies – investigating the impact of analytical performance of the test on clinical outcomes;

b. Done by indirect outcome studies – investigating the impact of analytical performance of the test on clinical classifications or decisions and thereby on the probability of patient outcomes, e.g., by simulation or decision analysis.

Model 2: Based on components of biological variation of the measurand.

Model 3: Based on state of the art of the measurement (i.e., the highest level of analytical performance technically achievable).

DE GRUYTER Clin Chem Lab Med 2017; 55(2): 189–194

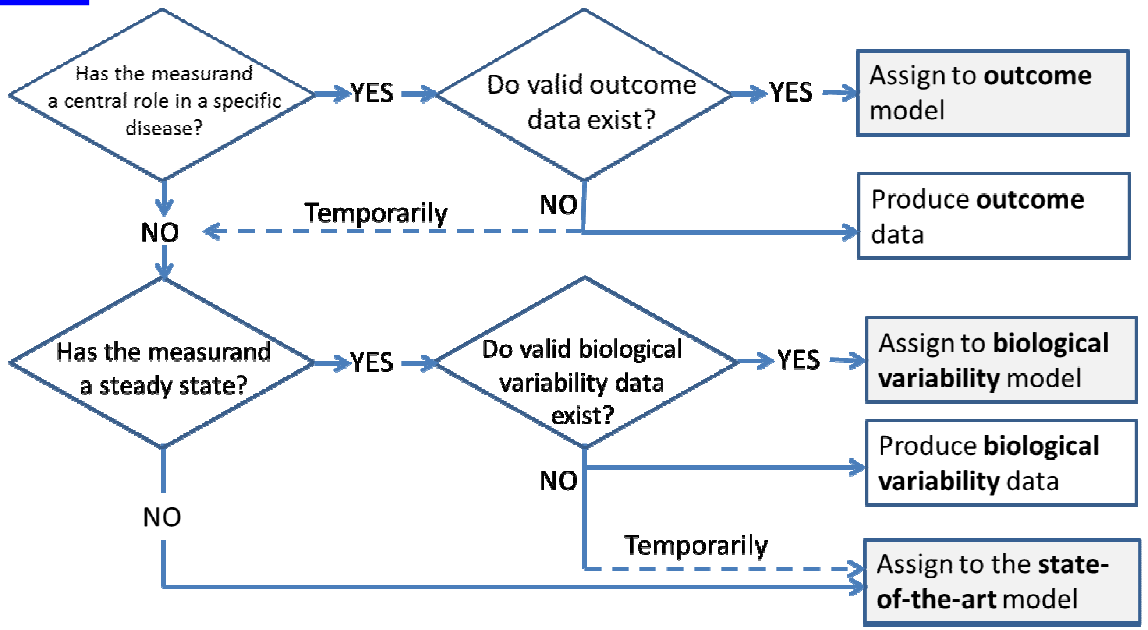
Opinion Paper

Ferruccio Ceriotti*, Pilar Fernandez-Calle, George G. Klee, Gunnar Nordin, Sverre Sandberg, Thomas Streichert, Joan-Lluis Vives-Corrons and Mauro Panteghini, on behalf of the EFLM Task and Finish Group on Allocation of laboratory tests to different models for performance specifications (TFG-DM)

Criteria for assigning laboratory measurands to models for analytical performance specifications defined in the 1st EFLM Strategic Conference

1. The measurand has a central role in diagnosis and monitoring of a specific disease
   ⇒ **outcome model** ➡ **Plasma Glucose**
2. The measurand has a high homeostatic control
   ⇒ **biological variability model**
3. Neither central diagnostic role nor sufficient homeostatic control
   ⇒ **state-of-the-art model**

Workflow for allocation of laboratory measurands to different models for performance specifications



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Analytical performance specifications for plasma glucose based on data of biological variability of the analyte

Model 2

- ***Minimum***

  $CV_A < 0.75 \times CV_I$ **4.05%**

  $B < 0.375 \times (CV_I^2 + CV_G^2)^{0.5}$ **3.0%**

  $TE < [1.65 \times 0.75 \times CV_I + 0.375 \times (CV_I^2 + CV_G^2)^{0.5}]$ **9.6%**

- ***Desirable***

  $CV_A < 0.50 \times CV_I$ **2.7%**

  $B < 0.250 \times (CV_I^2 + CV_G^2)^{0.5}$ **1.95%**

  $TE < [1.65 \times 0.50 \times CV_I + 0.250 \times (CV_I^2 + CV_G^2)^{0.5}]$ **6.4%**

- ***Optimum***

  $CV_A < 0.25 \times CV_I$ **1.35%**

  $B < 0.125 \times (CV_I^2 + CV_G^2)^{0.5}$ **1.0%**

  $TE < [1.65 \times 0.25 \times CV_I + 0.125 \times (CV_I^2 + CV_G^2)^{0.5}]$ **3.2%**

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Defining analytical performance specifications using *indirect* outcome data (Model 1b)

- Impact of analytical performance of test on clinical classifications or decisions and thereby on probability of outcomes (simulation or decision analysis).
- To model the clinical outcomes of misclassification requires clinical evidence about the consequences for patients.
- Where clinical evidence about these consequences is not available, the model estimates will be based on *assumptions* drawn from what evidence there is about disease prognosis, treatment benefits, harms, etc.

