

بنام خداوند جان آفرین که من در زبان آید

## اندازه گیری و تفسیر آزمایش HbA1c و معیارهای انتخاب روش

(Measurement / Interpretation of HbA1c & Method Selection Criteria)

دکتر محمدرضا بختیاری

دکتری علوم آزمایشگاهی تشخیص طبی

متخصص بیوتکنولوژی پزشکی



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### Case Studies:

Case 1: A 44 y/o female screened for diabetes:

- ✓ HbA1c= 46% (Biorad, HPLC)
- ✓ HbA1c= 2.9% (Bayer DCA 2000, Immunoassay)
- ✓ HbA1c= 4.2 (Primus, Affinity C.)

**Which one is correct?**

Case 2: A 20 y/o male patient

FBS= 165 mg/dL

HbA1c level= 18.2%

HbA0 level= 81.5%

Method: Ion-exchange HPLC (Agilent 1200 HPLC system)

**Should the HbA1c result be released?**



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## Learning Objectives

- ☐ Clinical importance of HbA1c measurement
- ☐ Biochemistry of HbA1c
- ☐ NGSP Standardization Program (1996)
- ☐ HbA1c Methodologies
- ☐ Interferences in HbA1c measurement
- ☐ Alternative measures of glycaemia
- ☐ How to choose a proper method
- ☐ Cases



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## Learning Objectives

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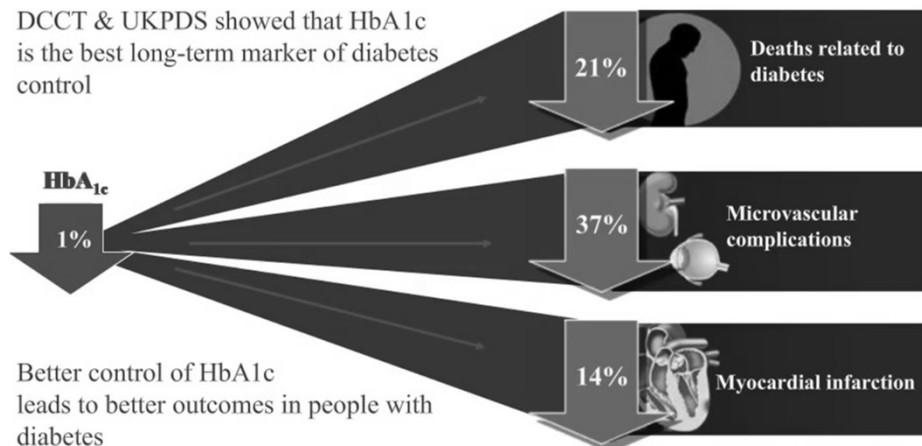
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## Why is HbA1c so Important?

DCCT & UKPDS showed that HbA1c is the best long-term marker of diabetes control



Stratton IM, et al. *BMJ* 2000; 321:405-412.

DCCT=Diabetes Control and Complications Trial

UKPDS=UK Prospective Diabetes Study



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## Diabetes Control Chart

	HbA <sub>1c</sub> test score	MEAN BLOOD GLUCOSE mg/dL	mmol/L
☹️ ↑ action suggested	14.0	380	21.1
	13.0	350	19.3
	12.0	315	17.4
	11.0	280	15.6
	10.0	250	13.7
↓ good ☺️ excellent	9.0	215	11.9
	8.0	180	10.0
	7.0	150	8.2
	6.0	115	6.3
	5.0	80	4.7
	4.0	50	2.6



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## DM Diagnosis based on Lab Tests

**TABLE 35.1** Criteria for Diagnosis of Prediabetes and Diabetes

Measurement	Units	Diagnostic of Prediabetes	Diagnostic of Diabetes <sup>a</sup>	Comments
Fasting plasma glucose (FPG)	mg/dL mmol/L	100–125 5.6–6.9	≥126 ≥7	After no calorie intake at least 8 hours
2-hour plasma glucose (2-hour PG)	mg/dL mmol/L	140–199 7.8–11	≥200 ≥11.1	2 hours after 75 g oral glucose
Random plasma glucose	mg/dL mmol/L	Not applicable	≥200 ≥11.1	Without oral glucose but with classic symptoms
HbA <sub>1c</sub>	% mmol/mol	5.7–6.4 39–47	≥6.5 ≥48	Measured in certified laboratory

<sup>a</sup>In the absence of unequivocal hyperglycemia, results should be confirmed by repeat testing.

Data from American Diabetes Association. Classification and diagnosis of diabetes. Standards of medical care in diabetes, 2018. *Diabetes Care*. 2018;41:S13–S27.

**HbA<sub>1c</sub> Recommended for Diagnosis by ADA in addition to Monitoring (2011)**



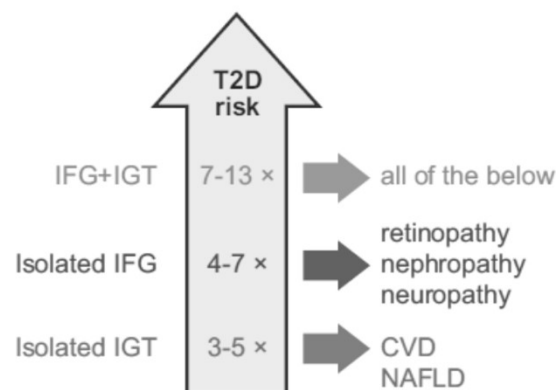
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### B Risk of developing diabetes and comorbidities by type of prediabetes



MAGKOS ET AL., 2023, *Physiol Rev* 103: 7–30



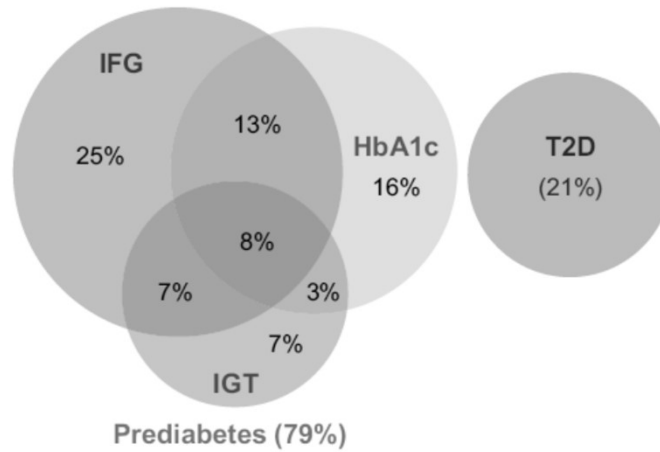
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**C** Prevalence of different types of hyperglycemia among people with hyperglycemia



MAGKOS ET AL., 2023, Physiol Rev 103: 7–30



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**D** Effect of obesity on prevalence of different types of prediabetes by OGTT



MAGKOS ET AL., 2023, Physiol Rev 103: 7–30



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	Advantages	Disadvantages
<b>Fasting and/or post challenge glucose measures</b>	<ul style="list-style-type: none"> <li>Established as the current means of diagnosing diabetes</li> <li>Directly measures the molecule thought to cause diabetes complications</li> <li>Not subject to misleading results due to non-glycaemic factors</li> <li>Smaller differences in results between laboratories compared to HbA<sub>1c</sub></li> <li>Less expensive to measure than HbA<sub>1c</sub></li> </ul>	<ul style="list-style-type: none"> <li>Requires patient to be tested in the fasting state and for the sample to be analysed promptly</li> <li>May require a glucose tolerance test for diagnosis</li> <li>A measurement of glucose at a single time-point</li> <li>Higher within-individual variability than that of HbA<sub>1c</sub></li> <li>Oral glucose tolerance testing laborious and time consuming</li> </ul>
<b>HbA<sub>1c</sub></b>	<ul style="list-style-type: none"> <li>Established as a means of monitoring patients already known to have diabetes</li> <li>Does not require a fasting sample and is more stable after sample collection than glucose</li> <li>A marker of glucose control over the previous weeks/months</li> <li>Lower within-individual variability than that of glucose</li> <li>Although more costly than glucose, overall cost as part of a screening/diagnostic pathway may not be</li> </ul>	<ul style="list-style-type: none"> <li>Measurement can be misleading in patients with haemoglobinopathies, anaemia or renal failure</li> <li>May differ between patients of different ages and ethnicity</li> <li>Larger differences in results between laboratories compared to glucose</li> <li>A surrogate marker of hyperglycaemia with between-individual discrepancies between glucose and HbA<sub>1c</sub></li> </ul>

ABCD position statement on haemoglobin A1c for the diagnosis of diabetes  
E. Kilpatrick, P. Winocour  
Published 1 September 2010  
Medicine Practical Diabetes International  
DOI:10.1002/PDI.1508



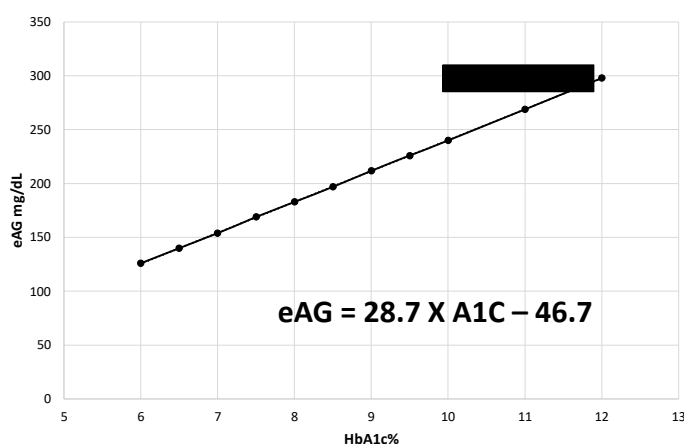
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## Estimated Average Glucose (eAG)

HbA <sub>1c</sub> %	eAG mg/dL
6	126
6.5	140
7	154
7.5	169
8	183
8.5	197
9	212
9.5	226
10	240
11	269
12	298



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## eAG/A1C Conversion Calculator

ADA is recommending the use of a new term in diabetes management, estimated average glucose, or eAG. Health care providers can now report A1C results to patients using the same units (mg/dl or mmol/l) that patients see routinely in blood glucose measurements. The calculator and information below describe the ADAG Study that defined the relationship between A1C and eAG and how eAG can be used to help improve the discussion of glucose control with patients.

**Choose source:**
☒ A1C to eAG  
☐ eAG to A1C  
Unit to calculate from

**To:**
☒ mg/dl  
☐ mmol/l  
Unit to calculate to

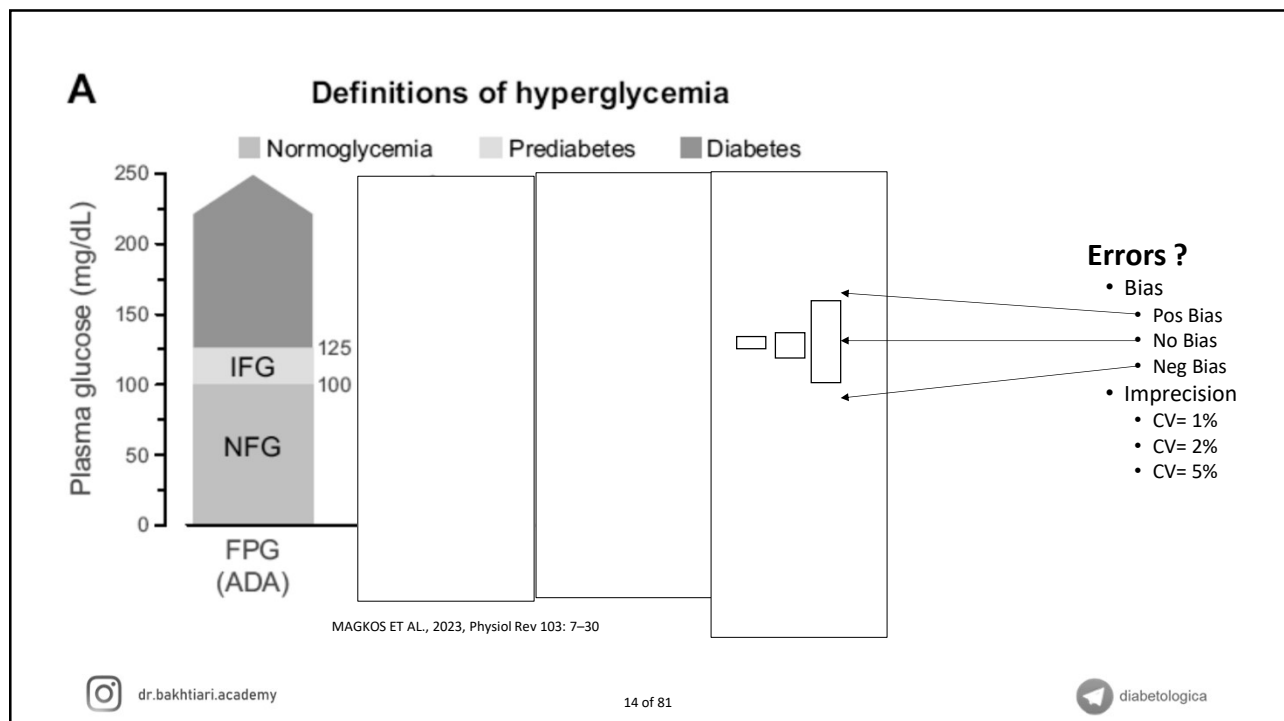
Source value \*

Calculate

Results

The relationship between A1C and eAG is described by the formula  $28.7 \times A1C - 46.7 = eAG$ .

