INQUADRAMENTO FISIOPATOLOGICO E DI LABORATORIO

Andrea Mosca

Centro Interdipartimentale per la Riferibilità Metrologica (CIRME)

Dip. di Fisiopatologia medico-chirurgica e dei trapianti

Universitá degli Studi di Milano

REGIONE AUTONOMA DELLA SARDEGNA
Azienda Ospedaliera Brotzu

MEDICINA di LABORATORIO
Direttore Dott. Marcello Angius

Incontro con il Laboratorio:
HbA_{1c} : La Clinica ed il Laboratorio
Diagnostica del diabete

Cagliari, 07 Giugno 2013 ore 11.00

Ospedale Brotzu Sala Ciccu

TOSOH

CONTENUTI

1. I riferimenti
2. Emoglobina glicata
3. Glucosio
4. Conclusioni

CONTENUTI

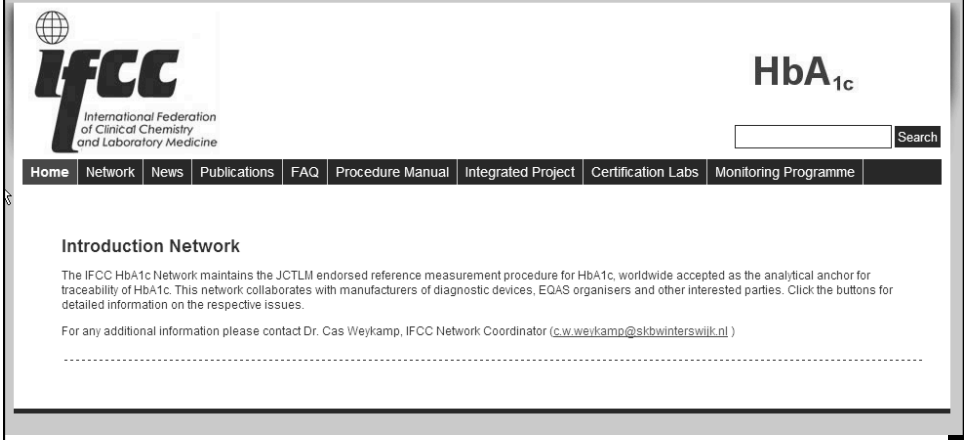
1. I riferimenti
2. Emoglobina glicata
3. Glucosio
4. Conclusioni

Clinical Chemistry 57:6
e1–e47 (2011)

Special Report

Guidelines and Recommendations for Laboratory Analysis in the Diagnosis and Management of Diabetes Mellitus

David B. Sacks,[1*] Mark Arnold,[2] George L. Bakris,[3] David E. Bruns,[4] Andrea Rita Horvath,[5] M. Sue Kirkman,[6] Ake Lernmark,[7] Boyd E. Metzger,[8] and David M. Nathan[9]



www.ifcchba1c.com

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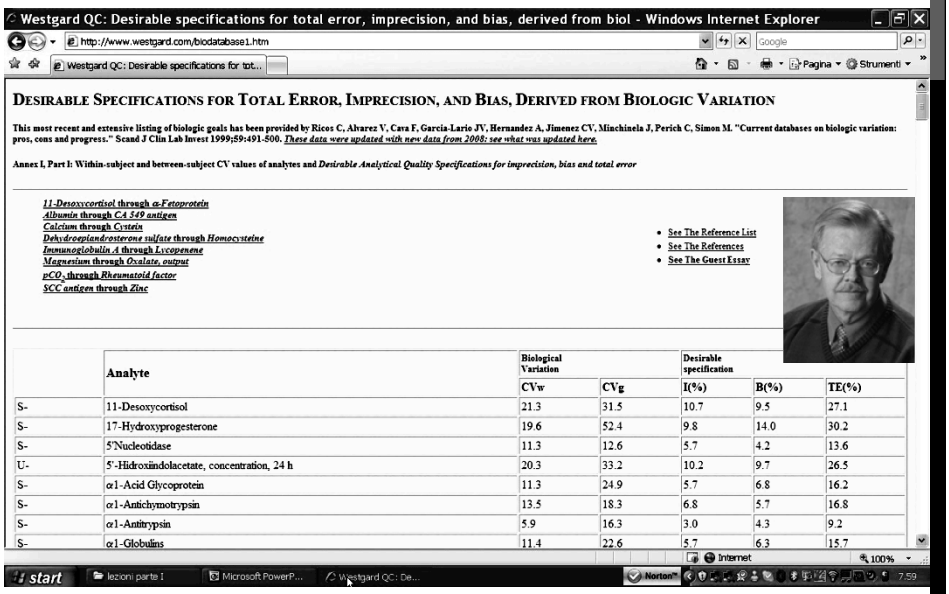
Westgard biodatabase