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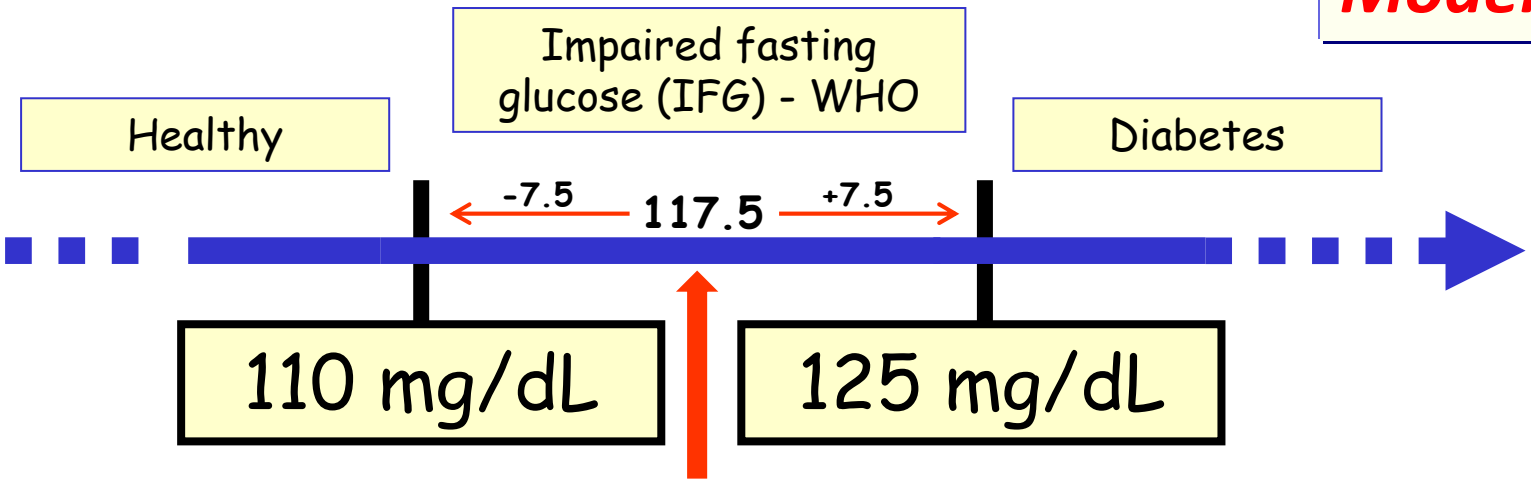
Defining analytical performance specifications for plasma glucose using *indirect* outcome data

Model 1b

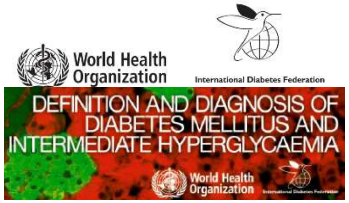


A subject with a FPG of 117.5 mg/dL must be differentiate from healthy condition (from one side) and a frank diabetes diagnosis (from the other side).

Therefore, TE of FPG measurement should be kept <7.5/117.5 = <6.38% so that a subject with an IFG cannot be misclassified as diabetic (FPG >125 mg/dL) or healthy (FPG <110 mg/dL).

Model 1b TEa = <6.38% **vs.** ***Model 2 Tea = <6.4%***

Equivalence of models 1 and 2 for measurands with well-defined biological and clinical characteristics



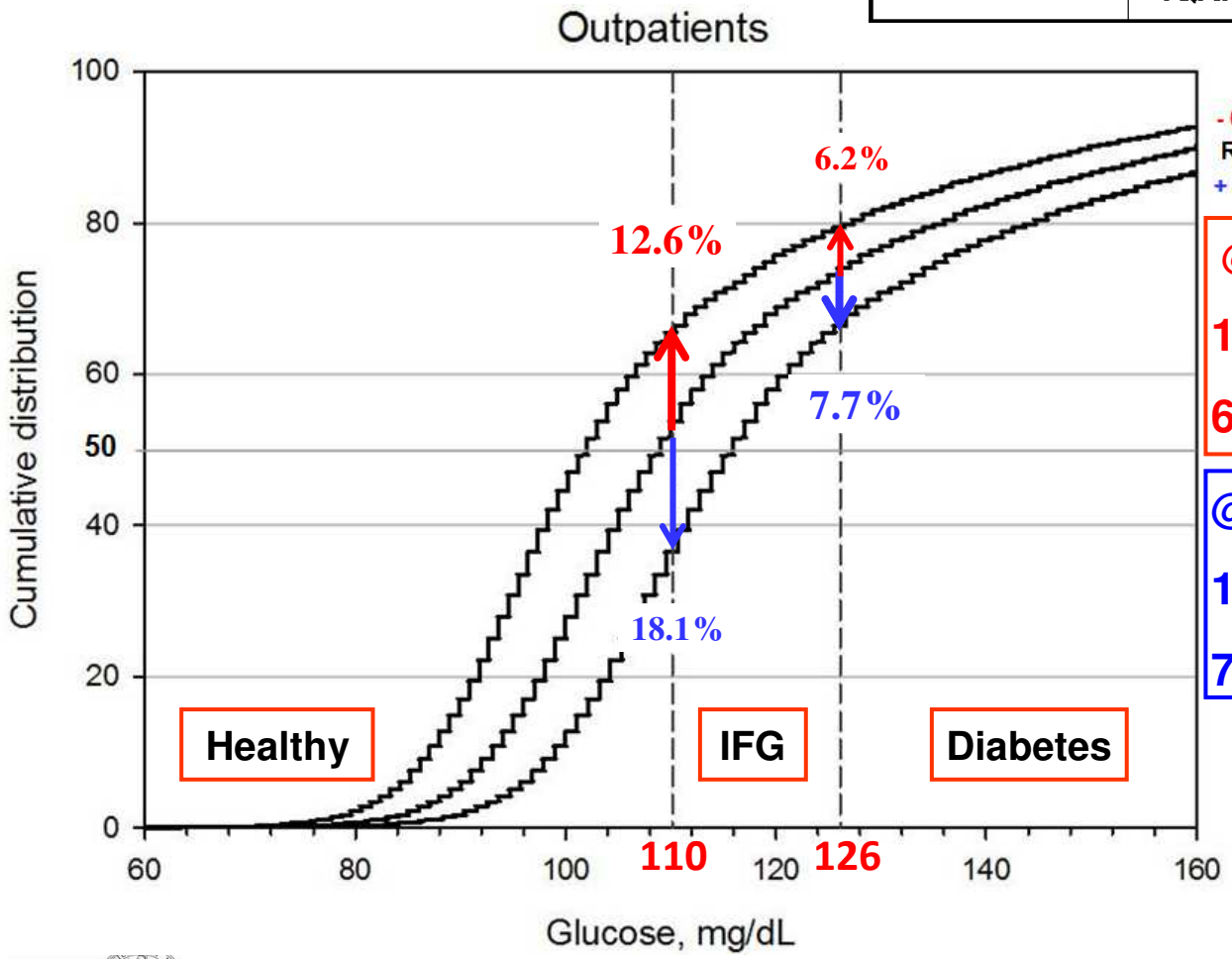
Global Reports on Diabetes 2016.
ISBN 978 92 4 156525 7