A1C		eAG	
%	mg/dl	mmol/l	logica



## Learning Objectives

- ✓ Clinical importance of HbA1c measurement
- ❑ **Biochemistry of HbA1c**
- ❑ NGSP Standardization Program (1996)
- ❑ HbA1c Methodologies
- ❑ Interferences in HbA1c measurement
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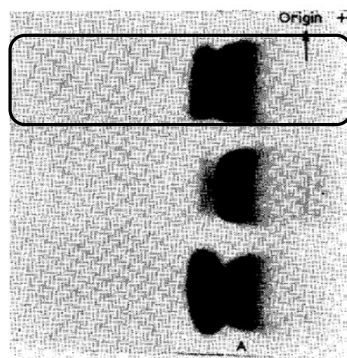


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## History of HbA1c

### An abnormal hemoglobin in red cells of diabetics.

Samuel Rahbar in Clinica Chimica Acta vol. 22(2) pp. 296-298 (1968)



Agar gel electrophoresis, pH 6.2.

a: Hb A+x;

b: normal;

c: Hb A+Hb F.



<https://archive.ph/20130414171216/http://www.diabetes.org/about-us/national-achievement-awards-2012/national-science-and-health/rahbar-outstanding-discovery-2012.html>



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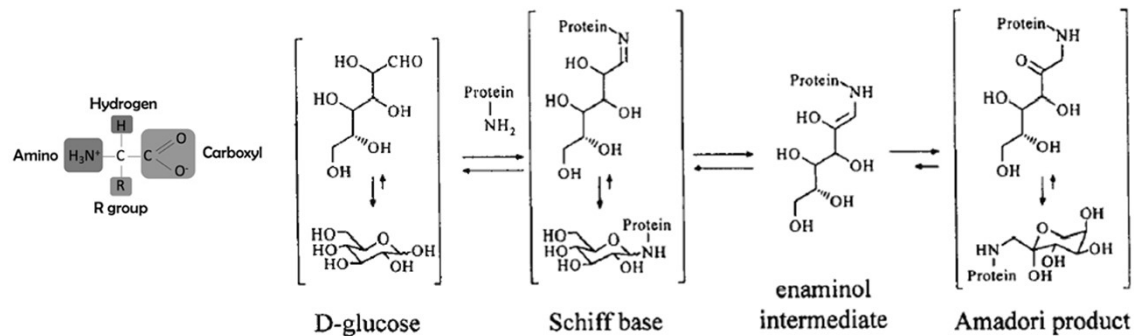
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# Protein Glycation



### Assumptions for use of HbA1c as a Monitor of Glycemia:

- ✓ Hb is present at a constant concentration
- ✓ RBC life span is constant
- ✓ Micro-Environment is constant

Glucose is the only Variable



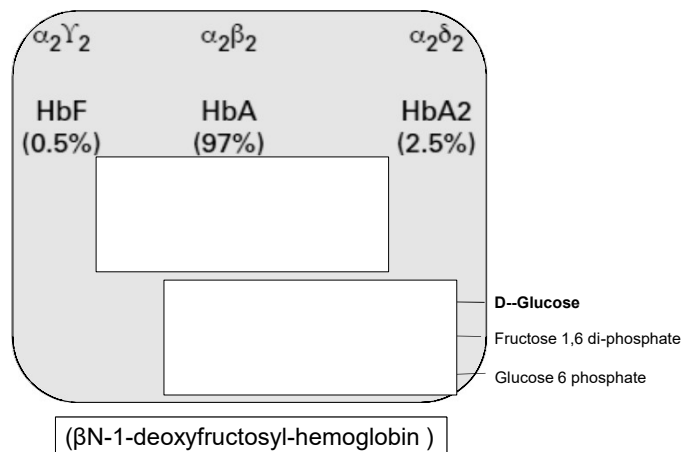
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## Hb Variety in Adults



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## Learning Objectives

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## HbA1c Standardization

### What: Equal Results

- ✓ Any Lab
- ✓ Anywhere in the World
- ✓ Irrespective of Technique or Manufacturer

### Achieved: Uniform Interpretation

- ✓ Clinical Guidelines
- ✓ Clinical Decision Limits
- ✓ Literature

### Benefits: Reliability

- ✓ Patient Safety
- ✓ ....

HbA <sub>1c</sub> test score	MEAN BLOOD GLUCOSE mg/dL	mmol/L
14.0	380	21.1
13.0	350	19.3
12.0	315	17.4
11.0	280	15.6
10.0	250	13.7
9.0	215	11.9
8.0	180	10.0
7.0	150	8.2
6.0	115	6.3
5.0	80	4.7
4.0	50	2.6



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Press F11 to exit full screen

Search NGSP



## Harmonizing Hemoglobin A<sub>1c</sub> Testing

*A better A1C test means better diabetes care*

<https://ngsp.org/>

Home News About the NGSP More About HbA<sub>1c</sub> Obtaining Certification Certified Methods and Laboratories CAP GH5 Data Enter Monitoring Data Links Contact Us

### Welcome to the NGSP Web Site

The purpose of the NGSP is to standardize Hemoglobin A<sub>1c</sub> test results to those of the Diabetes Control and Complications Trial (DCCT) and United Kingdom Prospective Diabetes Study (UKPDS) which established the direct relationships between HbA<sub>1c</sub> levels and outcome risks in patients with diabetes.

Download Certification Packets

The Relationship Between HbA<sub>1c</sub> and Estimated Average Glucose (eAG)

More about the DCCT | More about the UKPDS

### Convert between NGSP, IFCC and eAG

We have added a tool for converting between NGSP(%), IFCC (mmol/mol)

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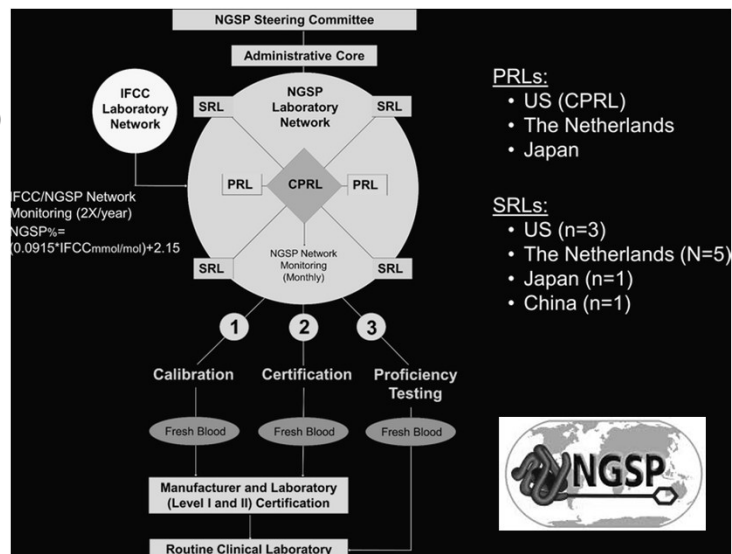
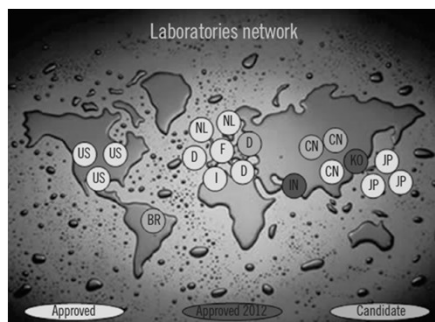
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## HbA<sub>1c</sub> Standardization

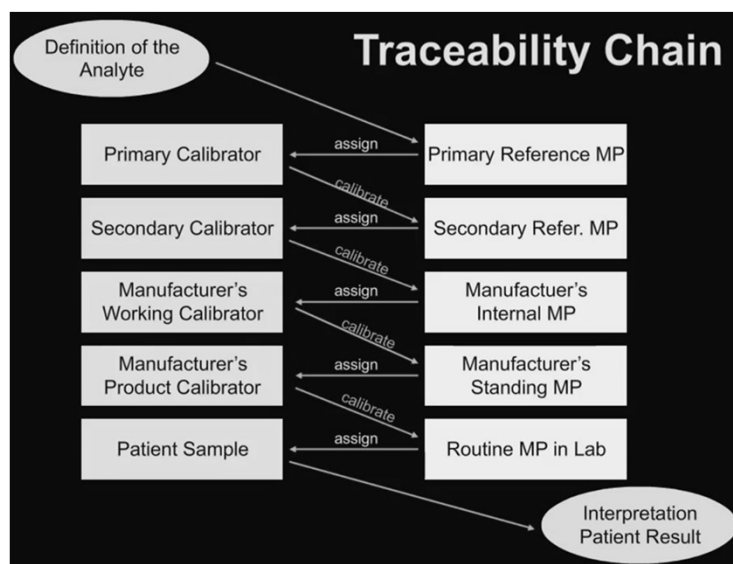
### NGSP Laboratory Network

- I. Administrative Core (NETCORE)
- II. Central Primary Reference Laboratory (CPRL)
- III. 2 Primary Reference Laboratories (PRL)
- IV. 10 Secondary Reference Laboratories (SRLs)





## HbA1c Standardization



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- ✓ The CPRL analyzes HbA1c by HPLC using Bio-Rex 70 resin following the existing CPRL method protocol.
- ✓ and sets the initial calibration for the standardization program based on the "set-point" used in the DCCT.
- ✓ The CPRL must document an accepted level of precision (total imprecision <3%) following NCCLS EP5-A2 guidelines.
- ✓ Back-up PRLs and SRLs calibrate their assays so that results from fresh blood specimens agree with the CPRL.
- ✓ The CPRL administers a monitoring program for all certified PRLs, SRLs, and Level 1 Laboratories.

## HbA1c Standardization

Milan 2007



### Consensus Statement on the Worldwide Standardization of the HbA1C Measurement

1. HbA1c test results should be standardized worldwide, incl. the ref. system and results reporting.
2. The IFCC reference system for HbA1c represents the only valid anchor to implement standardization of the measurement.
3. HbA1c results are to be reported by clinical labs worldwide in SI units (mmol/mol, no decimals) and derived NGSP units (% one decimal), using the IFCC-NGSP master equation (DCCT units).
4. HbA1c also reported as eAG.
5. The reportable term for glycated hemoglobin is HbA1c, although other abbreviations may be used in guidelines and educational material (A1C).



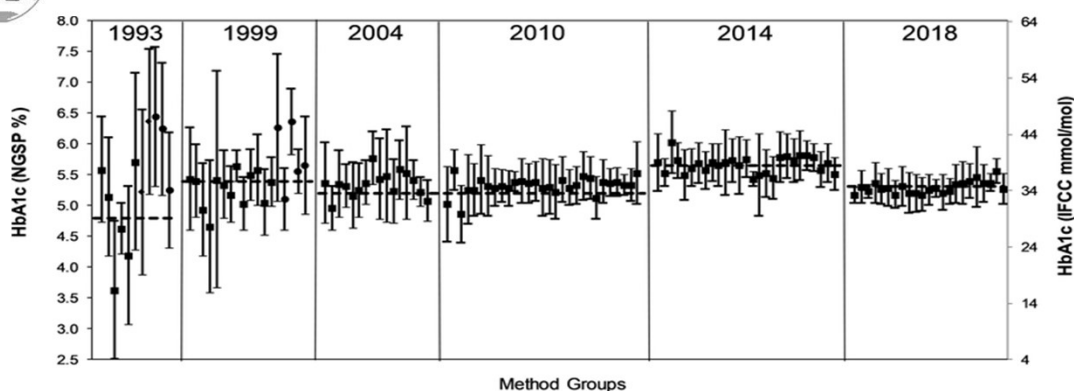
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## Over 20 Years of Improving Hemoglobin A1c Measurement



Clinical Chemistry 65:7 (2019)



