# ABC of Hb A1c

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MD MRCP UK

HBAIC USED IN DIAGNOSIS OF DM
A) TYPE 1 DM
B) type 2
c) gestational dm
D) DRUG INDUCED DM

The best investigation to consider failure of oral hypoglycemics
a)FBS
B) PPBS
C) RBS
0) HBAIC
One of your relatives in uk is diabetic send you this result;
hba1c 42 mmol/mol.
What will you inform him

a) Your dm is ok

b) Your dm is badly controlled
c) I don't know

# HISTORY OF Hb A1c

- KOENIG : THE FIRST TO SHOW THAT A1C LEVELS CORRELATED WELL WITH FASTING BLOOD GLUCOSE
- MORTENSEN & CHRISTOPHERSEN : DEMONSTRATED THAT THE FRACTION OF A1C DEPENDS ON THE GLUCOSE LEVELS OVER A PREVIOUS PERIOD(4 AND 12 WEEKS)
- 1978 – ASSAYS COMMERCIALLY AVAILABLE

# GLYCATION OF HEMOGLOBIN

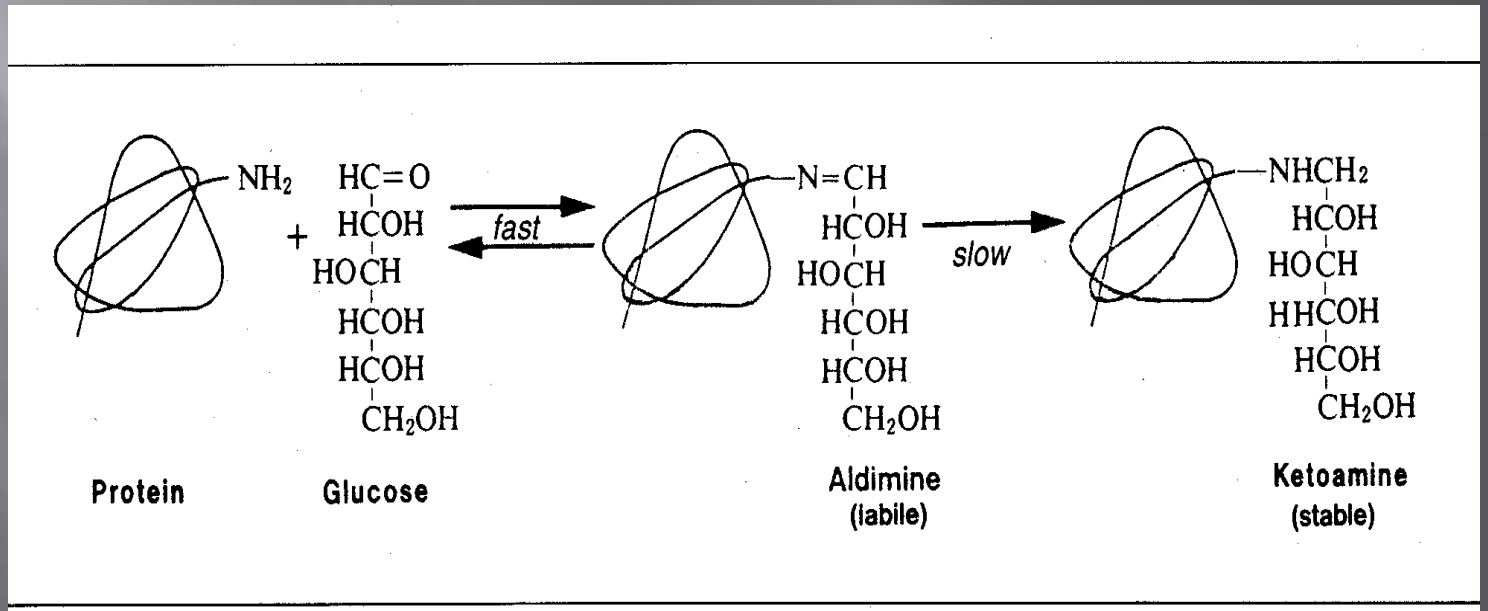
NON-ENZYMATIC ADDITION OF A SUGAR TO AMINO GROUPS OF PROTEINS

FORMATION OF GLYCATED HEMOGLOBIN IS <u>IRREVERSIBLE</u>

CONCENTRATION DEPENDS ON:

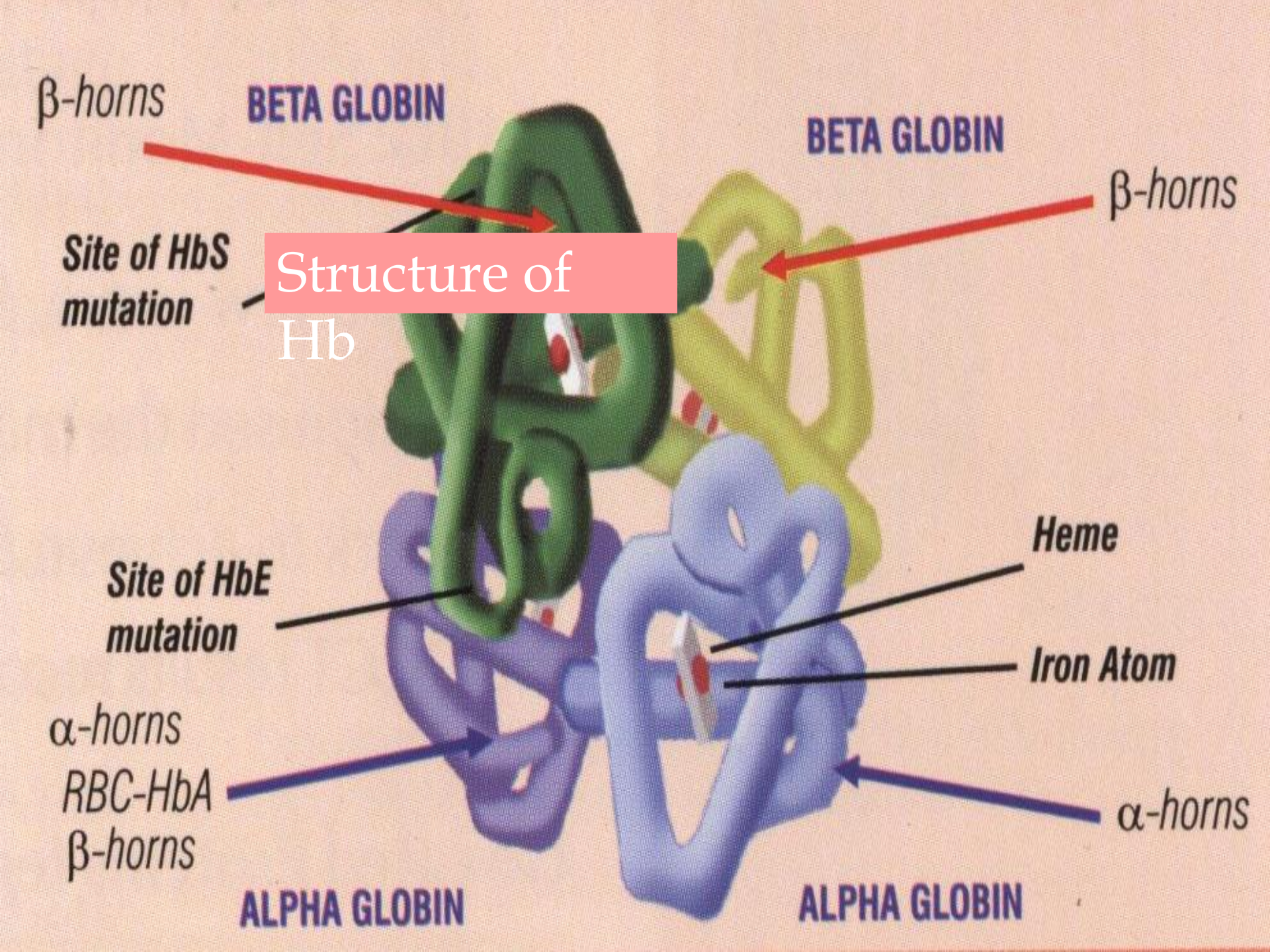
A. LIFE SPAN OF RBC

B. BLOOD GLUCOSE CONCENTRATION



# Different Hbs

1. Fetal Hemoglobin – Hb F
2. Adult Hemoglobin – Hb A
3. Sickle cell disease – Hb S
4. Hemoglobinopathies – Hb C, Hb E


β-horns
BETA GLOBIN
BETA GLOBIN
β-horns
Site of HbS mutation
Structure of Hb
Heme
Site of HbE mutation
Iron Atom
α-horns
RBC-HbA
β-horns
α-horns
ALPHA GLOBIN
ALPHA GLOBIN

**Haemoglobin electropharesis ;**

.