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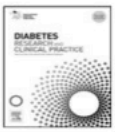
DIABETES RESEARCH AND CLINICAL PRACTICE 93 (2011) 310–311

Contents lists available at ScienceDirect

Diabetes Research and Clinical Practice

journal homepage: www.elsevier.com/locate/diabres



Commentary

Presidents' statement on WHO recommendation on HbA1c for diabetes diagnosis

WHO recommendation accepted by presidents of IDF, ADA & EASD

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Consensus autocontrollo

RACCOMANDAZIONI PER L'AUTOCONTROLLO DELLA GLICEMIA NEL PAZIENTE DIABETICO

Annunziata Lapolla, Padova – Coordinatore SID
Concetta Suraci, Roma – Coordinatore AMD
Maria Teresa Branca, Lecce – OSDI
Paolo Carraro, Padova – SIBioC
Mariarosa Carta, Vicenza - SIMeL
Valentino Cherubini, Ancona – SIEDP
Roberta Chiandetti, Udine – OSDI
Francesco Chiaramonte, Roma - AMD
Francesco Mario Gentile, Bari – AMD
Andrea Mosca, Milano- SIBioC
Roberto Testa, Ancona – SIMel
Elisabetta Torlone, Perugia – SID
Roberto Trevisan, Bergamo – SID

Rev. 22-12-12

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WHAT'S DIABETES IN 2013?

Professor David Leslie

Blizard Institute, University of London
and St Bartholomews Hospital

23 May 2013, 9:00 – 11:00
Room Gold
Symposium 13
Controversies in detection and monitoring of diabetes

EUROMEDLAB MILANO 2013 19-23 MAY

CONTENUTI

1. I riferimenti
2. Emoglobina glicata
3. Glucosio
4. Conclusioni

Vantaggi dell'HbA_{1c} per la diagnosi del diabete

1. Indicatore integrato della glicemia
2. Digiuno non necessario
3. Stabile nel campione
4. Bassa variabilità intra-individuale
6. Non alterata da fattori acuti (stress, febbre)
7. Ben standardizzata
8. Stretta correlazione con il rischio di complicanze microvascolari

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Table 2 Limitations in the use of HbA_{1c} for screening and diagnosis of diabetes.

Factors	Risk in the diagnosis
Analytical interferences (could be overcomed by appropriate sample handling or by choosing the most appropriate method for HbA_{1c} quantification)	
a) Hyperbilirubinaemia	overdiagnosis
b) Elevated serum triglycerides	overdiagnosis
c) Increased WBC	overdiagnosis
d) Presence of some hemoglobin variants (HbS, HbC, HbD, HbE)	misdiagnosis
In vivo effects due to physiological conditions (cannot be handled a-priori)	
a) Pregnancy	misdiagnosis
b) Seasonal variations	over- or mis-diagnosis
c) Age	overdiagnosis
d) Genetic determinants (including race)	over- or mis-diagnosis
e) Presence of other hemoglobin variants and/or thal. major	over- or mis-diagnosis
In vivo effects due to pathological conditions (cannot be handled a-priori)	
a) Type 1 diabetes under rapid development	misdiagnosis
b) Malaria	misdiagnosis
c) Haemolytic anaemia	misdiagnosis
d) Iron deficiency	overdiagnosis
e) Recent blood loss, recent transfusion	misdiagnosis
f) Splenectomy	overdiagnosis
g) Renal failure	overdiagnosis
h) HIV positive patients on anti-retroviral therapy	overdiagnosis
i) Erythropoietin and other drugs interacting with erythropoiesis	misdiagnosis
j) Chronic alcohol abuse	misdiagnosis
Other reasons	
a) HbA_{1c} may not be easily available in some countries	
b) Higher imprecision respect to plasma glucose determination	

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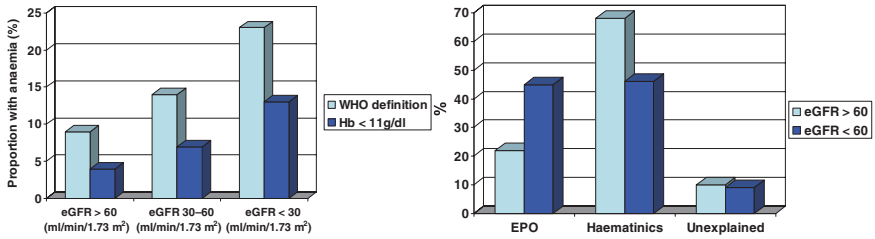
Lapolla et al, Nutr Met Cardiovasc 2011;21:467-75