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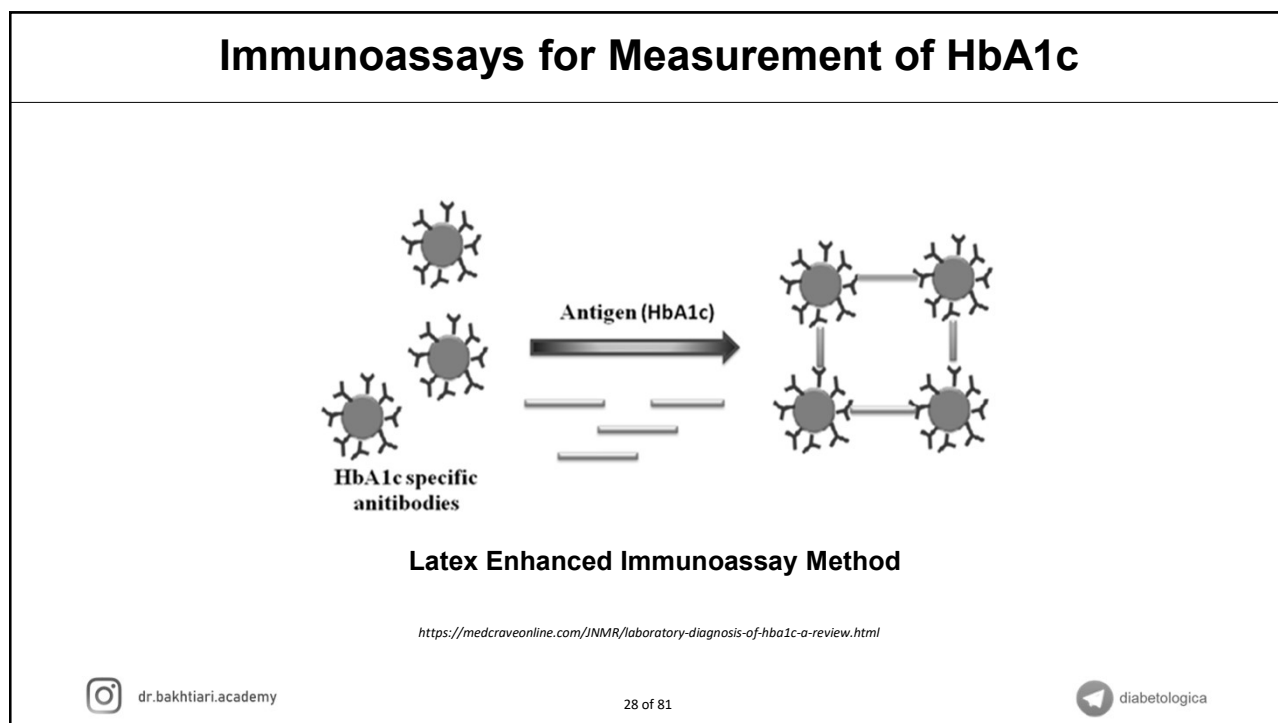
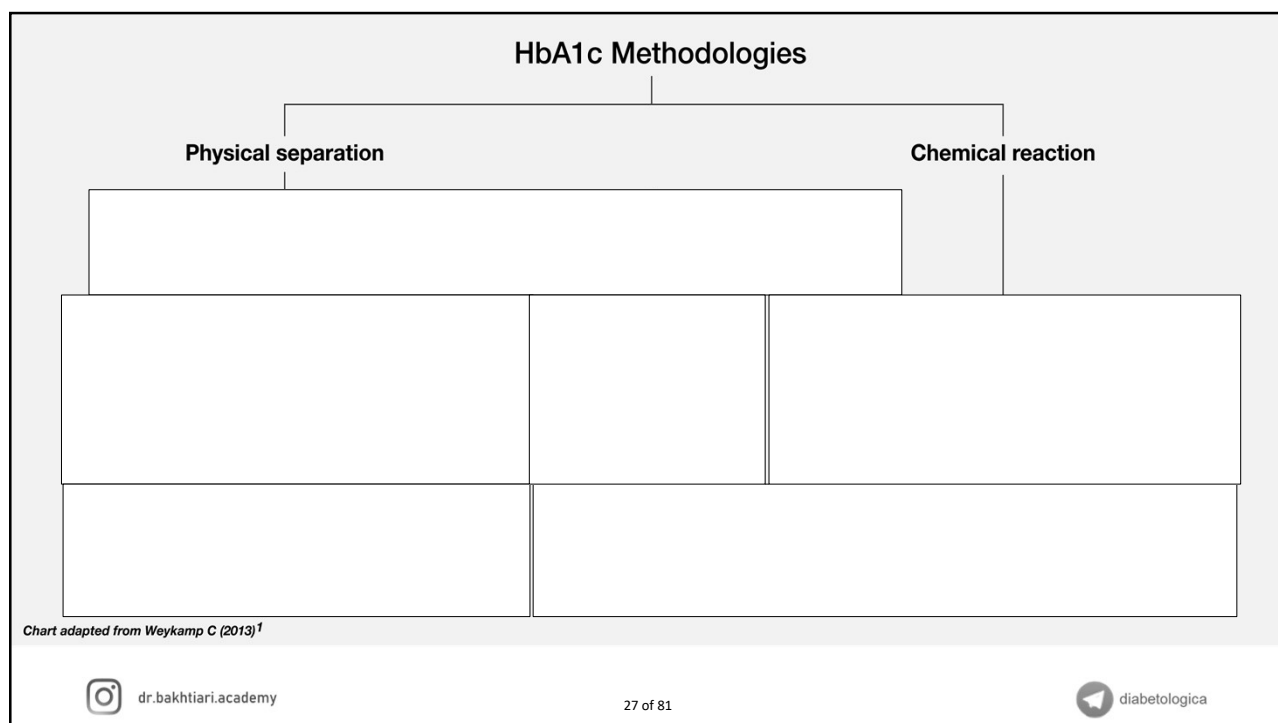


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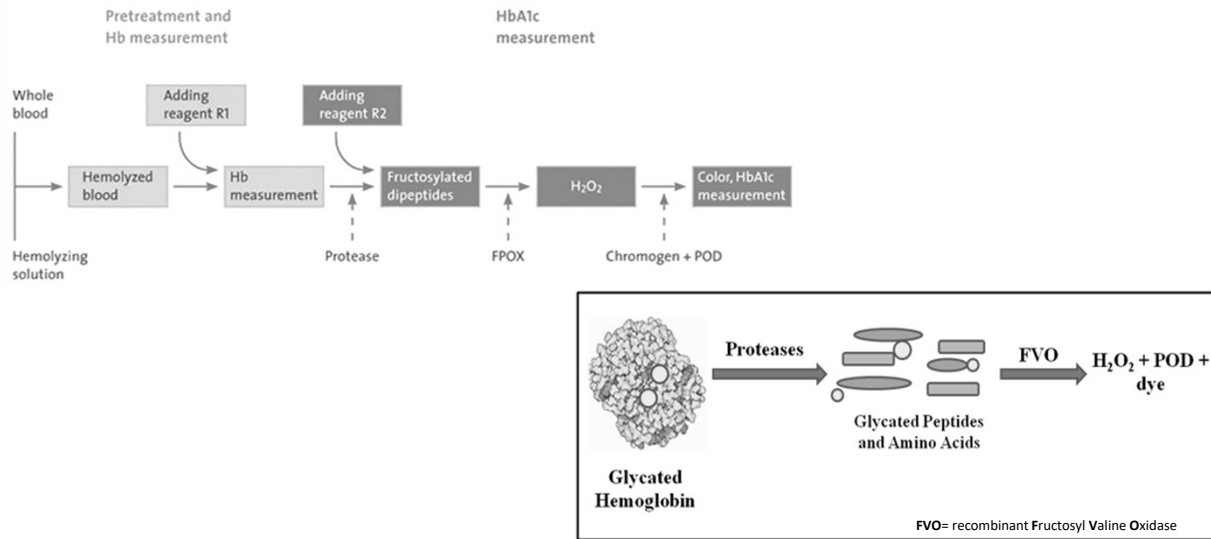
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## Enzymatic Measurement of HbA1c



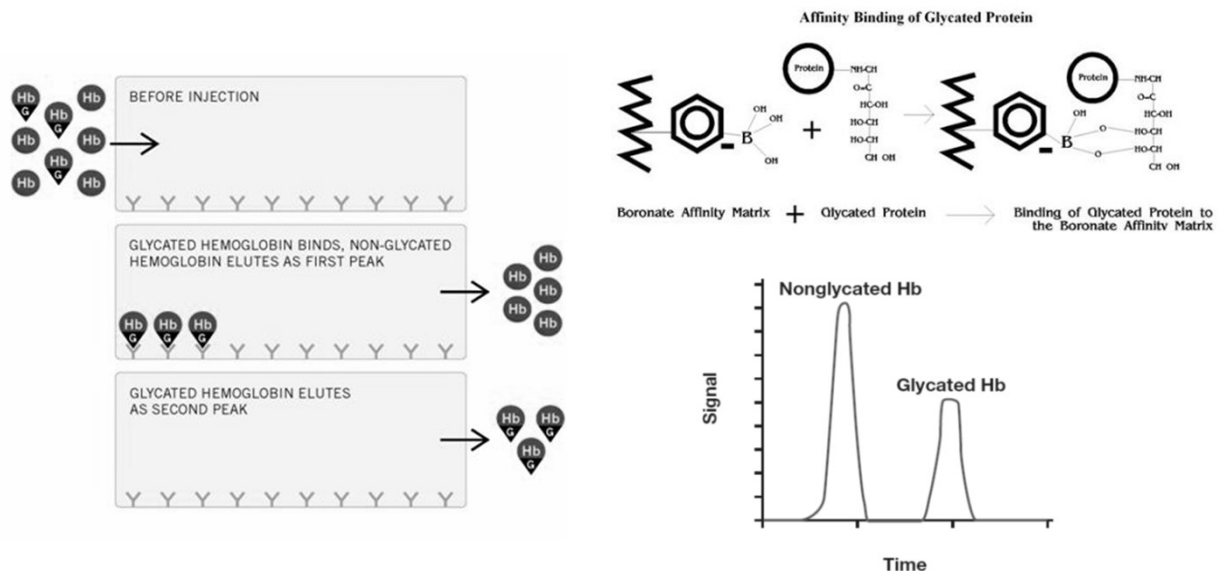
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## Boronate Affinity Chromatography for Measuring HbA1c



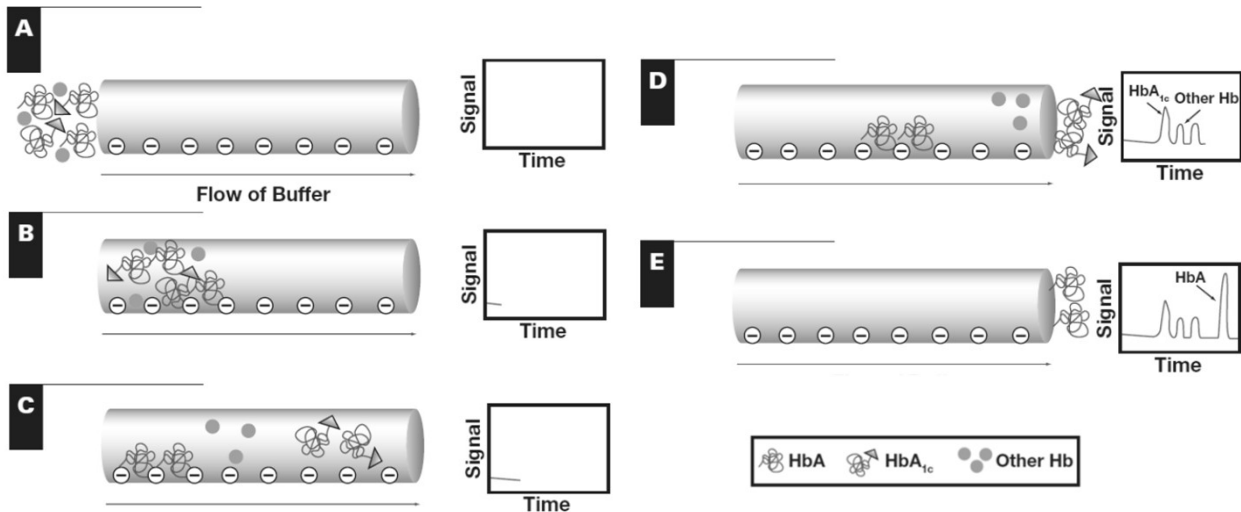
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### Ion-exchange High-Performance Liquid Chromatography (HPLC) for HbA1c measurement



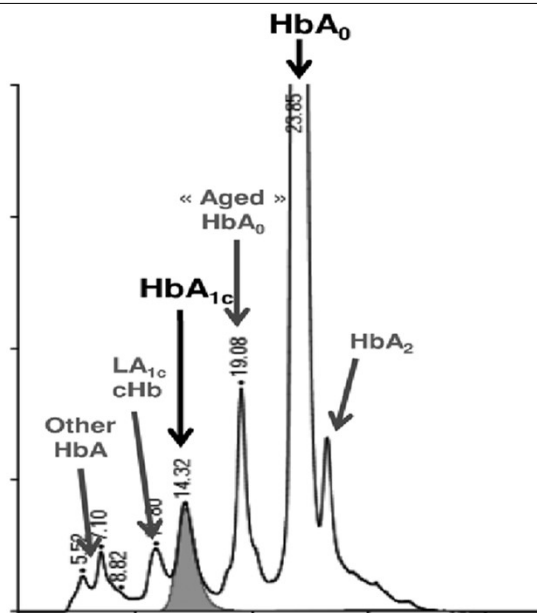
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### HPLC system for HbA1c



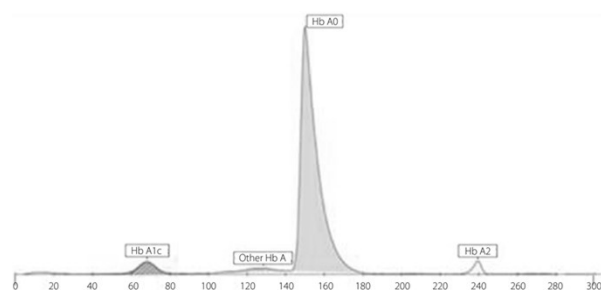
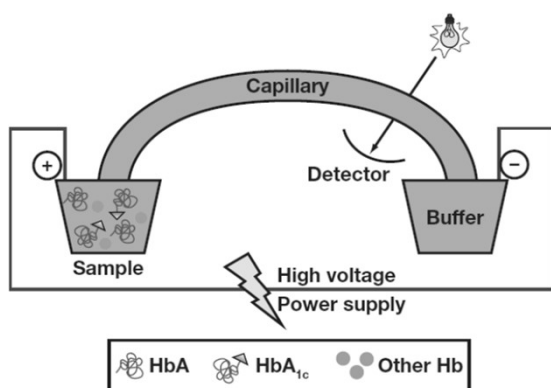
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## Capillary Electrophoresis (CE) for Measuring HbA1c



A1c Haemoglobin electrophoresis

Fractions	%	mmol/mol	Cal. %
Hb A1c	5.1	36	5.4
Other Hb A	2.4		
Hb A0	90.3		
Hb A2	2.5		