Haemoglobin HbA 97%,

$HbA_2$ 2.5 %

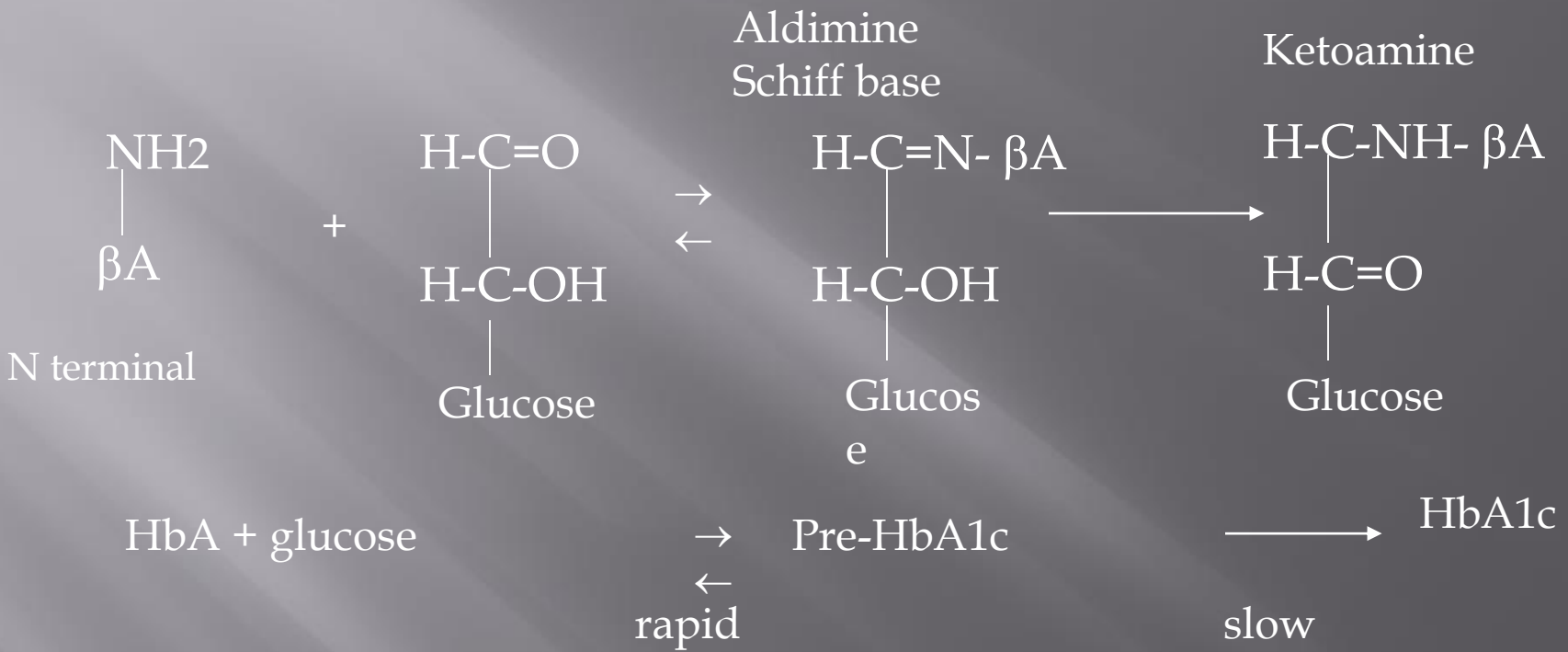
HbF 0.5%

Several minor haemoglobins migrate more rapidly than HbA in an electric field, called **HbA1, made up of HbA1a + HbA1b + HbA1c**.