PREVALENCE OF ANEMIA IN DIABETIC PATIENTS

UK EXPERIENCES (TEESSIDE)



Jones et al. Diabetic Medicine 2010; 27:655-9

n= 7331

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Red cell life span heterogeneity in hematologically normal people is sufficient to alter HbA1c

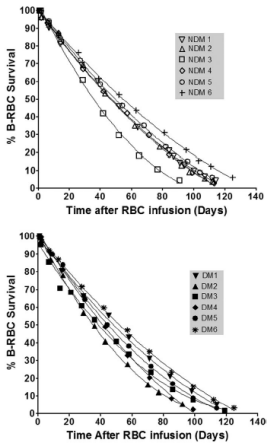


Figure 3. RBC disappearance in NDM and DM subjects. (Top) NDM subjects; (bottom) DM subjects. Because RBC disappearance curves were nonlinear, determination of RBC life span as the x-intercept is not the most meaningful approach to summarizing the exposure of the RBCs to plasma glucose. Mean cell age was therefore used, computed from the survival curves, fitted to cubic equations ("Analysis of RBC life span").

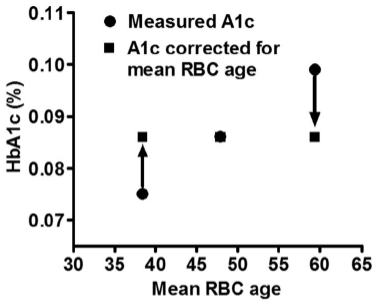
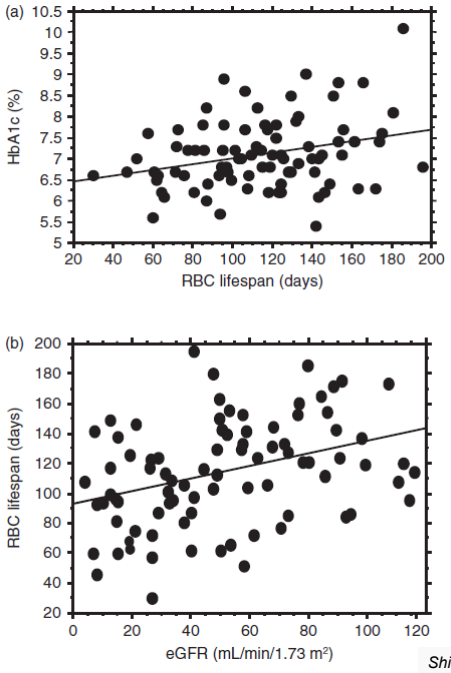


Figure 6. Impact of differences in mean RBC age on measured HbA1c. Three hypothetical patients with identical glycation rates would be expected to have similar HbA1c values (in this case 0.086, the average of the 6 DM subjects in our study). However, variable mean RBC age results in a measured HbA1c that ranges from lower to higher than expected. This could lead to inappropriate clinical management.

CV ~ 12 %

Cohen, Blood 2009;112:4284-91



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Shima et al, Ann Clin Biochem 2012;49:68-74

Common Variants at 10 Genomic Loci Influence Hemoglobin A_{1C} Levels via Glycemic and Nonglycemic Pathways

- Associations with HbA_{1c} in 46,368 nondiabetic adults of European descent
- 23 genome-wide association studies (GWAS) and 8 cohorts with de novo genotyped single nucleotide polymorphisms (SNPs)
- 6 new loci near FN3K (HFE, ANK1..)
- 4 known HbA1c loci (HK1, MTNR1B, GCK, G6PC2/ABCB11)
- Reclassification of 2% general population screened for diabetes

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Soranzo et al, Diabetes 2010;59:3229-39

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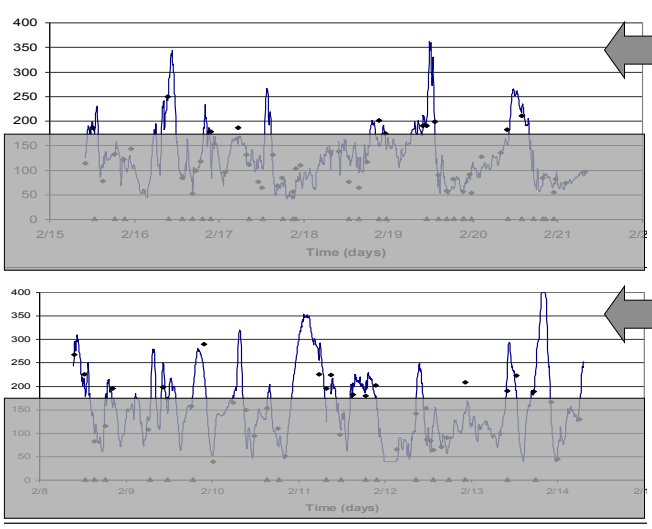
Despite similar A1c levels…