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## HbA1c Biosensors

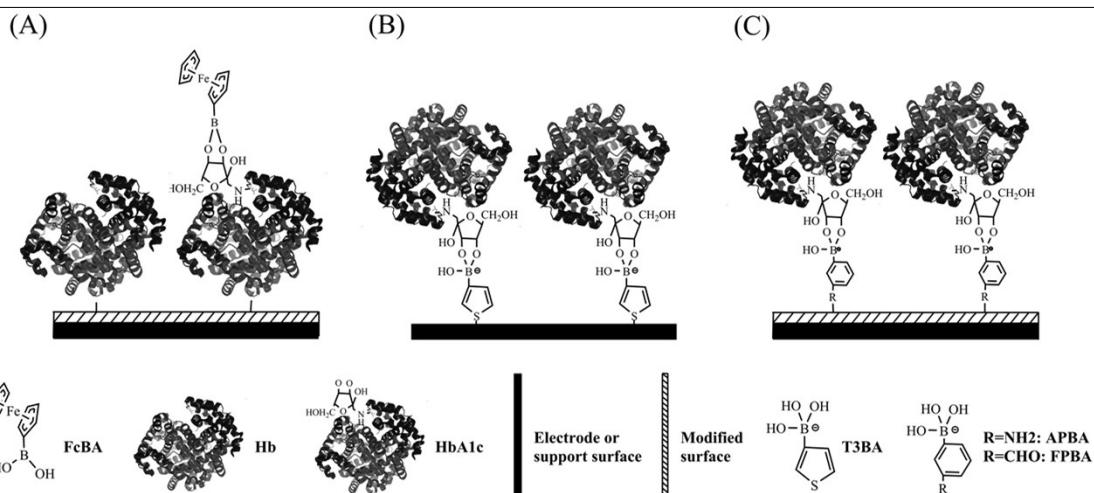


Figure 1. Schematic diagrams of HbA1c biosensors by using (A) FcBA; (B) T3BA; and (C) APBA or FPBA for HbA1c recognition. <https://doi.org/10.3390/s17081798>



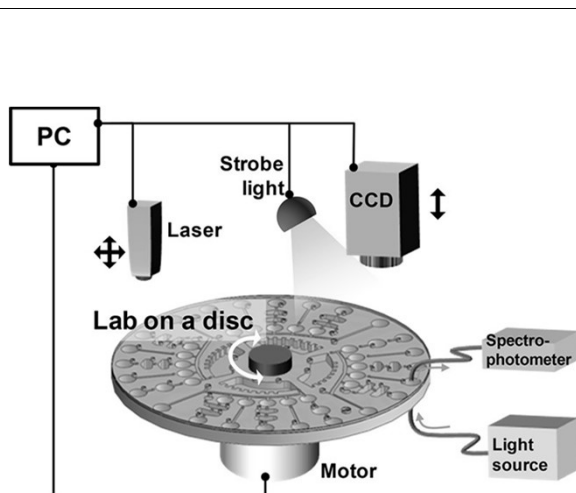
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# HbA1c Lab-on-a-Disk

ScienceDirect<sup>®</sup><https://doi.org/10.1016/j.talanta.2018.07.081>

Talanta

Volume 190, 1 December 2018, Pages 134–139



## Design and fabrication of a centrifugal microfluidic disc including septum valve for measuring hemoglobin A1c in human whole blood using immunoturbidimetry method

E. Mahmodi Arimand<sup>a</sup>, M. Saadatmand<sup>a</sup>, M.R. Bakhtiari<sup>b</sup>, M. Eghbal<sup>c</sup>

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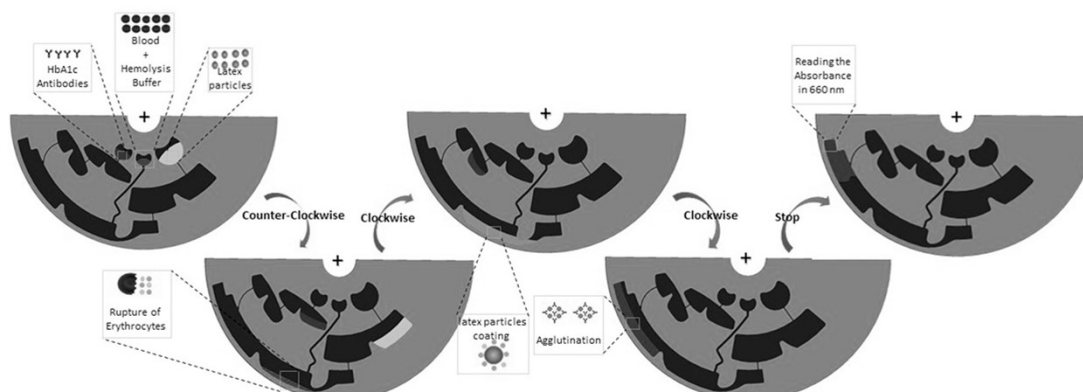
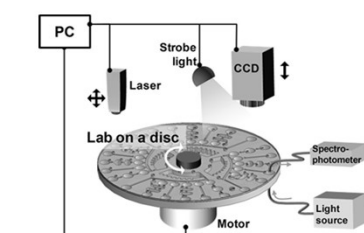


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### Abstract

Diabetes mellitus is a global endemic with a rapidly increasing prevalence in both



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## Commercial Methods Recommended for HbA1c

ASSAYS	ANALYTICAL WEAKNESS
<ul style="list-style-type: none"> <li>• Immunoassays <input type="text"/></li> <li>• Enzymatic assays <input type="text"/></li> <li>• Capillary electrophoresis</li> <li>• Ion-exchange chromatography</li> <li>• Boronate affinity chromatography</li> </ul>	<p>Interference by rare Hb variants</p> <p>No analytical interference by Hb variants</p> <p>No analytical interference by Hb variants</p> <p>Interference by all Hb variants including carbamylated Hb</p> <p>Measures total glycated hemoglobin, not just HbA1c</p>



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## Learning Objectives

- ✓ Clinical importance of HbA1c measurement
- ✓ Biochemistry of HbA1c
- ✓ NGSP Standardization Program (1996)
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- ☐ **Interferences in HbA1c measurement**
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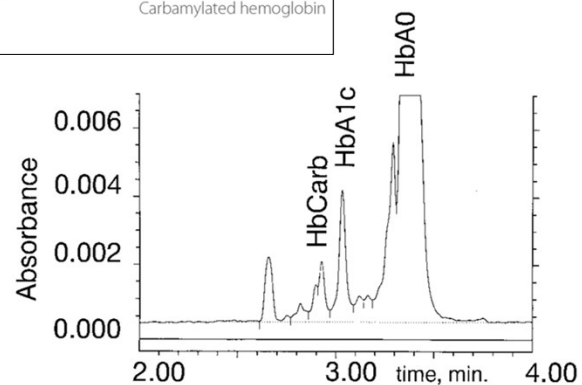
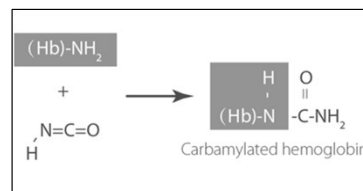
## Interferences in HbA1c measurement (Interpretation Challenging Factors)

1. Renal Disease (Uremia)
2. Anemias & RBC Turnover Changes
3. Hypertriglyceridemia
4. Drugs
5. Hb Variants (Hemoglobinopathies)

## Carbamylated Hemoglobin

### 1. Renal Disease (Uremia)

2. Anemias & RBC Turnover Changes
3. Hypertriglyceridemia
4. Drugs
5. Hb Variants (Hemoglobinopathies)



## False Variations of HbA1c (Confounding factors)

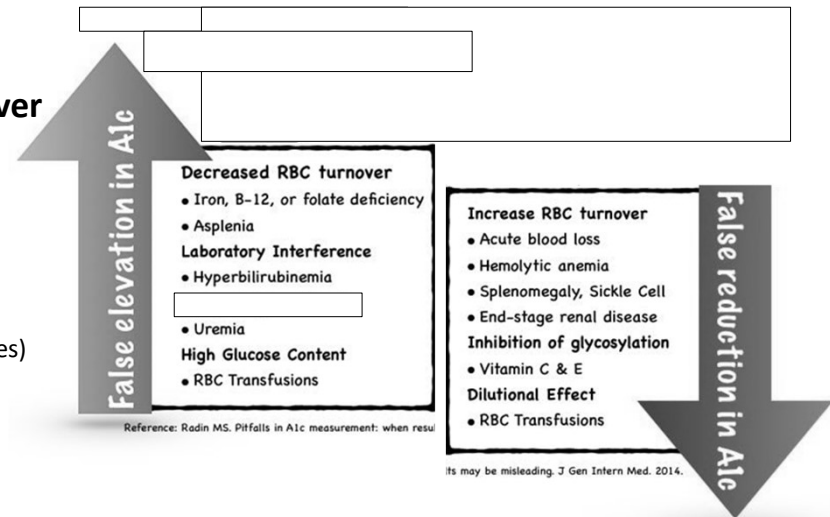
1. Renal Disease (Uremia)

**2. Anemias & RBC Turnover Changes**

3. Hypertriglyceridemia

4. Drugs

5. Hb Variants (Hemoglobinopathies)



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## False Variations of HbA1c (Confounding factors)

1. Renal Disease (Uremia)

**2. Anemias & RBC Turnover Changes**

3. Hypertriglyceridemia

4. Drugs

5. Hb Variants (Hemoglobinopathies)

**Table 1: Conditions causing inappropriately high or low HbA1c<sup>[10]</sup>**

Inappropriately Low HbA1c	Inappropriately High HbA1c	Variable Effect on HbA1c+
<ul style="list-style-type: none"> <li>• Hemolysis</li> <li>• Certain hemoglobinopathies</li> <li>• Recent blood transfusion</li> <li>• Acute blood loss</li> <li>• Hypertriglyceridemia</li> <li>• Drugs*</li> <li>• Chronic liver disease</li> </ul>	<ul style="list-style-type: none"> <li>• Iron deficiency</li> <li>• Vitamin B12 deficiency</li> <li>• Alcoholism</li> <li>• Uremia</li> <li>• Hyperbilirubinemia</li> <li>• Drugs*</li> </ul>	<ul style="list-style-type: none"> <li>• Fetal hemoglobin</li> <li>• Methemoglobin</li> <li>• Certain hemoglobinopathies</li> </ul>

\*Refer text and Table 2 + method-dependent

DOI:10.4103/2230-8210.98004



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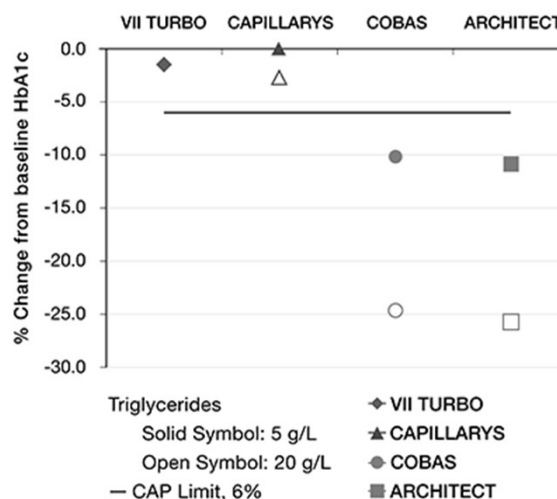
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## Investigation of Triglyceride Interference in HbA1c assays

1. Renal Disease (Uremia)
2. Anemias & RBC Turnover Changes
- 3. Hypertriglyceridemia**
4. Drugs
5. Hb Variants (Hemoglobinopathies)



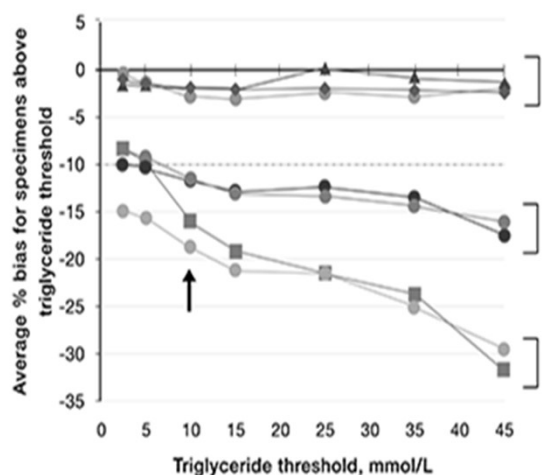
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## Investigation of Triglyceride Interference in HbA1c assays



Bio-Rad D-100,  
 Sebia CAPILLARYS  
 Siemens Dimension Vista®

HbA1c results did not show significant bias up to 65 mmol/L (57 g/L) triglycerides for 100%, 95% and 81% of specimens, respectively.

Beckman Coulter AU™  
 Roche COBAS®

HbA1c results showed significant bias above 10 mmol/L triglycerides (-12% for both), although these methods showed more random variation and/or a negative bias at low levels of triglycerides.

Ortho VITROS®  
 Abbott ARCHITECT™

HbA1c values were significantly depressed above a 10 mmol/L triglyceride threshold (-16% and -19% average bias, respectively), with bias increasing in proportion to the degree of lipemia.