**HbA1a1** is fructose-1, 6 diphosphate and **HbA1a2** is glucose-6-phosphate attached to the amino terminal of the beta chain.
**HbA1b** is pyruvic acid linked to the amino terminal valine of the beta chain
**HbA1c.** is Condensation of glucose and the N-terminal valine of each beta chain of haemoglobin

**HbA1c** makes up 80% of HbA1.

. Normally less than 6% of Hb is $HbA_{1c}$


Aldimine
Schiff base
Ketoamine
NH2
βA
N terminal
+
H-C=O
H-C-OH
Glucose
H-C=N- βA
H-C-OH
Glucos
e
H-C-NH- βA
H-C=O
Glucose
HbA + glucose
Pre-HbA1c
HbA1c
rapid
slow

**Interference:**

- Icterus
- Lipemia
- **Hemoglobin variants S and C** have no effect on the assay when they exist in the heterozygous forms (HbAS and HbAC.)

In homozygous **Hb SS or Hb CC** patients do not have HbA present or HbA1c thus criteria other than monitoring of HbA1c must be used to assess long term diabetic control in these patients.

HbF levels upto 30 % do not interfere

Altered red blood cell turnover eg haemolytic anaemia, major blood loss or blood transfusion

Carbamylated Hb from attachment of urea may also interfere

# Diagnosis of Diabetes

- Based exclusively on fasting glucose and oral Glucose Tolerance Test.

However, measuring blood glucose levels is associated With methodological, procedural and practical problems.

Day-to-day variation of blood glucose levels is considerable,
the concentration ex vivo falls quickly even when the blood sample is collected in a fluoride-oxalate tube,
Inter-laboratory levels can vary by at least 14% in a third of cases.

oral glucose tolerance test (OGTT)
requires
proper pretest preparation, including an appropriate diet for 3 days before the test and a satisfactory period of overnight fasting.

time-consuming,taking at least 2 hours.

The glucose load is poorly tolerated by a significant number of people, with nausea, vomiting, delayed gastric emptying and issues of venous access all potentially contributing to an invalid test result.

The test often needs to be repeated and has poor patient compliance.

# HbA1c as a diagnostic test for diabetes

# WHO Recommendation 2011

HbA1c can be used as a diagnostic test for diabetes providing that stringent quality assurance tests are in place and assays are standardized to criteria aligned to the international reference values, and there are no conditions present which preclude its accurate measurement.

An HbA1c of **48 mmol/mol (6.5%) is recommended as the cut point for diagnosing diabetes.**
**A value of less than 48 mmol/mol (6.5%) does not exclude diabetes diagnosed using glucose tests.**

In symptomatic adults with relatively slow onset of symptoms a single result ≥48 mmol/mol will suffice.

In patients without diabetes symptoms repeat venous HbA1c in the same lab within 2 weeks. If the second sample is <48 mmol/mol (6.5%) treat as high risk of diabetes and repeat the test in 6 months or sooner if diabetes symptoms develop.

**Situations where HbA1c must not be used as the sole test to diagnose diabetes**

HbA1c reflects glycaemia over the preceding 2 – 3 months so may not be raised if blood glucose levels have risen rapidly. Examples:

1

ALL symptomatic children and young people

Symptoms suggesting Type 1 diabetes (any age)

Short duration diabetes symptoms
Patients at high risk of diabetes who are acutely ill

Taking medication that may cause rapid glucose rise e.g. corticosteroids, antipsychotics ,

Acute pancreatic damage/pancreatic surgery

patients with any significant chronic medical disease, any anaemia or any abnormality of red blood cell structure.

If any of these conditions exist, the diagnosis should be based on measures of blood glucose levels using existing criteria (fasting or random glucose level, and OGTT). These

# IN THE CLINICAL MANAGEMENT OF DIABETES

- THE FASTING & THE POST-PRANDIAL GLYCEMIA REFLECT THE DAY TO DAY DIABETES CONTROL

- THE A1C MONITOR CHRONIC GLYCEMIA:

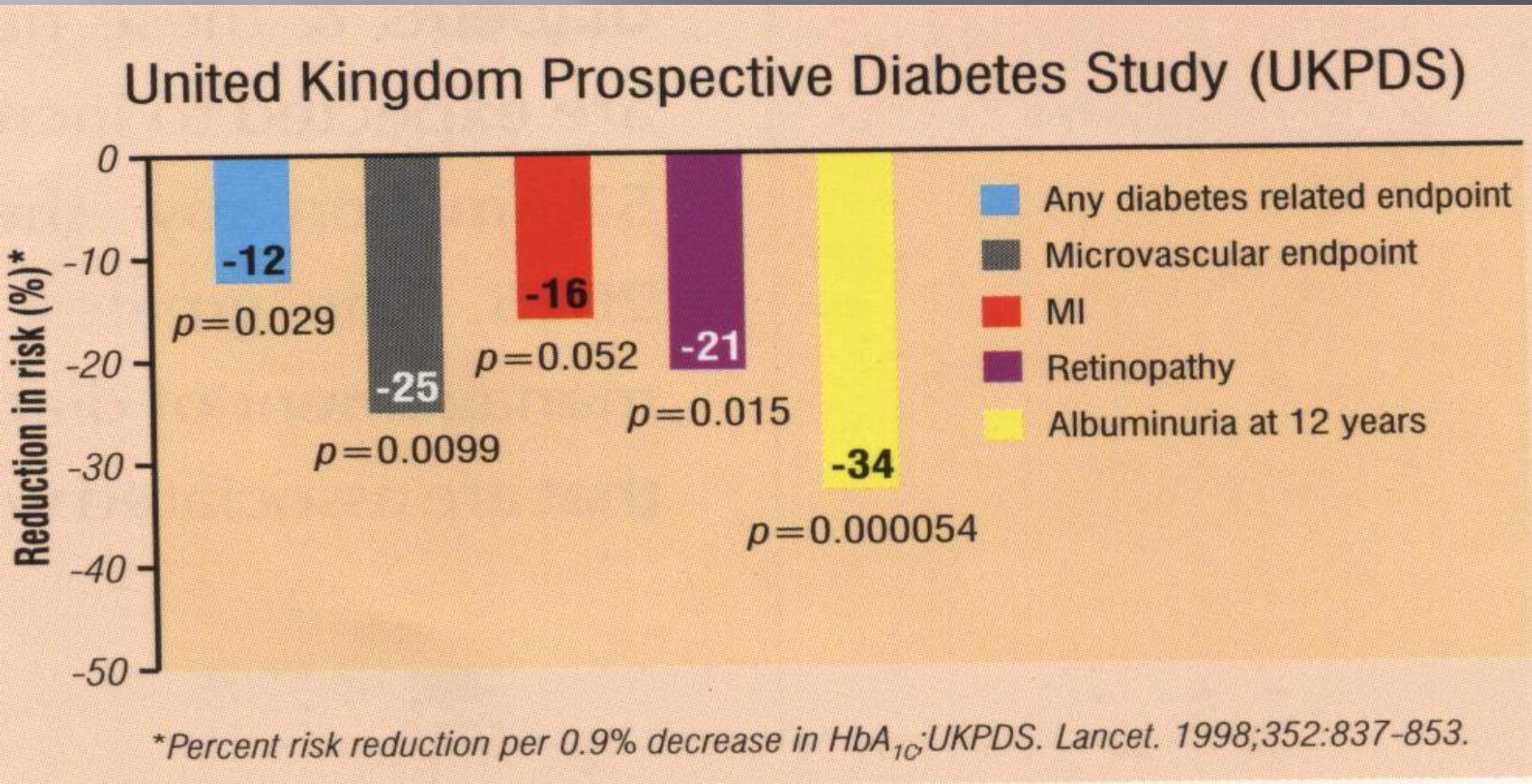
this assay is an essential tool to determine whether a patient has achieved the core goal of therapy to prevent or delay the development of long-term complications of diabetes

# HBA1C AS A TREATMENT TARGET

- AFTER PUBLICATION OF DCCT AND UKPDS, HBA1C WAS INTRODUCED AS A RISK PARAMETER FOR MONITORING THE POTENTIAL DEVELOPMENT OF LATE DIABETIC COMPLICATIONS.