Less Spiking

A1C 7.4%
1,5-AG 12.4 µg/ml
PPG max 195 mg/dL

More Spiking

A1C 7.3%
1,5-AG 4.5 µg/ml
PPG max 235 mg/dL

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Dungan et al. Diabetes Care 2006; 29 (6): 1214-9

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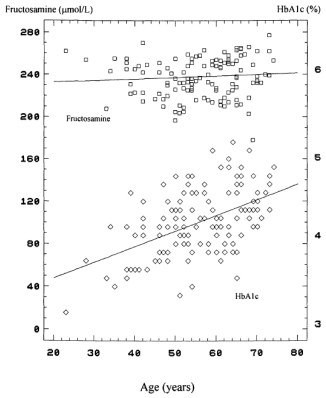

Figure 2. HbA_{1c} (diamonds) vs. age ($y = 0.018x + 3.23$, $r = 0.493$, $p < 0.00001$), and Fructosamine (squares) vs. age ($y = 0.140x + 230$. $r = 0.073$. $p = 0.42$) in 126 non-diabetic subjects.

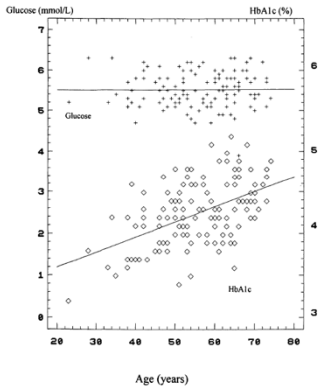

Figure 3. HbA_{1c} (diamonds) vs. age ($y = 0.018x + 3.23$, $r = 0.493$, $p < 0.00001$), and fasting plasma glucose (crosses) vs. age ($y = 0.0004x + 5.50$, $r = 0.009$, $p = 0.92$) in 126 non-diabetic subjects.

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Kilpatrick et al, QJMed 1996;89:307-12

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HEMOGLOBIN VARIANTS

Most common variants are HbS, HbC, HbE and HbD

Cannot use HbA_{1c} for diagnosis (or for monitoring) in individuals homozygous for HbS or HbC or with HbSC

Can measure HbA_{1c} accurately in most heterozygous Hb variants, if appropriate assay used (www.ngsp.org)

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ESSENTIAL STEPS FOR ACHIEVING THE STANDARDIZATION OF HBA_{1C}

Table 1 Essential steps in order to provide HbA_{1c} - IFCC standardized results.

actions	*tools*
Choice of the method	Evaluate the IFCC certificate (ask the manufacturer)
Calibration	Enter the IFCC target values provided by the manufacturer (ask the manufacturer for traceability to the IFCC reference system)
Reporting the HbA_{1c} result	Use the mmol/mol units (eventually transform afterwards in % units) Report decisional limits (not the reference intervals)
Monitoring the long-term imprecision	Internal Quality Control with two levels materials Calculate the CVs per month (or over a longer time frame)
Evaluating the trueness	Regular participation to EQAS exercises (commutable materials, IFCC target values assigned by the IFCC reference measurement procedures)

Mosca et al, Clin Chem Lab Med 2013, in press

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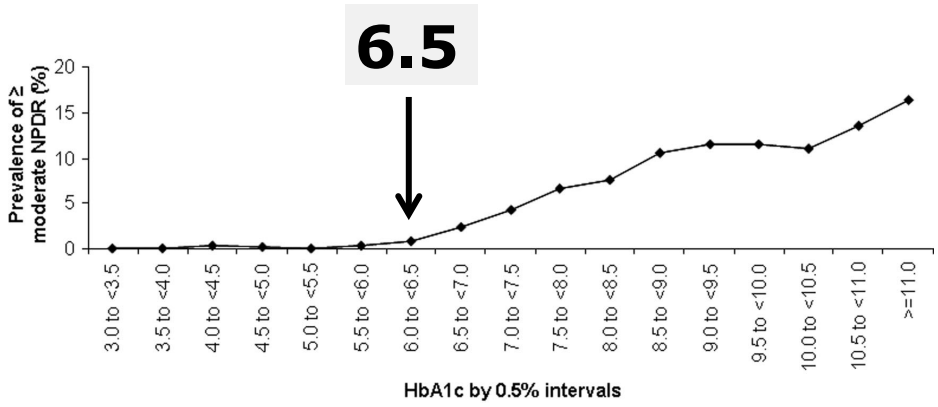
NGSP units, %



4.0-6.0

int. rif.