Pasqualetti S et al, Clin Biochem 2017. pii: S0009-9120(17)30116-9.doi: 10.1016/j.clinbiochem.2017.03.009.

Impact of measurement error of plasma glucose on clinical classification

simulation analysis

PG distribution	Reference	@ -6.38%	@ +6.38%
n 6537	$\tilde{x}$ = 109 mg/dL IQR: 99-128	$\tilde{x}$ = 102 mg/dL IQR: 93-120	$\tilde{x}$ = 116 mg/dL IQR: 105-136



@ - 6.38% TE

12.6% IFG misclassified as healthy

6.2% Diabetics misclassified as IFG

@ + 6.38% TE

18.1% Healthy misclassified as IFG

7.7% IFG misclassified as diabetics

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Pasqualetti S, et al. Clin Biochem 2017. pii: S0009-9120(17)30116-9. doi: 10.1016/j.clinbiochem.2017.03.009.

PREDIABETICS CLINICAL PATHWAYS

IFG defines a set of individuals at increased risk to develop diabetes

Early intervention lowering plasma glucose over time for:

1 delaying the onset of diabetes

2 preserving β-cell function [hepatic (and muscle) insulin resistance plus defective insulin secretion environment] and the likelihood that vascular hyperglycaemia-related complications will be delayed or prevented

CLINICAL OUTCOME

IFG subjects misclassified as normoglycemic (FALSE NEGATIVE) represent the most impacting results

Measuring FPG with a TEa of -6.38% would imply that 12.6% of individuals will miss the interventions necessary to stop the progression to DM and the worsening of vascular hyperglycemia-related outcomes (clinical and economical evaluation to show the acceptability of this misclassification rate is needed)

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Analitical aspects of glucose testing

$$V_{TOT} = (V_P^2 + V_A^2 + V_I^2)^{1/2}$$

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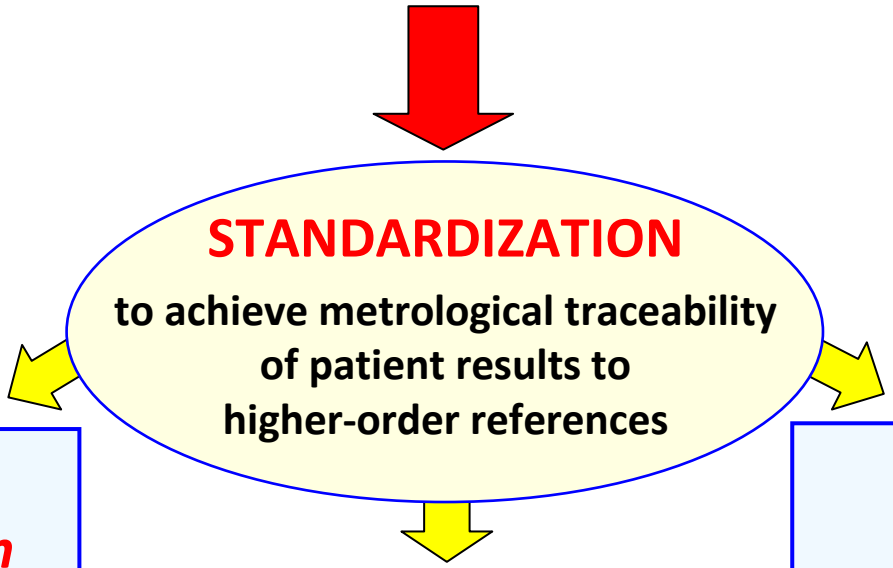
Laboratory customers (i.e., doctors and patients) expect lab results to be equivalent and interpreted in a reliable and consistent manner



Unbroken Traceability Chain

Definition of higher order references ***in order to*** *implement the appropriate trueness transfer process* ***to commercial calibrators***