# How well it measures ?

Lowering Hb A1c reduces risk of complications



**Reference Ranges**

< 6.5 % normal

6.5-7.0 % target in diabetic patients

7.0 -9.0% suboptimal  diabetic control

> 9.0 % poor diabetic control

# CURRENT TREATMENT GOALS FOR $HBA_1C$

| | |
|---|---|
| ADA | 7% |
| Diabetes UK | <7% |
| IDF | <6.5% |
| AACE | <6.5% |
| EASD | <6.5% |

# Advantages of $HbA_{1c}$

- Index of long-term control over 120 days and not a snap shot like PG
- Can be done at any time of day
- Not influenced by diet, exercise, emotional disturbances on test day
- Useful index in clinical trials
- Useful if missed drugs / default diet
- Useful in DD of stress hyperglycemia

relied on for significant management decisions, such as initiation of insulin therapy.

The strength of its relationship with diabetes-related complications was demonstrated( in an analysis of the combined data from eight studies conducted between 1988 and 2004, which reported that HbA1c levels were at least as strongly related to the presence of diabetic retinopathy as were blood glucose levels.

It is also strongly associated with macrovascular outcomes and mortality.)

# Limitations of $HbA_{1c}$

- Cannot be an emergency room test to titrate Insulin or OHA dosage
- Cannot register hypoglycemia
- – if it is elevated it confirms poor control, if it is boarder line, it cannot assure good control in the recent past.
- Not sensitive enough for use in GDM
- ↓ Anaemia, Uraemia, Pregnancy

# DISADVANTAGES OF HEMOGLOBIN $A_1C$

1-LACK OF STANDARDIZATION IN HEMOGLOBIN A1C ASSAYS

2-MANY PATIENTS STILL DO NOT UNDERSTAND THE RELEVANCE OF THE $A_1C$ NAME AND UNITS :

DIFFICULT TO EXPLAIN TO PATIENTS CONCEPT OF % IS NOT INTUITIVE

3-DOES NOT REFLECT THE FLUCTUATIONS OF GLYCEMIA

# 1-LACK OF STANDARDIZATION IN HEMOGLOBIN $A_1C$ ASSAYS

NATIONAL GLYCOHEMOGLOBIN STANDARDIZATION PROGRAM (NGSP)

DEVELOPED IN 1996 TO STANDARDIZE HEMOGLOBIN TEST RESULTS SO THAT CLINICAL LABORATORY RESULTS ARE COMPARABLE TO THOSE REPORTED IN DCCT.

# STANDARDIZATION OF HB A1C

- THE THREE MAJOR **HBA1C HARMONISATION (STANDARDIZATION, *COMPARABILITY*)** SCHEMES INCLUDE:

  (1) THE **NATIONAL GLYCOHEMOGLOBIN STANDARDISATION PROGRAM (NGSP)** IN THE **UNITED STATES**

  (2) THE SCHEME OF THE **JAPANESE DIABETES SOCIETY (JDS)**

  (3) THE **MONOS-METHOD (SWEDEN)**

# IN 1995 :THE IFCC  CREAT A NEW GLYCATED HEMOGLOBIN METHOD & STANDARDS

# THE NEW IFCC GLYCATED HEMOGLOBIN ASSAY

- **VERY SPECIFIC:** PRECISELY MEASURES GLYCATED $HBA_1C$, WITHOUT OTHER COMPONENTS

- BUT : *VERY COMPLICATED

  * REQUIRES COSTLY EQUIPMENT (A MASS SPECTROMETER)

# Comparison of NGSP and IFCC $HbA_1c$ Levels

| NGSP (%) | IFCC (mmol/mol) |
|---|---|
| 4.0 | 2.1 |
| 6.0 | 4.3 |
| 7.0 | 5.3 |
| 8.0 | 6.4 |
| 8.56 | 7.0 |
| 10.0 | 8.6 |

# INTERNATIONAL WORKING GROUP ON HBA1C ASSAY

IDF,ADA,EASD AND IFCC MET IN JULY 2003:

## 3 OPTIONS:

1. CONTINUE TO REPORT RESULTS IN DCCT (%) EQUIVALENT (NGSP)
2. CHANGE TO IFCC UNITS (MMOL/L)
3. CHANGE TO/ADD OTHER UNITS SUCH AS AVERAGE GLUCOSE