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## False Variations of HbA1c (Confounding factors)

1. Renal Disease (Uremia)
2. Anemias & RBC Turnover Changes
3. Hypertriglyceridemia

### 4. Drugs

5. Hb Variants (Hemoglobinopathies)

**Table 2: Drugs causing inappropriately low or high HbA1c**

Postulated Mechanism	Falsely Low HbA1c	Falsely High HbA1c
Increased erythrocyte destruction	Dapsone <sup>[11-16]</sup> Ribavirin <sup>[17]</sup> Antiretrovirals <sup>[18]</sup> Trimethoprim-Sulfamethoxazole <sup>[14]</sup>	
Altered hemoglobin	Hydroxyurea <sup>[19]</sup>	
Altered glycation	Vitamin C <sup>[10]</sup> Vitamin E <sup>[10]</sup> Aspirin (small doses) <sup>[10]</sup>	
Interference with assays		Aspirin (large doses) <sup>[20]</sup> Chronic opiate use <sup>[21]</sup>



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DOI:10.4103/2230-8210.98004

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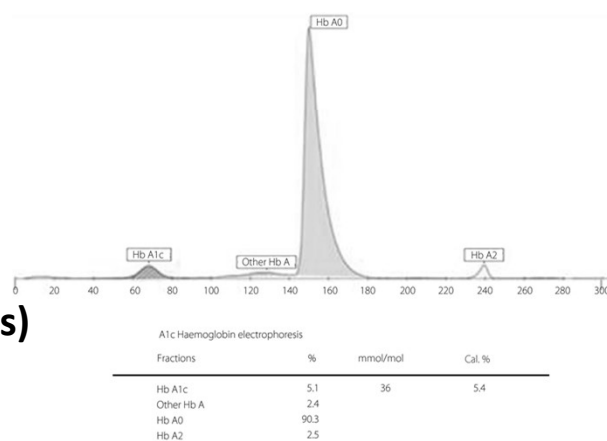
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## Interferences in HbA1c measurement (Interpretation Challenging Factors)

1. Renal Disease (Uremia)
2. Anemias & RBC Turnover Changes
3. Hypertriglyceridemia
4. Drugs

### 5. Hb Variants (Hemoglobinopathies)

A2, F, C, D, E, S



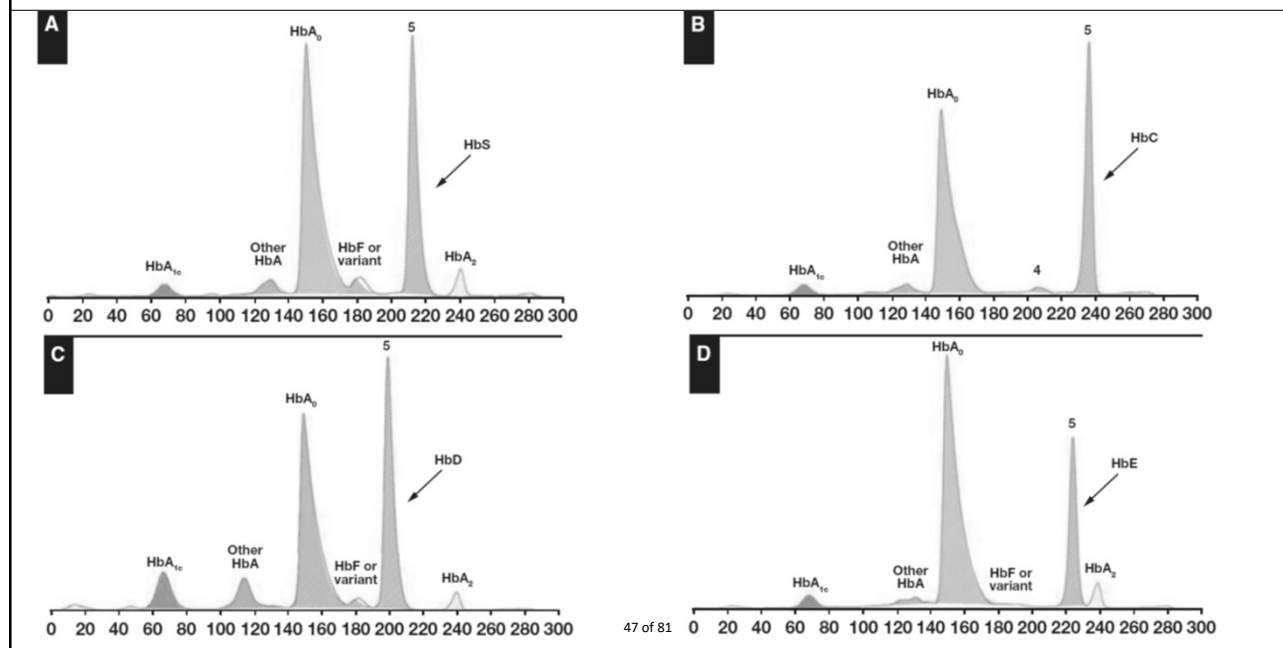
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### Capillary Electrophoresis system for HbA1c



### How are Hb variants detected?

1. Patients may already be known to have variants
2. HbA1c results discordant from prevailing blood Glucose
3. Abnormal Chromatograms
  - ✓ More than 700 known Hb Variants
  - ✓ Many clinically silent
    - ✓ Variable interference with HbA1c



## How Hb Variants can affect HbA1c?

### 1. Analytical Interference (Method Dependent)

- ✓ Does the result match the prevailing Glucose?
- ✓ At first test by an alternative method (Different Principle)
- ✓ Check Hb, Indices, Blood Film
- ✓ Hb Electrophoresis
- ✓ Mass Spectrometry
- ✓ DNA sequencing

### 2. Altered RBC Turnover

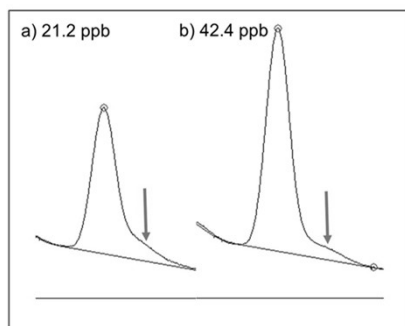
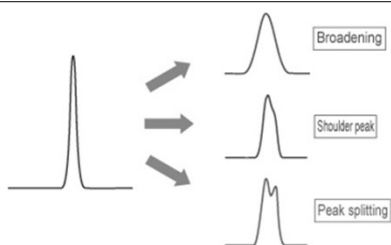
Alere Afinion	No	No	No	No	\$
Arkay ADAMS A1c HA-8180V (Menarini)	No	No	HbA1c not quantified (no for ver. EU 1.41)	HbA1c not quantified (no for ver. EU 1.41)	No <30%
Beckman HbA1c Advanced B00389 Manual Application on DxC 700 AU AU system	No	No	No	No	\$
Beckman HbA1c Advanced B93009 Online Application on DxC 700 AU	No	No	No	No	\$
Beckman Synchron System Unicel DxC	No	No	No	No	\$
Bio-Rad D-100 (A1c program)	No	No	No	No	-
Bio-Rad Variant II Turbo 2.0	No	No	No	No	No <25% HbF
Ortho-Clinical Vitros	No	No	No	No	\$
Roche Cobas c513	No	No	No	No	\$
Sebia Capillarys 2 Flex Piercing	No	No	No	No	No <15% HbF
Siemens DCA Vantage	No	Yes/ No*	Yes/ No*	No	No <10% HbF
Siemens Atellica	No	No	No	No	\$
Siemens Dimension	No	No	No	No	\$
Tosoh G8 ver. 5.24, 5.28	No	No	No	No	No <30% HbF
Trinity HPLC	No	No	No	No	No <15% HbF



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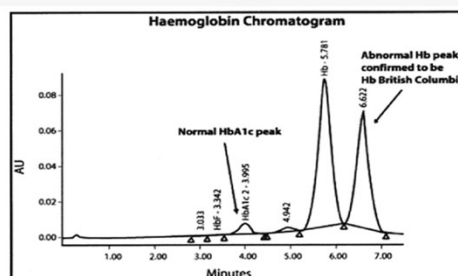
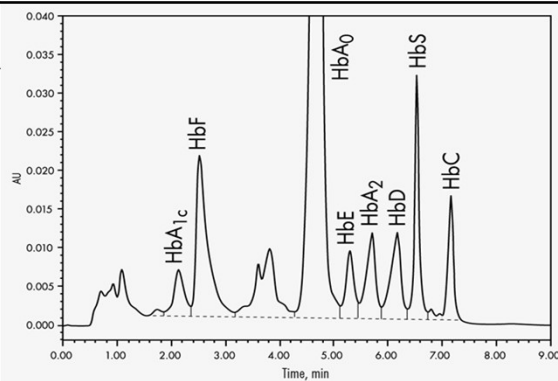
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## Analytical Interference



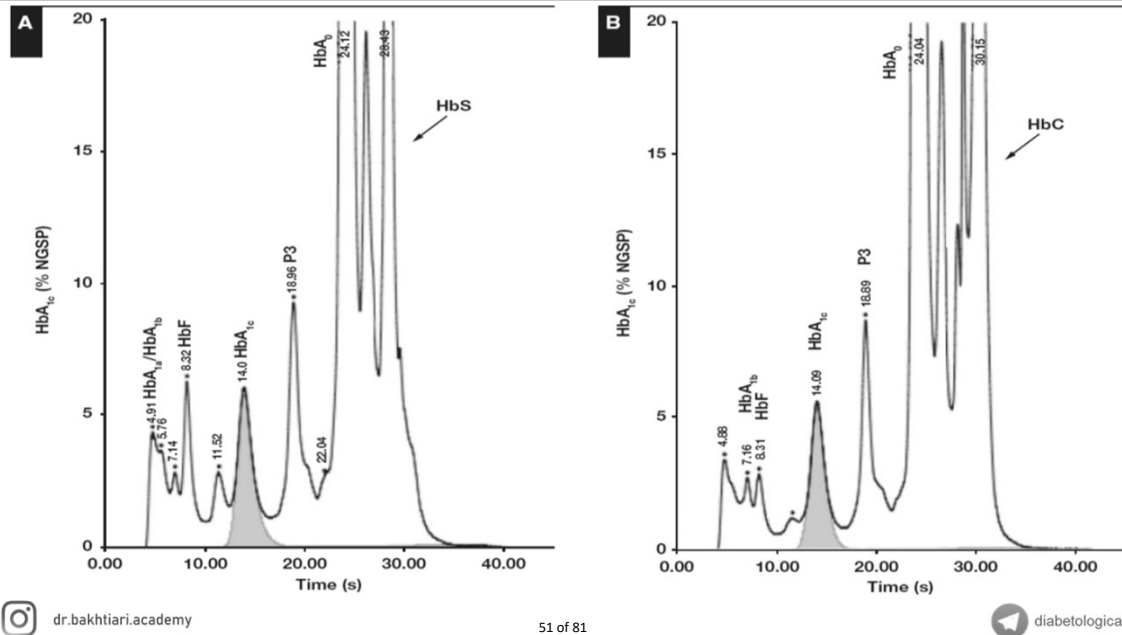
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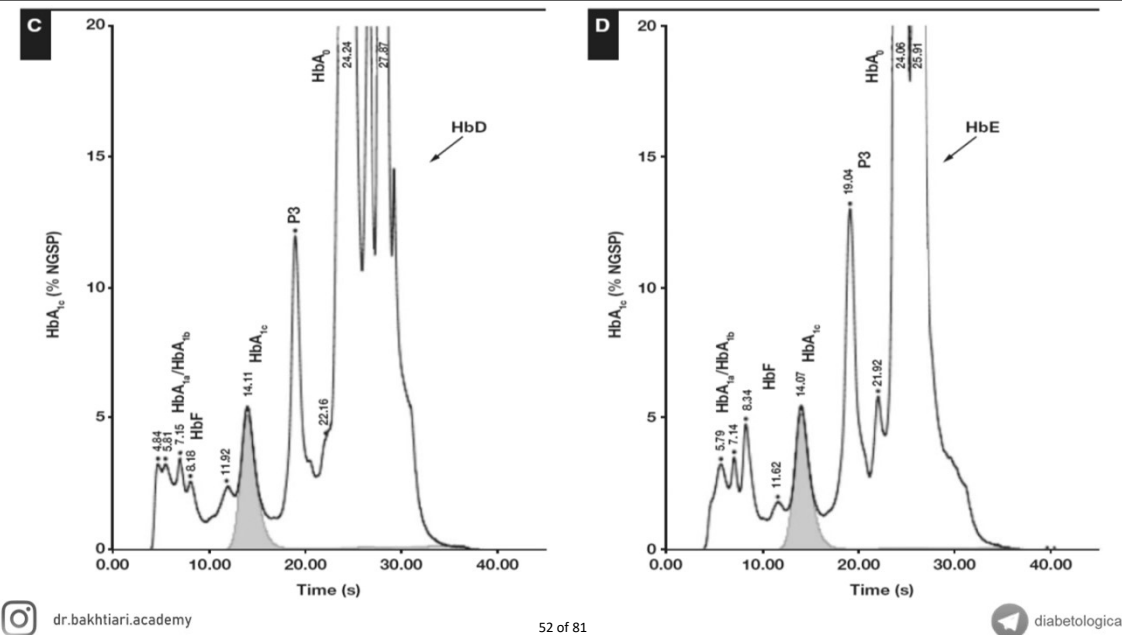


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### HbA1c chromatograms on the Bio-Rad D-100 for some Hb variants



### HbA1c chromatograms on the Bio-Rad D-100 for some Hb variants



# Effects of Hemoglobin Variants and Chemically Modified Derivatives on Assays for Glycohemoglobin

LYNN BRY, PHILIP C. CHEN, and DAVID B. SACKS\*

Department of Pathology, Brigham & Women's Hospital, Harvard Medical School, 75 Francis St., Boston, MA 02115.