Measurement uncertainty budget

Definition of allowable limits for uncertainty

Post-market surveillance

Survey - *suitability of assays and laboratory performances*

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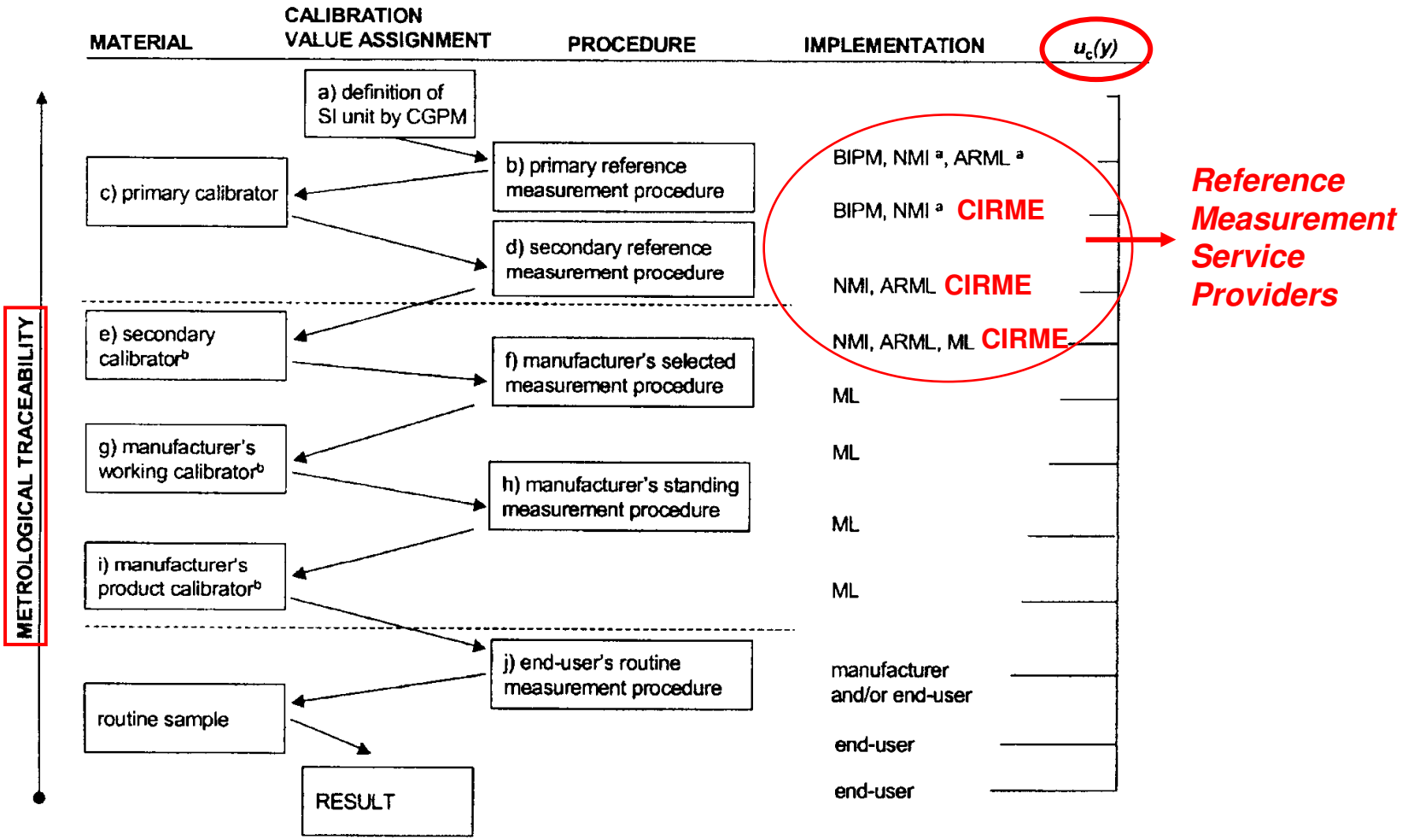
Panteghini M. Clin Chem Lab Med 2012;50:1237-41

TRACEABILITY ESTABLISHMENT



ISO 17511:2003. In vitro diagnostic medical devices - Measurement of quantities in biological samples – Metrological traceability of values assigned to calibrators and control materials.

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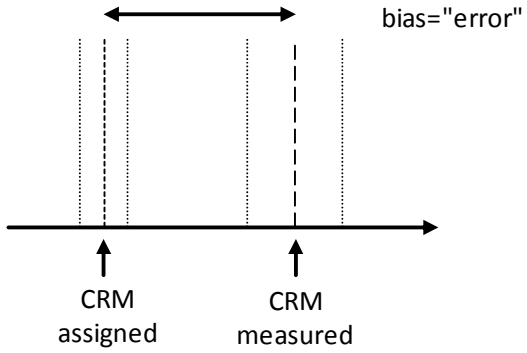
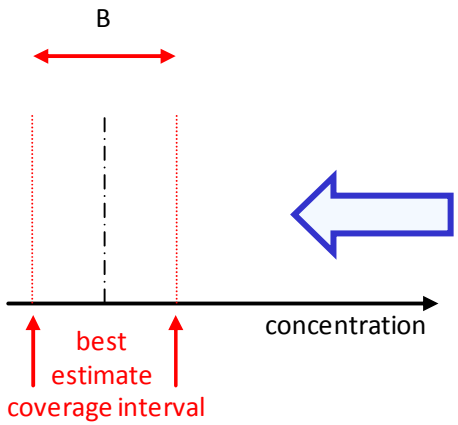
MEASUREMENT UNCERTAINTY AND BIAS CORRECTION

"Non-negative parameter characterizing the dispersion of the quantity values being reasonably attributed to a measurand, based on the information used"



***Bias,* systematic measurement error**

Bias correction*, *realignment of measuring system by adjusting the value assigned to the calibrator