7.0 **9.0**

DCCT 1993

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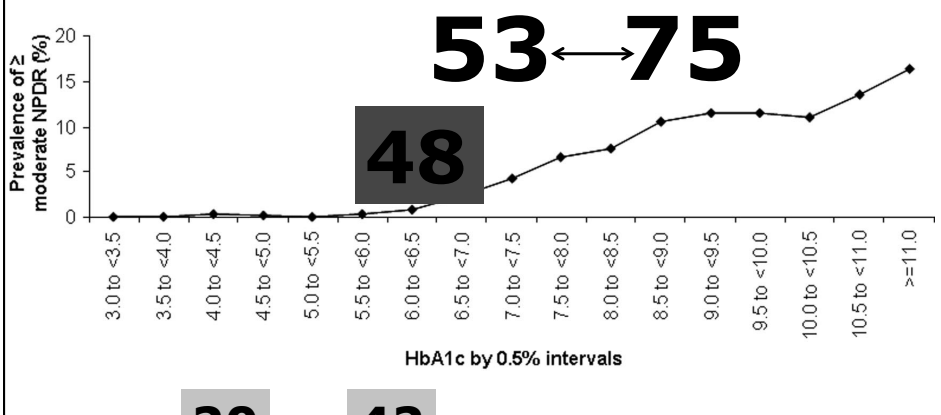
SI units, mmol/mol



20 **42**

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HBA$_{1C}$ METHOD EFFECT ON OPTIMAL DIABETES CARE

VEQ
SANGUE FRESCO INTERO

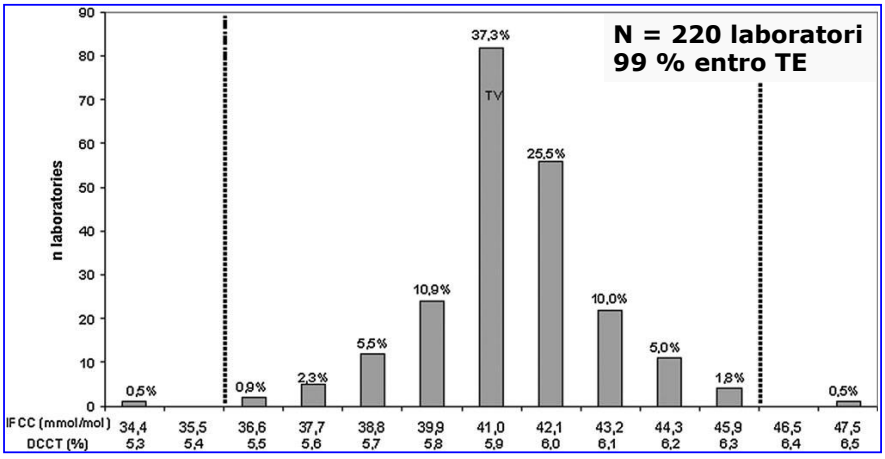

FIG. 1. Measured hemoglobin A_{1c} results compared with the target value (TV) of 41.0 mmol/mol (5.9%-Diabetes Control and Complications Trial [DCCT]) ± allowable total error of 6%. IFCC, International Federation of Clinical Chemistry.

Lenters-Westra et al, Diab Technol Therap 2011; 13:1-5

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Table 3 A summary of the clinical utility of HbA_{1c} testing in the diagnosis of diabetes, as from the recent literature available.