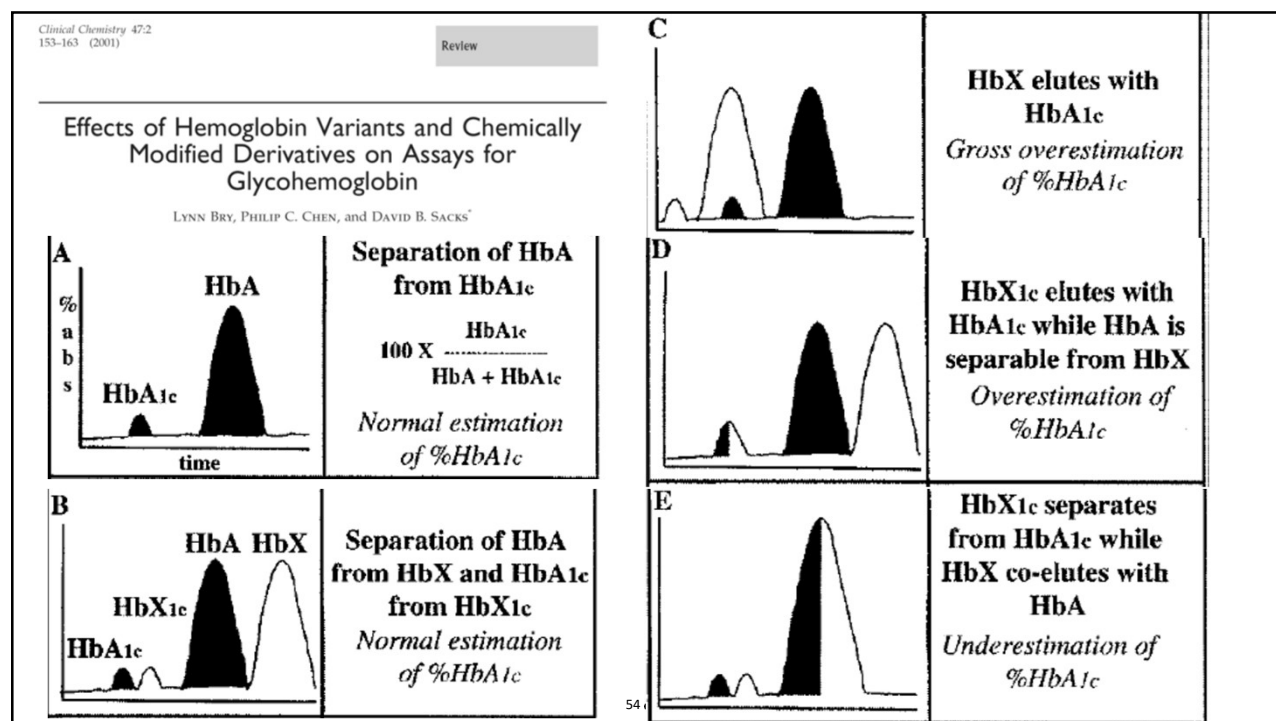


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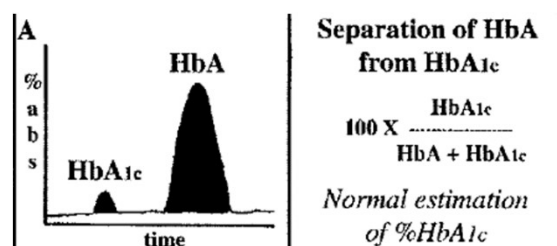
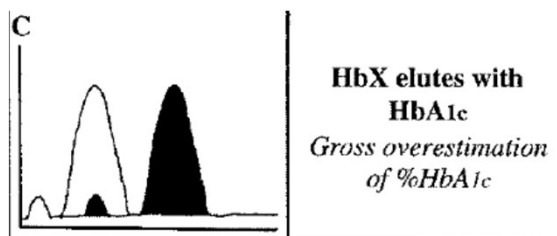


## A Case Study: How variants can affect HbA1c?

A 44 y/o female screened for diabetes:

1. HbA1c= 46% (Biorad, HPLC)
2. HbA1c= 2.9% (Bayer DCA 2000, Immunoassay)
3. HbA1c= 4.2 (Primus, Affinity C.)

Which one is correct?



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Hb H Interference on Measurement Of HbA1c With Ion-Exchange HPLC

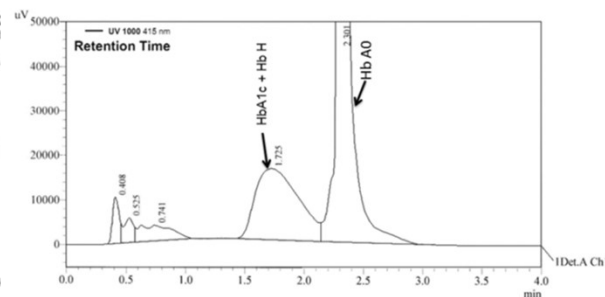
ACTA INFORM MED. 2013 Sep; 21(3): 216-218

doi: 10.5455/aim.2013;  
Received: 05 May 2013 • Accepted

## Hb H Interference on Measurement Of HbA1c With Exchange HPLC

Mehmet Agilli<sup>1</sup>, Halil Yaman<sup>1</sup>, Fevzi Nuri Aydin<sup>1</sup>, Tuncer Cayci<sup>1</sup>, Irfan Sener<sup>1</sup>  
Department of Medical Biochemistry, Gulhane Military Medical Academy, Ankara, Turkey<sup>1</sup>

Correspondence Author: Mehmet Agilli, M.D. Department of Medical Biochemistry, Gulhane Military Medical Academy, 06018 Etli Turkey. mehmetagilli@yahoo.com Phone: +90 505 6829819 Fax: +90 312 3043300



A 20-year-old male patient

FBS= 165 mg/dL

HbA1c level= 18.2%

HbA0 level= 81.5% (ion-exchange HPLC method) (Agilent 1200 HPLC system)

Hemoglobin electrophoresis was performed with Interlab G26 agarose electrophoresis automated compact system.

HbA0= 87.9%, Hb H= 10.8%, Hb A2= 1.3%

CBC= Values were within reference ranges except MCV (79.6 fL) .

It is important to keep in mind that HbA1c might be falsely high with ion-exchange HPLC method because of Hb H containing sample.



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Factors that Interfere with HbA1c Test Results			
		HbA1c Falsely Lowered	HbA1c Falsely Elevated
Biological Factors	Erythropoiesis	<ul style="list-style-type: none"> <li>Increased erythropoiesis:               <ul style="list-style-type: none"> <li>Erythropoietin/Iron/Vitamin B12 Therapy;</li> <li>Reticulocytosis;</li> <li>Chronic Liver Disease</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>Decreased erythropoiesis               <ul style="list-style-type: none"> <li>Fe / Vitamin B12 Def.</li> </ul> </li> <li>Renal Failure</li> </ul>
	Glycation	<ul style="list-style-type: none"> <li>Aspirin (small doses);</li> <li>High doses of vitamin C or vitamin E;</li> <li>Certain hemoglobinopathies<sup>1</sup></li> </ul>	<ul style="list-style-type: none"> <li>CKD</li> <li>Alcoholism</li> </ul>
	Erythrocyte Life Span	<ul style="list-style-type: none"> <li>Shortened erythrocyte life span<sup>1,3,5</sup>:               <ul style="list-style-type: none"> <li>Hemolytic anemia;</li> <li>Splenomegaly;</li> </ul> </li> <li>Rheumatoid arthritis;<sup>1</sup></li> <li>Nephritis;<sup>3</sup></li> <li>Liver Disease<sup>3</sup>;</li> <li>Sepsis<sup>3</sup>;</li> <li>Certain hemoglobinopathies;</li> <li>Paroxysmal nocturnal hemoglobinuria</li> <li>Drugs such as antiretrovirals, ribavirin and dapsone</li> </ul>	<ul style="list-style-type: none"> <li>Increased erythrocyte life span</li> <li>Splenectomy</li> </ul>
Analytical Factors	Assay Method Interference	<ul style="list-style-type: none"> <li>HyperTriglyceridemia</li> <li>Certain hemoglobinopathies</li> </ul>	<ul style="list-style-type: none"> <li>Hyperbilirubinemia</li> <li>Carbamylated Hb</li> <li>Certain hemoglobinopathies</li> <li>Chronic opiate usage</li> </ul>



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## Learning Objectives

- ✓ Clinical importance of HbA1c measurement
- ✓ Biochemistry of HbA1c
- ✓ NGSP Standardization Program (1996)
- ✓ HbA1c Methodologies
- ✓ Interferences in HbA1c measurement
- ☐ **Alternative measures of glycaemia**
- ☐ How to choose a proper method
- ☐ Cases



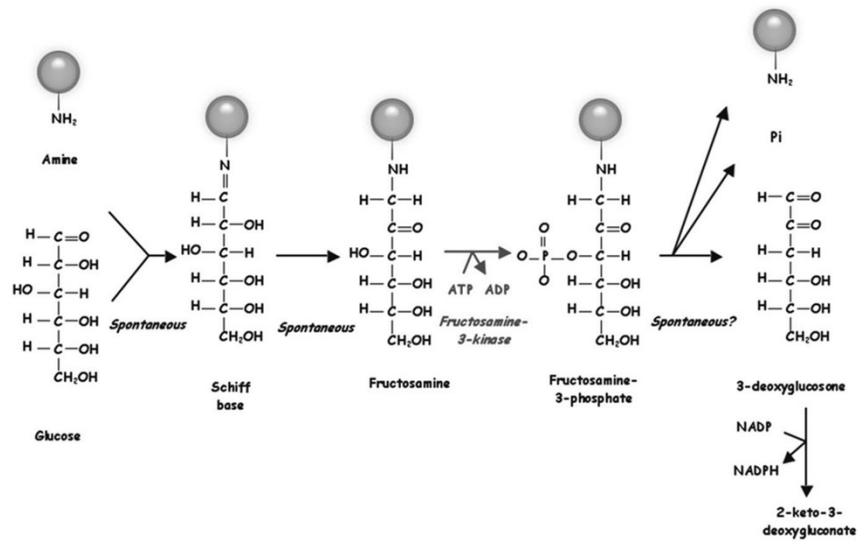
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## Mechanism of Fructosamine formation



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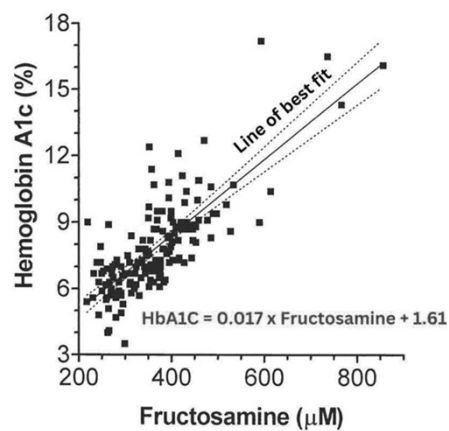
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## Fructosamine HbA1c Relationship

Glucose (mg/dl)	A1c (%)	Fructosamine (μmol)
90	5	212.5
120	6	250
150	7	287.5
180	8	325
210	9	362.5
240	10	400
270	11	437.5
300	12	475
330	13	512.5
360	14	550
390	15	587.5



<https://myendoconsult.com/learn/fructosamine-to-a1c-conversion-calculator/>



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## Learning Objectives

- ✓ Clinical importance of HbA1c measurement
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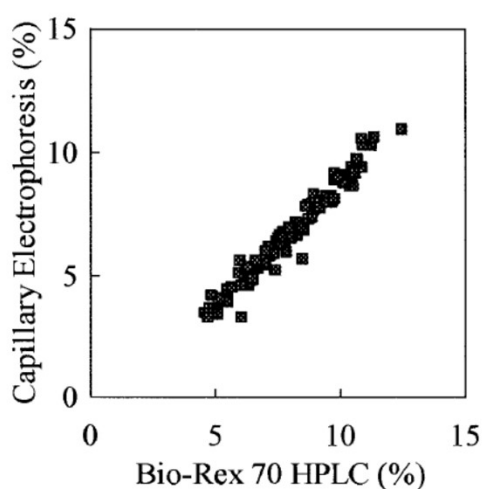
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## Comparison of Bio-Rex 70 HPLC results and those by CE (n=100)



**Table 2. Cost-effectiveness of CE and Bio-Rex 70 HPLC assays of Hb A<sub>1c</sub>.**

	Bio-Rex 70 HPLC	CE
Equipment, \$ per year	3000	6000
Maintenance, \$ per year	1200	6000
Sample throughput		
Tests per day	5	40
Tests per year	1000	8000
Technician, \$ per year	48 000	48 000
Reagents, estimated, \$ per year	600	23 500
Costs per sample, \$	52.80	10.45

The HPLC method is not suitable for routine use because of its low throughput (5 samples a day). Multichannel CE systems, when available, should have greatly increased sample throughput, making them attractive for routine use.



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ورود ثبت نام جستجو...