Uncertainty of calibrator

Uncertainty

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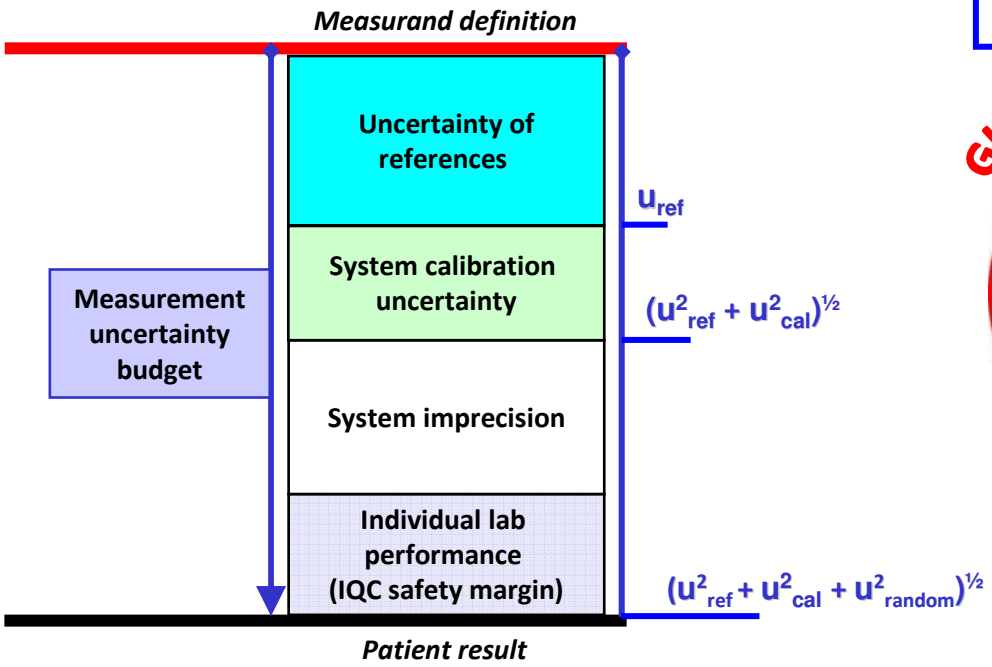
ALLOWABLE UNCERTAINTY BUDGET

Three main components of uncertainty:

1. ***Uncertainty of references*** - reference materials, reference procedures;
2. ***Uncertainty of commercial system calibrators*** - manufacturer 's calibrator values [transfer process];
3. ***Uncertainty of random sources*** – system imprecision, individual lab performance.



... FOR PLASMA GLUCOSE

From MODEL 2

4.05% minimum

2.70% desirable

1.35% optimum

MESUREMENT UNCERTAINTY GOAL
[for unbiased results]

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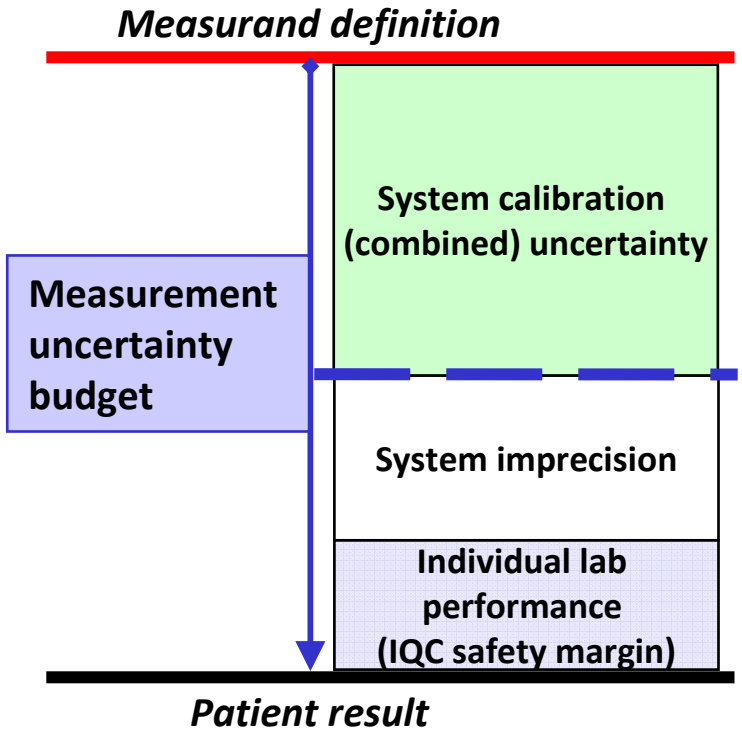
Braga F et al. Clin Chem Lab Med 2015;53:905-12

Need to define criteria for manufacturers that can be achieved for their calibrators leaving enough uncertainty budget for the laboratories to produce clinically acceptable results.



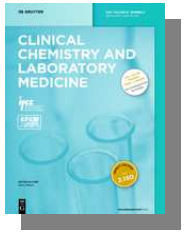
→ **The allowable limit for the combined uncertainty of manufacturer's commercial calibrators @ 50% of the goals**

Opinion Paper

Clin Chem Lab Med 2013; 51:973-9

Renze Bais*, Dave Armbruster, Rob T. P. Jansen, George Klee, Mauro Panteghini, Joseph Passarelli and Ken A. Sikaris on behalf of the IFCC Working Group on Allowable Error for Traceable Results (WG-AETR)

Defining acceptable limits for the metrological traceability of specific measurands