Author, year of publication	N°/study	Country	Ref test	HbA_{1c} cut off	Sensitivity	Specificity
Saudek et al., 2008 [61]	NHANES 1999–2004	US	FPG	≥6.5	44.3%	99.6%
	NHANES III	US	FPG	≥6.5	42.8%	99.6%
Ng et al., 2010 [62]	1135	UK	OGTT	19.9% of positive OGTT present HbA1c <6.0%		
Lu, 2010 [66]	2494 MP	Australia	OGTT	≥6.5	–	88.8%
	6014 Aus Diab	Australia	OGTT	≥6.5	–	99.9%
Van't Riet, 2010 [63]	2753 new Hoorn Study	Netherland	OGTT	≥6.5	24%	99%
Carson, 2010 [65]	6890 subgroup of NHANES 99-06	US	FPG	≥6.5	49.9%	99.5%
Kramer, 2010 [64]	2107 Rancho Bernardo Study	US	OGTT	≥6.5	44%	79%
	Age quartiles		OGTT	≥6.5	52%	95%
Zhou, 2010 [67]	2332	China	OGTT	≥5.6	64.4% M	61.6% M
				≥5.6	62.3 % F	63.3% F
Christensen, 2010 [68]	Inter99 Study	Denmark	OGTT	≥6.5	42.6%	–
	Whitehall II Study	UK	OGTT	≥6.5	25%	–
	Aus Diab	Australia	OGTT	≥6.5	17%	–
	Inuit Health in Transition Study	Greenland	OGTT	≥6.5	29.6%	–
	Black population	Kenya	OGTT	≥6.5	20%	–
	CURES Study	India	OGTT	≥6.5	78%	–
	23094	All	OGTT	≥6.5	43.5%	–
Olson, 2010 [69]	SIGT (1581), NANHES III (2057), NANHES 2005-2006 (1154)	US	OGTT	≥6.5	30 %	88-97%

Lapolla et al, Nutr Met Cardiovasc 2011;21:467-75

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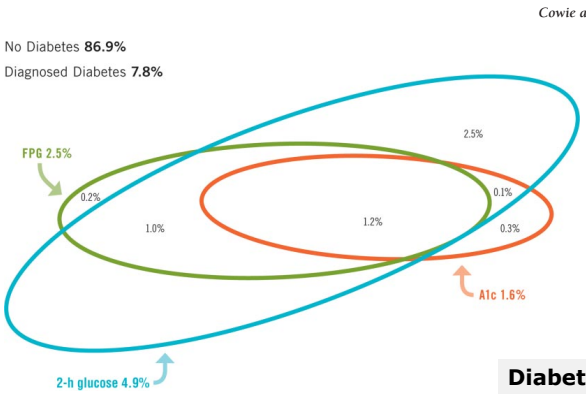
Undiagnosed diabetes in the U.S. population aged ≥20 years by three diagnostic criteria—NHANES 2005–2006



Diabetes

FPG: 2.5 %
2h PG: 4.9 %
HbA1c: 1.6 %

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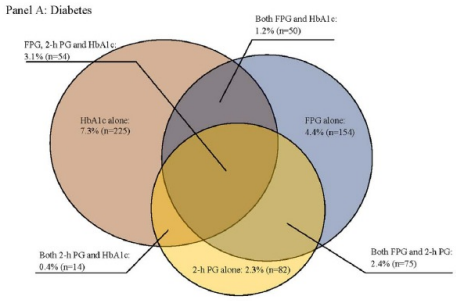
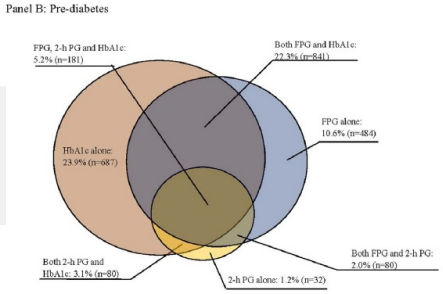
Cowie et al, Diabetes Care 2010;33:562-8

Diabetes

FPG: 4.4 %
2h PG: 2.3 %
HbA1c: 7.3 %





Pre-diabetes

FPG: 10.6 %
2h PG: 1.2 %
HbA1c: 23.9 %

Figure 1. The prevalence of diabetes and pre-diabetes using different screening methods.
doi:10.1371/journal.pone.0037260.g001

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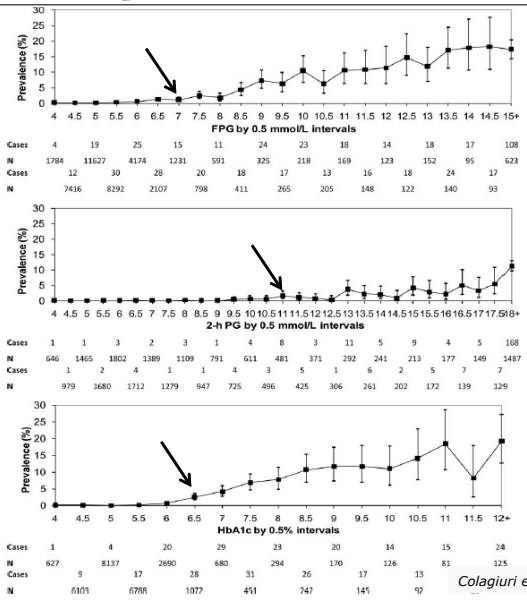
Zhang et al, PLOSone 2012;7(5):e37260