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موضوع: بررسی نقش بیوشیمی در تشخیص و درمان بیماری های مزمن

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دکتر محمدرضا بختیاری  
درمانگاه شفا

منوی اصلی

صفحه نخست

مشخصات آزمایشگاه

دوره های ارزیابی خارجی

لیست نام دوره های جدید

فاکتورهای خرید

خروج

نمونه	شرح	گروه
B1	نمونه سرم مجهول یوشیمی	(گروه 1)
B3	نمونه مجهول پروتئین انرژا B3	(گروه 1)
B4	نمونه مجهول B4.2 HBA1C	(گروه 2)
B4	نمونه مجهول B4.2 HBA1C	(گروه 2)
U1	نمونه مجهول ادرار	(گروه 1)
H2	شمارش افراتی گلبولهای سفید و بررسی مورفولوژی سلولهای	(گروه 1)
H3	شمارش افراتی گلبولهای سفید و بررسی مورفولوژی سلولهای	(گروه 1)
H4	شمارش رتیکولوسیت ها	(گروه 1)
H1	شمارش رتیکولوسیت ها	(گروه 1)
H3	شمارش افراتی گلبولهای سفید و بررسی مورفولوژی سلولهای	(گروه 1)
H4	شمارش رتیکولوسیت ها	(گروه 1)
C1	نمونه مجهول پانسمای انعقادی	(گروه 1)
S1.1	نمونه مجهول ایمنوسورولوژی برای آزمایش HIVAB, HBSAG	(گروه 1)
S3	نمونه مجهول سورولوژی خانواده رابت	(گروه 1)
M1	تشخیص میکروارگانیسم مربوط به M2	(گروه 3)
M1	تشخیص میکروارگانیسم مربوط به M2	(گروه 3)
M2	تشخیص میکروارگانیسم باکتری	(گروه 1)
P1	نمونه مجهول PARASITOLOGY P1.1	(گروه 1)
P1	نمونه مجهول PARASITOLOGY P1.2	(گروه 2)

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درمانگاه شفا
  
منوی اصلی
  
صفحه نخست
  
مشخصات آزمایشگاه
  
دوره های ارزیابی خارجی
  
فاکتورهای خرید
  
خروج

پرگشت
  
B4 Results
  
نمونه مجهول HbA1c B4.2
  
کد نمونه 1222
  
EQAP-10013
  
آزمایشگاه درمانگاه شفا
  
تور: چهل و پنجم تابستان 1402
  
نام تست: HbA1c
  
واحد: %
  

عملکرد عالی (رنگ آبی)	DI	< 0.5
عملکرد مطلوب (رنگ سبز)	DI	0.5 - 1.0
نتیجه قابل قبول اما در حدود مرز (رنگ زرد)	DI	1.0 - 2.0
نیاز به بررسی روش و کنترل کالیبراسیون (رنگ نارنجی)	DI	2.0 - 3.0
وجود نقص و نیاز به پیگیری فوری (رنگ قرمز)	DI	> 3.0
نتیجه گزارش شده خارج از محدوده است (Out Of Range)	DI	> 10.0

Kit_Maker	PeerGroup Count	PeerGroup Mean	PeerGroup %CV	Lab Result	Lab DI
A1c Care	20	10.42	14.89		
Aptec	48	9.10	7.12		
Archem	6	10.97	11.54		
AUDICAM	4	10.16	18.39		
Bionik	3	9.87	6.52		
Biorex Fars	139	9.95	9.70		
Biosystems	6	8.87	11.38		
Capillarys / Sebia	82	9.81	2.09		
Dia Lab	31	9.88	5.99		
Diaxim	192	9.19	8.20	8.90	0.39
DIAZMA TEB	5	11.40	18.07		
Genui	16	9.84	18.36		

خروج

نام تست: HbA1c
  
واحد: %
  

عملکرد عالی (رنگ آبی)	DI	< 0.5
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Diaxim	192	9.19	8.20	8.90	0.39
DIAZMA TEB	5	11.40	18.07		
Genui	16	9.84	18.36		
Genus	3	9.38	3.53		
Ichroma	14	9.31	9.09		
Ideal Tashkhis	128	9.33	9.84		
LabCard	58	8.98	7.52		
Man	15	9.83	6.38		
Novin Bio KIT	20	10.14	12.72		
NycoCard	71	8.91	7.24		

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Table Tools

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Illustrations

Recommended Charts Charts

Maps PivotChart 3D Map Tours

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Slicer Timeline Filters

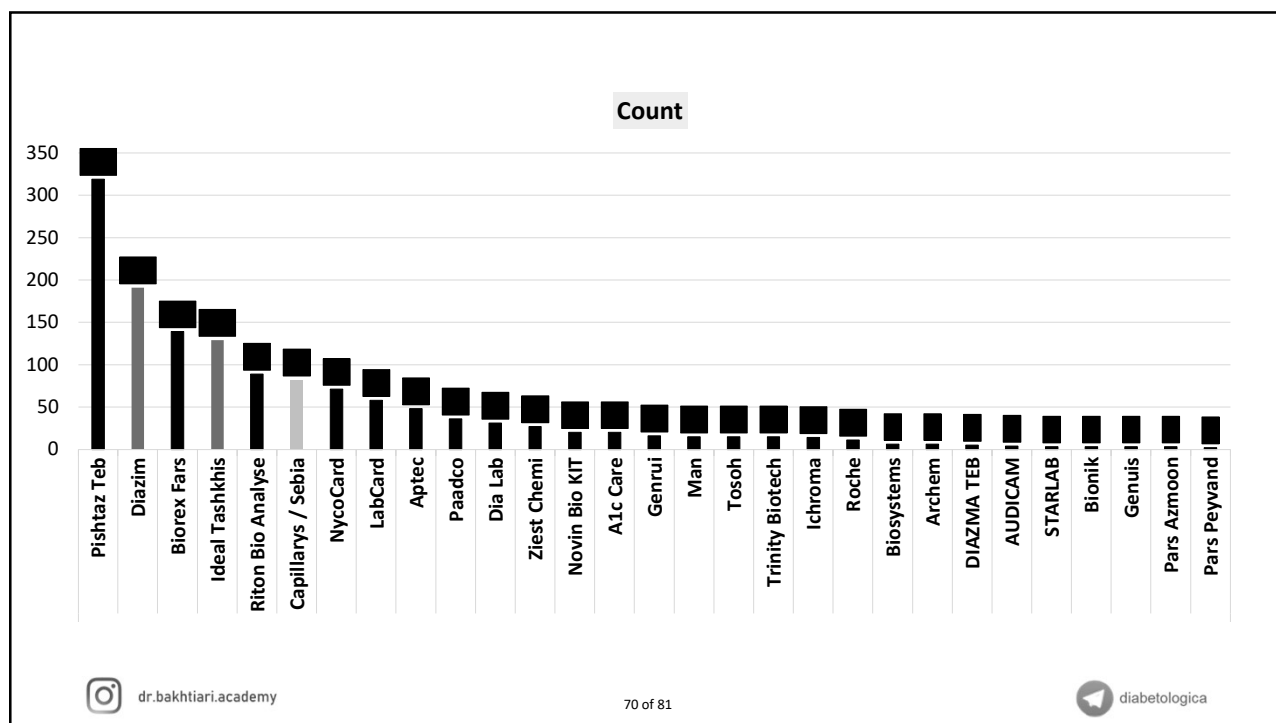
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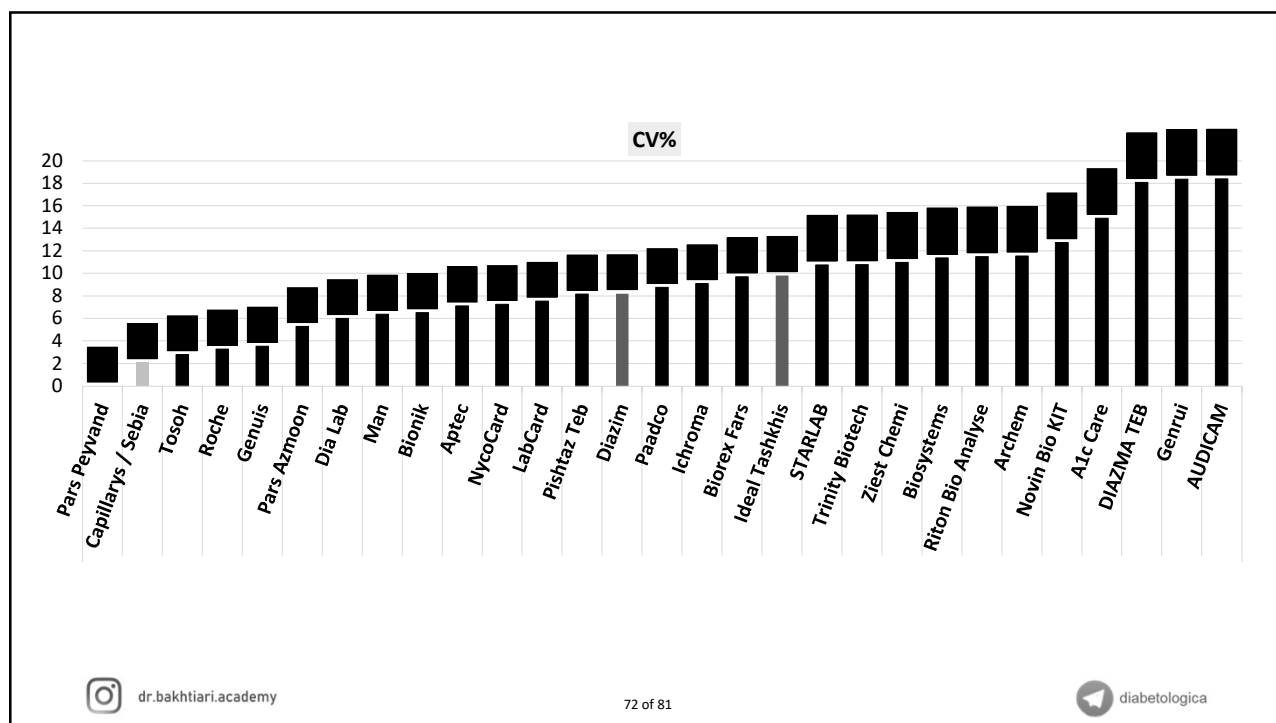
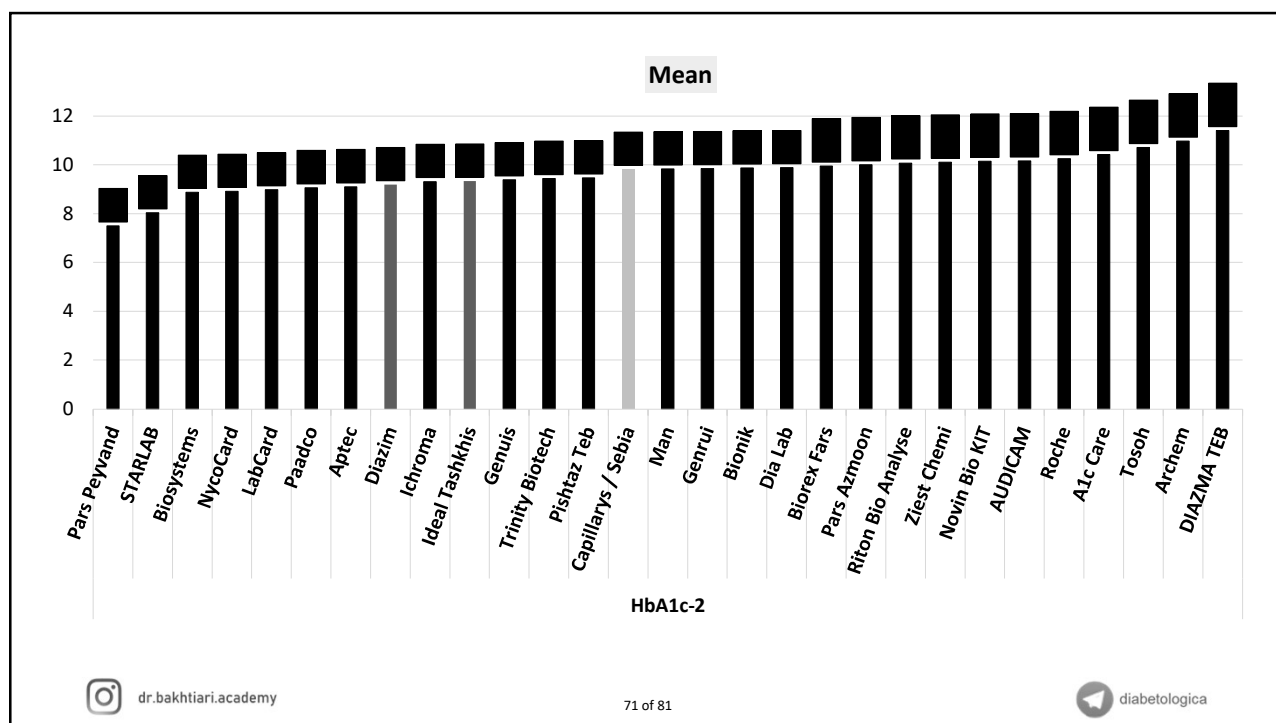
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	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
	Kit_Maker	PeerGroup Count	PeerGroup Mean	PeerGroup %CV	Course											
2	Advia	25.0	12.8	18.3	39											
3	Architect	37.0	11.9	8.3	39											
4	Autobio CLIA	10.0	11.8	8.6	39											
5	Bio Merieux	119.0	13.5	12.1	39											
6	Dia Plus	8.0	14.4	18.4	39											
7	Dia Sorin	42.0	8.6	13.3	39											
8	Diametro	4.0	16.6	21.4	39											
9	DiaZist	25.0	18.7	20.0	39											
10	Edehal System	3.0	15.1	25.5	39											
11	Erba	3.0	35.7	26.5	39											
12	IBT	2.0	30.1	44.2	39											
13	Ideal Tashkhis	64.0	14.8	24.8	39											
14	Immullite	50.0	7.4	11.6	39											
15	Mindray	26.0	13.1	12.2	39											
16	Monobind	59.0	15.3	22.3	39											
17	Pad Tan Elm	17.0	17.9	40.8	39											
18	Pad Tan Teb	9.0	13.6	36.1	39											
19	PGI	67.0	13.2	25.1	39											
20	Pishgaman	27.0	18.9	15.4	39											
21	Pishtaz Teb	228.0	17.8	24.3	39											
22	Roche	106.0	15.6	10.8	39											
23	Maglumi	16.0	11.6	7.3	39											
24	Tosoh	21.0	10.6	10.2	39											
25	Vitros	3.0	13.6	3.2	39											
26	Advia	14.0	28.5	8.1	39											
27	Architect	37.0	33.7	8.1	39											
28	Arka	5.0	37.1	12.5	39											