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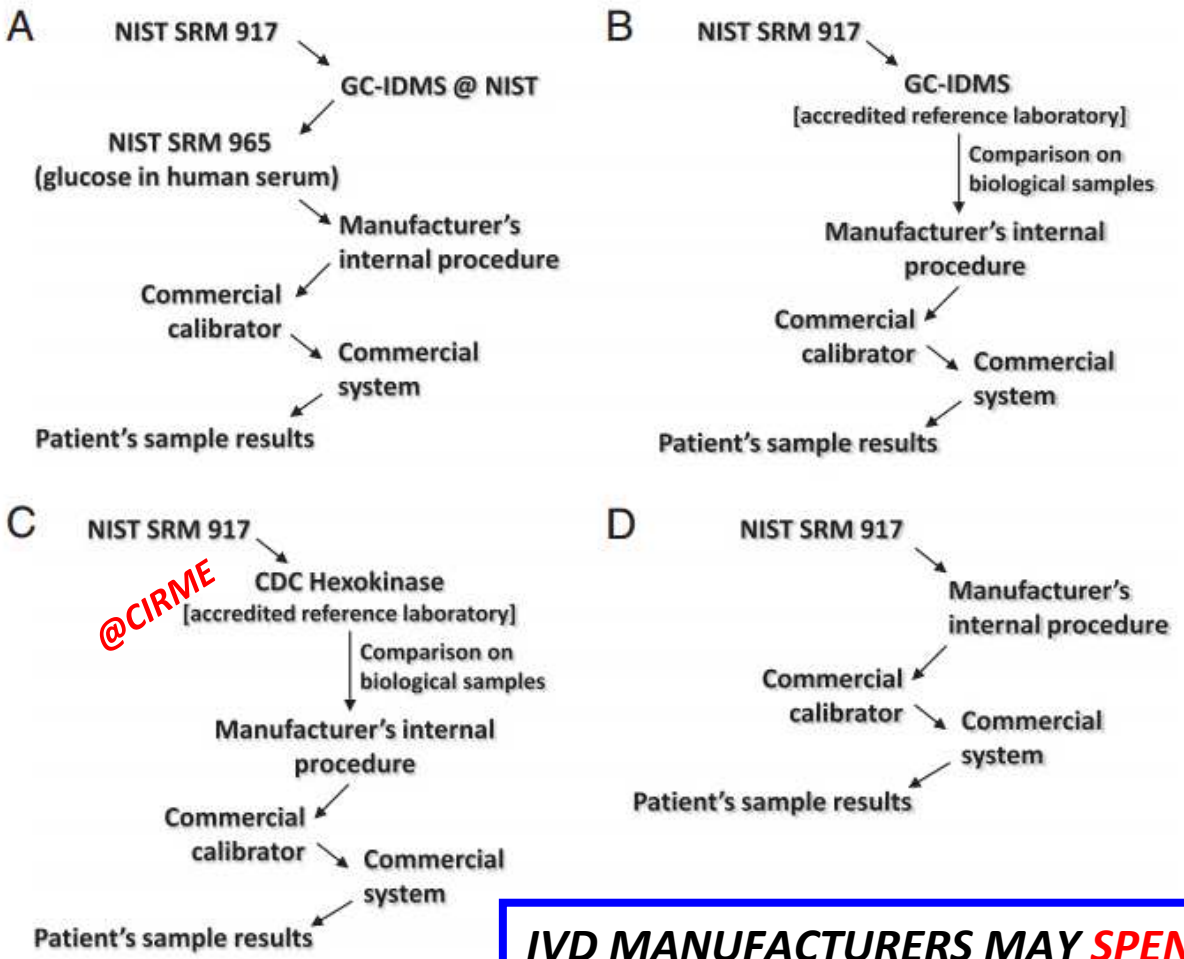
THE TRACEABILITY CHAINS AVAILABLE TO IVD MANUFACTURERS FOR GLUCOSE



IVD MANUFACTURERS MAY SPEND DIFFERENT AMOUNTS OF THE TOTAL UNCERTAINTY BUDGET TO ALLOW TRACEABILITY OF THEIR ANALYTICAL SISTEM TO HIGHER ORDER REFERENCES

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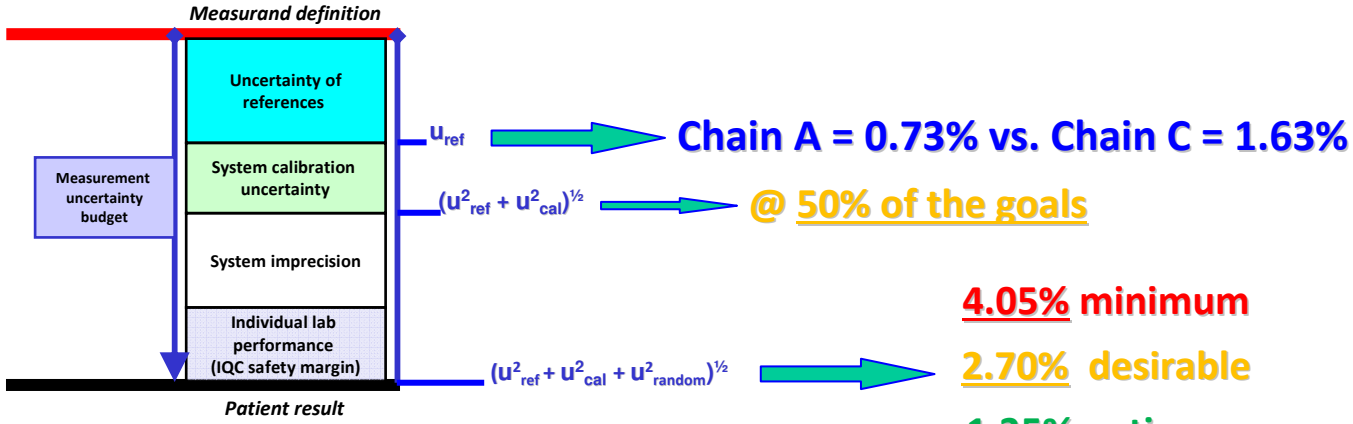
Braga F et al. Clin Chim Acta 2014;432:55-61

Are the analytical system commercially available for glucose determination able to achieve the desirable limit for combined uncertainty in a clinical setting (fit for purpose)?