Glycemic Thresholds for Diabetes-Specific Retinopathy

Implications for diagnostic criteria for diabetes



DETECT-2
9 studies
n = 27933

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Colagiuri et al, Diabetes Care 2011;34:145-50

COMMENTARY
HbA1c in type 2 diabetes diagnostic criteria: addressing the right questions to move the field forwards

Unimportant questions	Important questions
Does HbA1c diagnose the same group of patients as glucose-based criteria?	**Which glycaemia measurement is the best predictor of microvascular disease?**
How does the cardiovascular risk factor profile differ in individuals identified by various diabetes diagnostic criteria in cross-sectional studies?	**Does use of HbA1c as a diagnostic measure lead to earlier diagnosis of type 2 diabetes and thereby improve clinical outcomes?**
Should oral glucose tolerance testing be part of future diagnostic algorithms?	**In which patients should HbA1c measurement be combined with fasting glucose?**
	What is the impact of using HbA1c for diabetes diagnosis on laboratory costs and what are the potential savings elsewhere?
	How best can diabetes and cardiovascular risk screening be combined?

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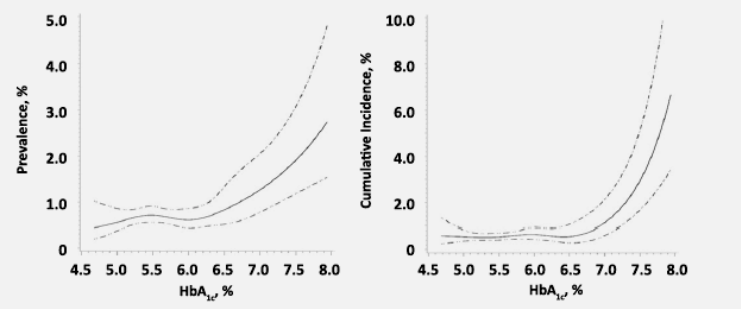
Sattar et al, Diabetologia 2012;55:1564-7

New Diabetes Diagnostic Threshold of Hemoglobin A_{1c} and the 3-Year Incidence of Retinopathy



Tsugawa Y et al, Diabetes 2012;61:3280

n = 19897 Japanese adults
2006-2009

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CONTENUTI

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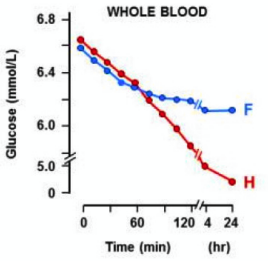
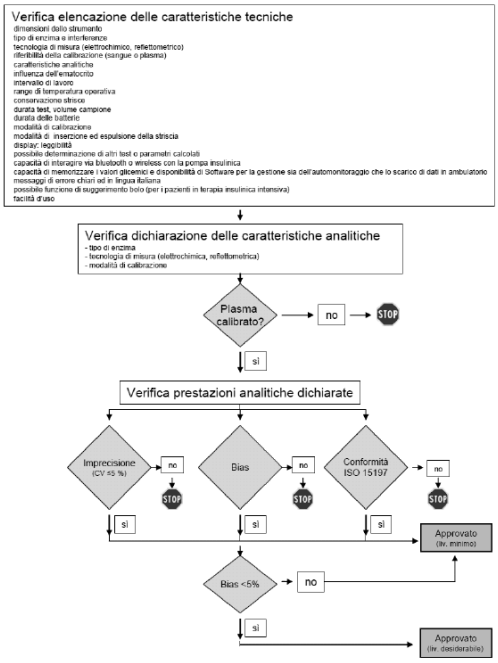
Glicemia a digiuno, criticità

- Digiuno da ≥8 ore (variazione durante la giornata)
- Ampia variabilità biologica
  - Intra-individuale CV 4,6 – 8,3 %
  - Tra-individui CV 7,5 – 12,5 %
- Instabilità
- Fattori acuti
- Differenze plasma/siero/sangue intero
- FPG meno legata alla complicanze diabetiche (vs. HbA_{1c})



Chan et al Clin Chem 1989;35:315



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PRESTAZIONI ANALITICHE

I limiti minimi vengono così definiti:

- Imprecisione: CV inferiore a 5% per valori medi di glicemia compresi tra 75 e 300 mg/dl
- Inesattezza (bias): calibrazione riferita al glucosio plasmatico (dichiarazione del produttore).