Calculate

Sheet2 count mean cv Report





## کنترل کیفی آماری

### در آزمایشگاه تشخیص پزشکی

ثبت نام در این دوره آموزش مجازی = یک تیر و چند نشان

شروع دوره: تیرماه ۱۴۰۱  
مهلت استفاده از تخفیف: ۲۰ خرداد

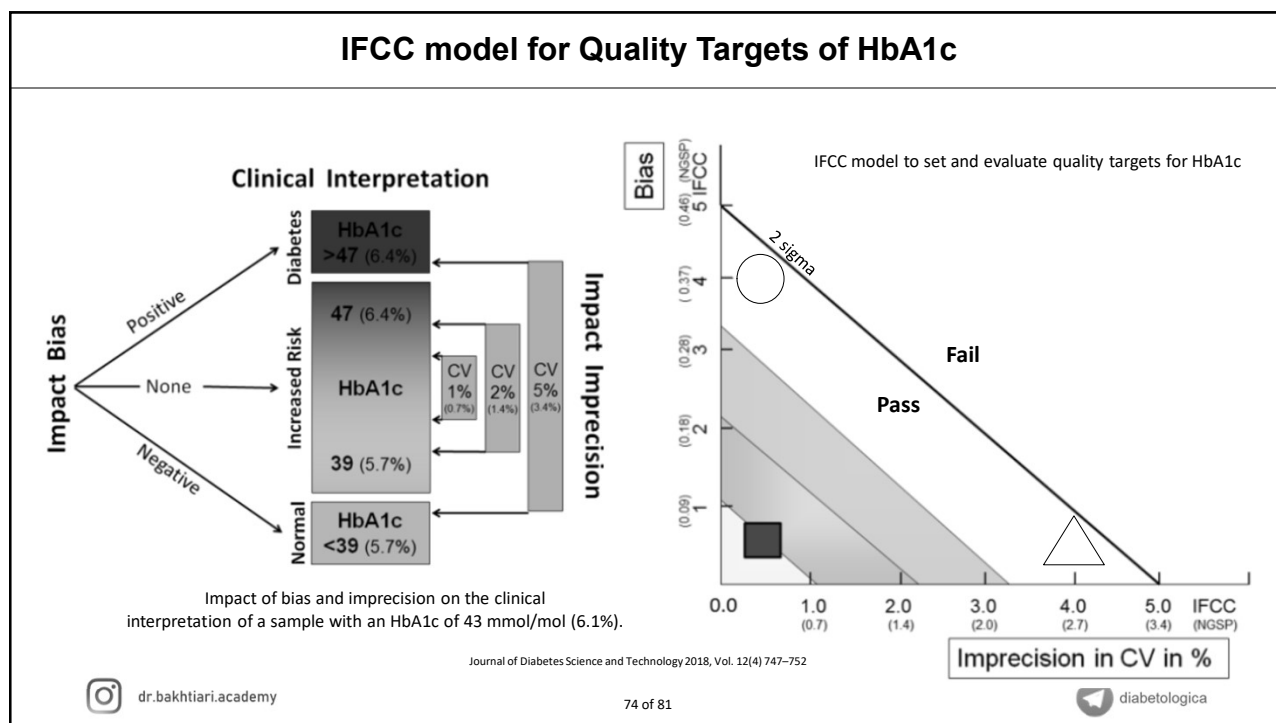
دوره نظری و عملی با حل تمرین ✓  
 تسلط بر کنترل کیفی داخلی و خارجی در آزمایشگاه ✓  
 کسب مهارت و دانش لازم برای استقلال در طراحی، اجرا و تفسیر برنامه QC در آزمایشگاه ✓  
 تسلط بر نرم افزار اکسل ✓  
 تسلط بر محاسبات ریاضی و آماری با اکسل و XLSTAT ✓



با سرمایه گذاری بر آموزش خود، متفاوت و حرفه ای تر شوید.

مدرس: دکتر ممد رضا بفتیاری



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## Biological Variation of HbA1c & Analytical Performance Specification

### Haemoglobin A1c (NGSP)

[Analytical Performance Specification](#)
[RCV Calculation](#)

Matrix	BV Estimate	median CV estimate	lower CI limit	higher CI limit	Comments	Date Updated
Whole Blood		1.2	0.2	1.9		21/9/2023
Whole Blood		4.8	3.3	6.8		21/9/2023

Specification	Imprecision (CVa)	BIAS	MAU	Total Error
Minimum	0.9	1.9	1.8	3.3
Desirable	0.6	1.2	1.2	2.2
Optimal	0.3	0.6	0.6	1.1



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## Biological Variation of HbA1c & Analytical Performance Specification

### Haemoglobin A1c (IFCC)

[Analytical Performance Specification](#)
[RCV Calculation](#)

Matrix	BV Estimate	median CV estimate	lower CI limit	higher CI limit	Comments	Date Updated
Whole Blood		1.6	1.3	2.4		21/9/2023
Whole Blood		7.1	6.7	7.5		21/9/2023

Specification	Imprecision (CVa)	BIAS	MAU	Total Error
Minimum	1.2	2.7	2.4	4.7
Desirable	0.8	1.8	1.6	3.1
Optimal	0.4	0.9	0.8	1.6



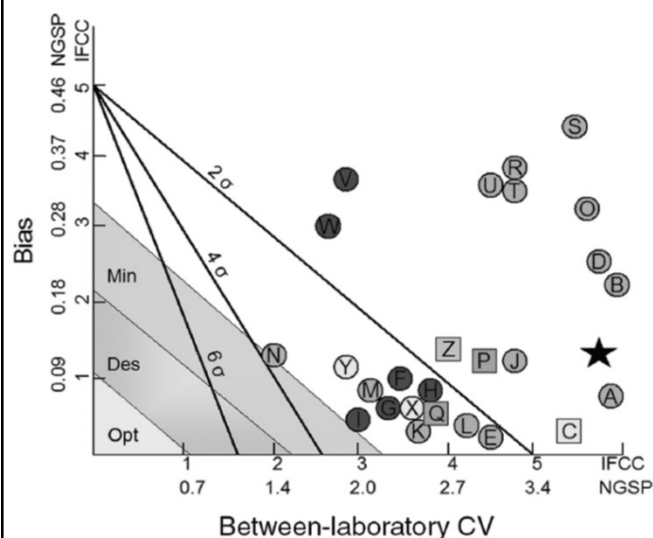
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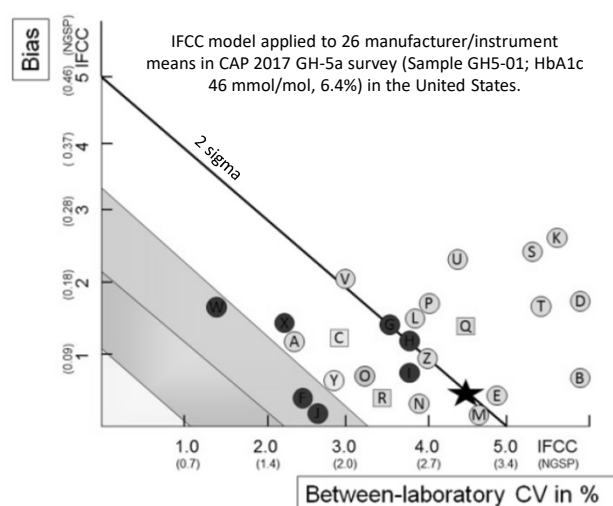
## Models applied to 26 manufacturer/instrument means in CAP 2014 GH2-A survey



- **Black star** represents the **overall mean** of all laboratories
- Specific manufacturers with colors for analytical principles:
  - **green** (immunoassays)
  - **red** (ion exchange HPLC)
  - **yellow** (boronate affinity HPLC)
  - **blue** (capillary electrophoresis)
  - **gray** (dry Chemistry)
- **Circles** (laboratory instruments)
- **Squares** (POCT/ dry chemistry instruments)

Clinical Chemistry 61:5 752–759 (2015)

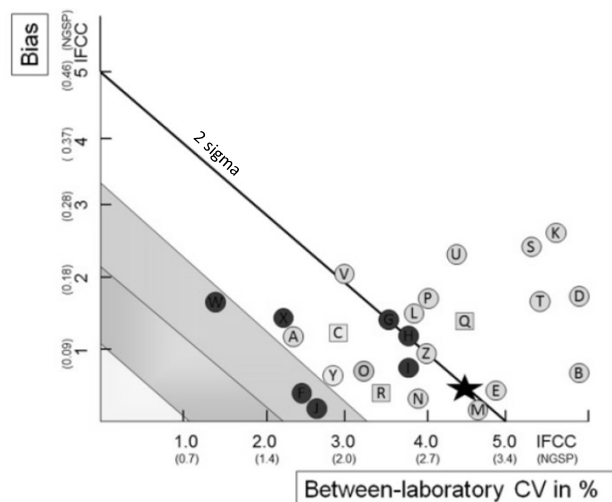
## Evaluation of Performance of Laboratories and Manufacturers Within the Framework of the IFCC model for Quality Targets of HbA1c (2017)



- **Black star** represents the **overall mean** of all laboratories
- Specific manufacturers with colors for analytical principles:
  - **green** (immunoassays)
  - **red** (ion exchange HPLC)
  - **yellow** (boronate affinity HPLC)
  - **blue** (capillary electrophoresis)
  - **pink** (enzymatic assays)
- **Circles** (laboratory instruments)
- **Squares** (POCT/ dry chemistry instruments)

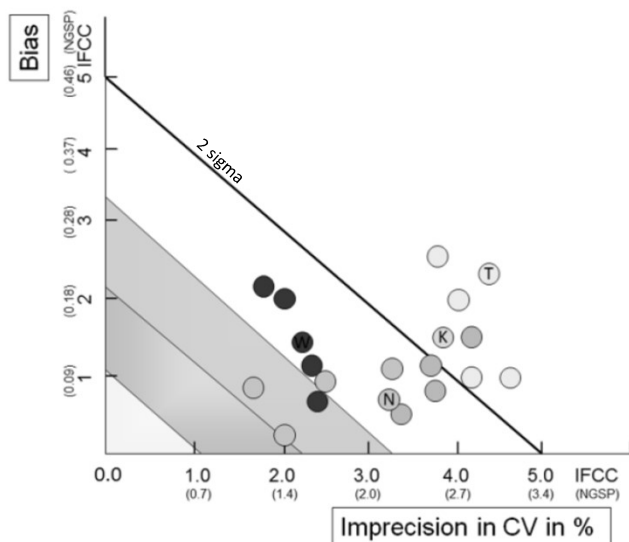
Journal of Diabetes Science and Technology 2018, Vol. 12(4) 747–752

## Evaluation of Performance of Laboratories and Manufacturers Within the Framework of the IFCC model for Quality Targets of HbA1c



(A) Abbott Architect c enzymatic,	Pass
(B) Abbott Architect I System,	Fail
(C) Axis-Shield Afinion,	Pass
(D) Beckman AU systems,	Fail
(E) Beckman UniCel Dx C Synchron,	Fail
(F) Bio-Rad D100,	Pass
(G) Bio-Rad D10,	Pass*
(H) Bio-Rad Variant II,	Pass*
(I) Bio-Rad Variant II Turbo,	Pass
(J) Bio-Rad Variant Turbo 2.0,	Pass
(K) Roche Cobas c311,	Fail
(L) Roche Cobas c500 series,	Fail
(M) Roche Cobas Integra 400,	Pass
(N) Roche Cobas Integra 800,	Pass
(O) Sebia Capillarys 2 Flex Piercing,	Pass
(P) Siemens Advia Chemistry Systems,	Fail
(Q) Siemens DCA 2000/20Q0+,	Fail
(R) Siemens DCA Vantage,	Pass
(S) Siemens Dimension ExL,	Fail
(T) Siemens Dimension RxL,	Fail
(U) Siemens Dimension Vista,	Fail
(V) Siemens Dimension Xpand	Fail
(W) Tosoh G7 Auto HPLC,	Pass
(X) Tosoh G8 Auto HPLC,	Pass
(Y) Trinity Biotech Premier Hb9210,	Pass
(Z) Ortho Clin Diag Vitros 5,1 FS, 4600, 5600 Chem System.	Pass

## Evaluation of Performance of Laboratories and Manufacturers Within the Framework of the IFCC model for Quality Targets of HbA1c



○ Sebia Capillarys 2 Flex Piercing,	Pass
○ Tosoh G7 Auto HPLC,	Pass
○ Roche Cobas Integra 400,	Pass
○ Siemens Dimension Vista,	Fail