Company	Platform	Principle of commercial method	Calibrator	Declared standard uncertainty[a]	Higher-order reference employed		Type of traceability chain used[b]	Combined standard uncertainty associated with the used chain[c] *(Expanded)*
					Method	Material		
Abbott	Architect	ND	Multiconstituent calibrator	2.70%	IDMS	NIST SRM 965	A	1.22–1.45%[d]
Beckman	AU	Hexokinase	System calibrator	ND	ND	NIST SRM 965	A	1.22–1.45%[d]
	Synchron	Hexokinase	Synchron multicalibrator	ND	ND	NIST SRM 917a	D	1.60–3.00%[e]
Roche	Cobas c	Hexokinase	C.f.a.s.	0.84%	IDMS	ND	B	1.70%
	Integra	Hexokinase	C.f.a.s.	0.62%	IDMS	ND	B	1.70%
	Modular	Hexokinase	C.f.a.s.	0.84%	IDMS	ND	B	1.70%
		GOD	C.f.a.s.	0.84%	IDMS	ND	B	1.70%
Siemens	Advia	Hexokinase	Chemistry calibrator	1.30%	Hexokinase	NIST SRM 917a	C	1.88–3.26%[f]
		GOD	Chemistry calibrator	0.80%	Hexokinase	NIST SRM 917a	C	1.88–3.26%[f]

Braga F, Panteghini M. Clin Chim Acta 2014;432:55-61



The uncertainty of (glucose) measurement my be dependent on the type of traceability chain selected for trueness transferring, making therefore difficult (e.g. chain C) to achieve the acceptable limits for measurement uncertainty on clinical sample

POST-MARKET SURVEILLANCE

Requirements for the applicability of EQAS results in the evaluation of the performance of participating laboratories in terms of traceability of their measurements

Feature	Aim
EQAS materials value-assigned with reference procedures by an accredited reference Laboratory	To check traceability of commercial system to reference systems
Proved commutability of EQAS materials	To allow transferability of participating laboratory performance to the measurement of patient samples
Definition and use of the clinically allowable measurement error (EQAS category 1/2A or 1/2B)	To verify the suitability of laboratory measurements in clinical setting

i.e. Glucose @CIRME (CDC reference procedure)

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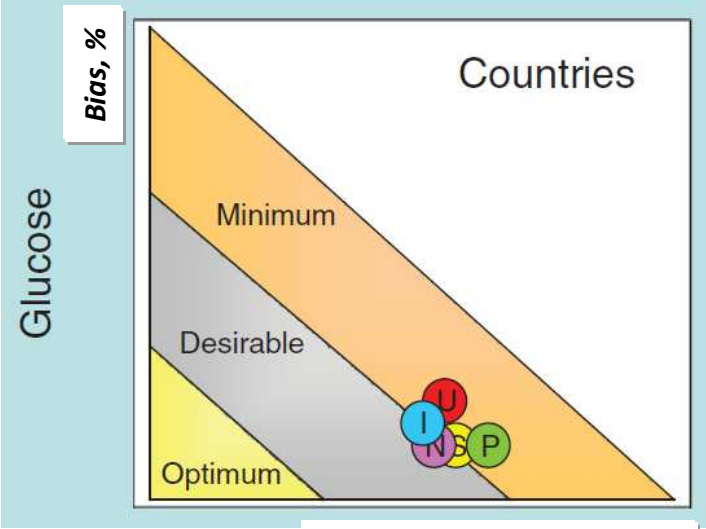
Panteghini M. Clin Chem Lab Med 2010;48:7
Infusino I et al. Clin Chem Lab Med 2010;48:301
Braga F, Panteghini M. Clin Chem Lab Med 2013;51:1719
Braga F, Panteghini M. Clin Chim Acta 2014;432:55
Infusino I et al. Clin Chem Lab Med 2017;55:334-40

Trueness-Based EQAS – Example 1

DE GRUYTER Clin Chem Lab Med 2017; 55(2): 203–211

Cas Weykamp*, Sandra Secchiero, Mario Plebani, Marc Thelen, Christa Cobbaert, Annette Thomas, Nuthar Jassam, Julian H. Barth, Carmen Perich, Carmen Ricós and Ana Paula Faria

Analytical performance of 17 general chemistry analytes across countries and across manufacturers in the INPUtS project of EQA organizers in Italy, the Netherlands, Portugal, United Kingdom and Spain



References (materials and procedure)
- frozen human serum
- GC-IDMS reference procedure

Performance specifications for TEa derived from biological variation

Analyte	Countries					
	ES	IT	PT	UK	All	NL
Glucose	7.7	6.8	8.3	8.0	7.5	6.7

Glucose TE

From MODEL 2

EQAS Category 1/2A

9.6% minimum

6.4% desirable

3.2% optimum

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Trueness-Based EQAS - Example 2

Trueness Assessment **for serum glucose measurement in different** ***Commercial Systems*** **through the preparation of** ***Commutable Reference Materials***

Chang Yu et al. Ann Lab Med 2012;32:243-9

References (materials and procedure)

- Pooled sera
- US Centers for Disease Control (CDC) reference procedure

Table 1. Relative bias for glucose measurement using 6 commercial systems

System code	Manufacturer	Method	Stated traceability for the reference method	Analyzer type	Relative bias (%)				
					RM1	RM2	RM3	RM4	RM5
GOD01	Beckman	GOD-oxygen electrode	HK	DxC800 (N=2), DxC20 (N=1)	2.88	-0.17	1.39	1.38	2.82
GOD02	Roche	GOD-POD	ID-MS	Modular P800 (N=3)	3.19	1.66	3.96*	3.43*	4.58*
GOD03	Ortho	GOD-dry chemistry	HK	Vitros 250 (N=3)	1.92	-0.17	2.68	1.38	3.14
HK01	Beckman	HK-G6PD	HK	DxC800 (N=2), DxC20 (N=1)	-1.92	-3.48*	-2.78	-2.77	-0.85
HK02	Roche	HK-G6PD	ID-MS	Modular P800 (N=3)	-3.83*	-1.82	-0.11	-1.6	-0.11
HK03	Dade Behring	HK-G6PD	ID-MS	RXL-MAX (N=3)	-1.28	-1.82	-1.28	-0.73	-0.27

Most *BUT NOT ALL* of the measurement systems met the minimum quality specifications for bias.