Sono altresì valutati come desiderabili le seguenti caratteristiche analitiche, dedotte da più recenti raccomandazioni (90):

- Imprecisione: CV inferiore a 5% (tra 75 e 300 mg/dl)
- Inesattezza (bias): calibrazione riferita al glucosio plasmatico con scostamento medio inferiore al 5% rispetto a metodo di riferimento su plasma per valori ≥ 75 mg/dL ed inferiore a 15 mg/dl (per valori sotto i 75 mg/dl)

da aggiornare per nuova soglia (100 mg/dL)

CONTROLLO DI QUALITÀ

…. prove di letteratura dimostrano un deterioramento delle prestazioni quando uno stesso strumento viene utilizzato da operatori non esperti, quali pazienti non opportunamente preparati (95).

Anche variazioni di lotto di materiale possono determinare scostamenti significativi.

In assenza di una supervisione da parte di un Organismo di Riferimento nazionale la Struttura di Diabetologia deve quindi sviluppare un programma di verifica periodica della precisione e della concordanza rispetto a metodi di riferimento, in collaborazione con il laboratorio accreditato di riferimento (vedasi Appendice).

CQI: utile anche per valutare eventuali problemi di conservazione delle strisce

Box 10

Si raccomanda alle Aziende produttrici di distribuire i materiali di controllo insieme alle strisce e a favorirne l'impiego con adeguate politiche dei prezzi.

Il Centro di Diabetologia deve disporre di materiali di controllo da impiegare a conferma delle misure ottenute, secondo specifici protocolli prodotti dalle Società Scientifiche.

(Livello di evidenza VI, forza della raccomandazione B)

VALUTAZIONE ESTERNA DI QUALITÀ (VEQ)

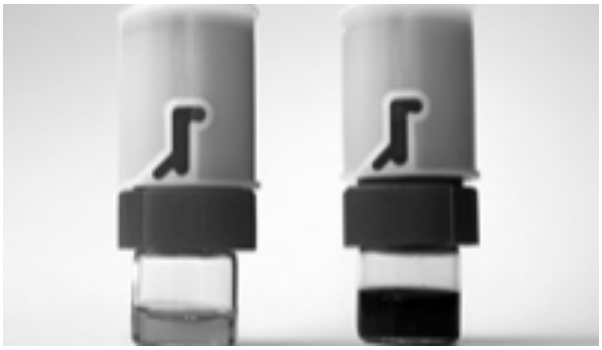
… sono ancora poco diffusi programmi di Valutazione Esterna di Qualità dedicati esplicitamente agli strumenti portatili e quelli operativi presentano problemi ancora non risolti di commutabilità dei materiali.

E' comunque opportuno che le strutture di riferimento diabetologico e di laboratorio scelgano una strategia a questo proposito, basata su un programma di VEQ o su confronto tra dati. A questo scopo, le Società Scientifiche scriventi sono impegnate nella stesura di idonei protocolli (vedasi Appendice)

… Ha la finalità di misurare l'inesattezza (confrontabilità)
Va gestita in diretta collaborazione con la struttura del laboratorio delegata alla gestione delle analisi decentrate elaborando una strategia gestionale idonea allo specifico contesto locale. L'orientamento è quello di mantenere il sistema analitico allineato ai criteri minimi proposti dalla norma ISO 15197.

La riferibilità di ogni tipologia di glucometro può essere ottenuta attraverso il dosaggio periodico di materiali di controllo interni. Nella gestione dei risultati di tale programma devono essere definiti a priori i limiti di accettabilità e le eventuali azioni correttive.

STABLE WHOLE BLOOD CONTROL MATERIAL



A whole blood glucose Quality Control (CueSee) that is stable for >2 months.

Conclusioni

1. ≈50 % dei pazienti ha un diabete non diagnosticato
2. L'HbA_{1c} predice lo sviluppo delle complicanze microvascolari
3. HbA_{1c} e glicemia a digiuno per la diagnosi del diabete: maggiore sensibilità? (HbA_{1c} da sola adeguata per lo screening)
4. HbA_{1c} utilissima per il pre-diabete
5. Traguardi analitici per l'HbA_{1c} almeno agli standard minimi basati sulla variabilità biologica
   (imprecisione ≤1.9 %, bias ≤±2.8 %, ET ±5.9 %)
6. Si auspica una sperimentazione delle raccomandazioni del documento di consenso in collaborazione tra team diabetologici e professionisti di laboratorio

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