From MODEL 2

EQAS Category 1/2A

3.0% minimum

2.0% desirable

1.0% optimum

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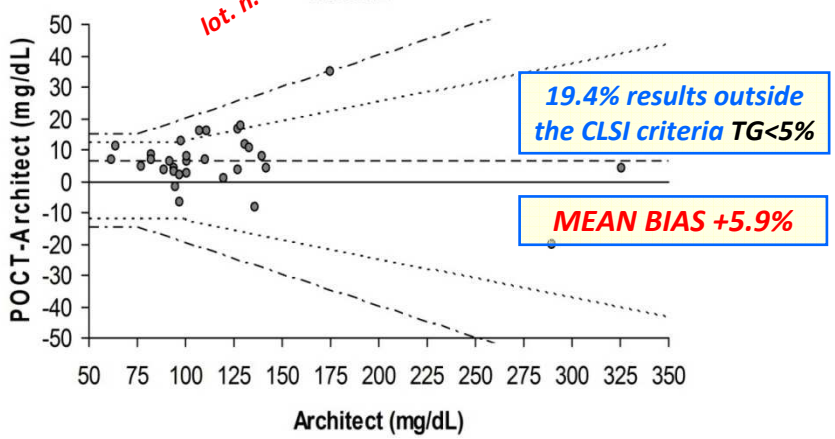
Verification of the accuracy of three glucose point-of-care testing (poct) devices for their use in a hospital setting

Elena Aloisio, Erika Frusciante, Alberto Dolci, Mauro Panteghini
Research Centre for Metrological Traceability in Laboratory Medicine (CIRME), University of Milan, Italy

- **Comparison with a standardized automated system (Abbott, ref. n. 3L82, mean bias 0.2% *vs* CDC ref. procedure performed @CIRME)**
- **CLSI acceptability criteria (POCT12-A3)**

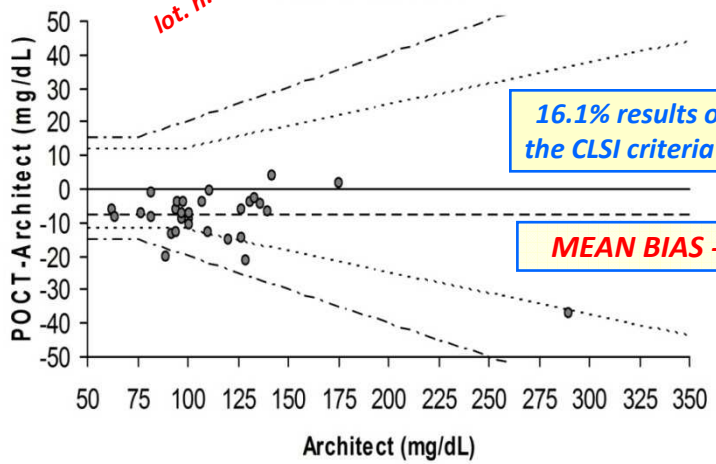
AccuCheck lot. n. 20812648



19.4% results outside the CLSI criteria TG<5%

MEAN BIAS +5.9%

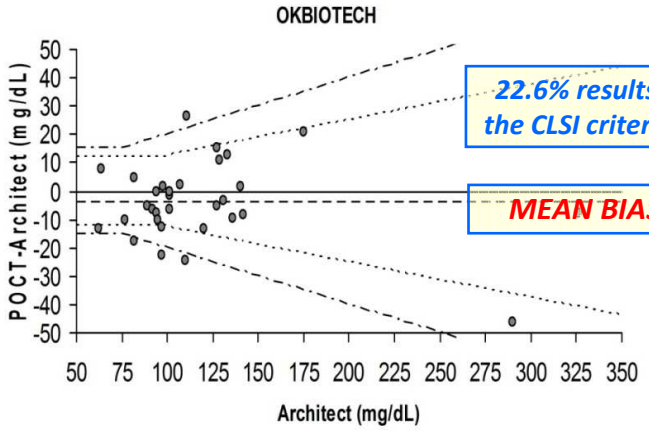
NovaPro lot. n. 1890215125



16.1% results outside the CLSI criteria TG<5%

MEAN BIAS -7.7%

OKmeterDirect lot. n. S141223-1



22.6% results outside the CLSI criteria TG<5%

MEAN BIAS -3.9%

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Aloisio E et al. Bioch Clin 2017;41:79-84

…DESPITE MANY EFFORTS BY THE PROFESSION…

…QUANTIFICATION OF A SIMPLE MOLECULE LIKE GLUCOSE IS NOT SIMPLE…

…BUT WE ARE WELL ON THE WAY !

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CIRME - Centre for Metrological Traceability in Laboratory Medicine

http://users.unimi.it/cirme

Thank you !!

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EFLM EUROPEAN FEDERATION OF CLINICAL CHEMISTRY AND LABORATORY MEDICINE

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MEASUREMENT UNCERTAINTY IN MEDICAL LABORATORIES: FRIEND OR FOE?

MILANO, ITALY
November 30th, 2017

AULA MAGNA - LITA SEGRATE
Via Fratelli Cervi, 93 - Segrate, Milano