# CHANGING VALUES

# Before 2009

- HbA1C results would have appeared as:

  Haemoglobin A1c  7.0% total Hb (3.6-6.8)

The HbA1c is expressed as a percentage of the Total Haemoblobin relative to a calibrator aligned to the DCCT method

# After June 2009

- HbA1c results have appeared as:

Haemoglobin A1c 7.0% total Hb (3.6-6.8)

HbA1c (IFCC) 53 mmol/mol

The second result has been obtained using the IFCC reference standard

# From June 2011

- HbA1c results appeared as:

HbA1c (IFCC)  53 mmol/mol (<48)

| DCCT –HbA1c (%) | IFCC-HbA1c (mmol/mol) |
|---|---|
| 6.0 | 42 |
| 6.5 | 48 |
| 7.0 | 53 |
| 7.5 | 59 |
| 8.0 | 64 |
| 9.0 | 75. |

Consensus by ADA,EASD, IFCC and IDF for worldwide standardization

# Relationship between old and new units

- IFCC-HbA1c (mmol/mol) = [DCCT-HbA1c (%) – 2.5] x 10.929

# THE A1C-DERIVED AVERAGE GLUCOSE (ADAG) STUDY

INTERNATIONAL STUDY DESIGNED TO:

- LOOK CAREFULLY AT RELATIONSHIP BETWEEN HBA1C AND AVERAGE GLUCOSE
- DETERMINE THE MATHEMATICAL RELATIONSHIP BETWEEN THE TWO FOR RELIABLE CONVERSION
- ESTABLISH THAT THE RELATIONSHIP IS VALID ACROSS:
  - DIABETES TYPES
  - A WIDE RANGE OF HBA1C LEVELS AND AGE
  - DIFFERENT RACES/ETHNICITIES

*NATHAN ET AL, DIABETES CARE 31:1473, 2008*

# INTERNATIONAL $A_1C$-DERIVED AVERAGE GLUCOSE (ADAG) STUDY

## STUDY DESIGN:

- 11 INTERNATIONAL CLINICAL CENTERS
- 300 TYPE 1 DIABETES
- 300 TYPE 2 DIABETES
- 100 HEALTHY VOLUNTEERS

- ✓ 48-HOUR CGMS EVERY MONTH FOR 4 MONTHS
- ✓ 8-POINT GLUCOSE PROFILES DURING CGMS DAYS
- ✓ AT LEAST 7-POINT GLUCOSE PROFILES 3 DAYS PER WEEK
- ✓ $HBA_1C$ MEASURED 5 TIMES OVER 4 MONTHS IN CENTRAL LAB

# ADAG STUDY EXCLUDED KNOWN SOURCES OF "INACCURACY" OF HB A1C

- HEMOGLOBINOPATHY
- ANEMIA
- PREGNANCY
- HEPATIC OR RENAL DISEASE

# ADAG STUDY: OTHER FACTORS EXAMINED

- **DOES THE HBA1C-AVERAGE GLUCOSE RELATIONSHIP DIFFER BY:**
  - **TYPE 1 OR TYPE 2 DIABETES NO**
  - **DIABETES OR NO DIABETES NO**
  - **AMOUNT OF GLUCOSE VARIABILITY NO**
  - **GENDER NO**
  - **AGE NO**
  - **ETHNICITY/RACE NO**

***(BUT TREND TOWARD HIGHER HBA1C PER AG IN AFRICAN AND AFRICAN-AMERICAN PARTICIPANTS VS. WHITES, P=0.07)***

- **SMOKING NO**

# Current Translation of $HbA_1c$ vs. Average Glucose (DCCT)

| AG mmol/l (mg/dL) | $HbA_1c$ (%) |
|---|---|
| 5.6 (100) | 5 |
| 7.5 (135) | 6 |
| 9.4 (170) | 7 |
| 11.4 (205) | 8 |
| 13.3 (240) | 9 |
| 15.3 (275) | 10 |
| 17.2 (310) | 11 |
| 19.2 (345) | 12 |

# Correlation of MPG - $HbA_{1c}$

Mean Plasma Glucose =

$(33.3 \times HbA_{1C\%}) - 86$

(Nathan et. al. NEJM, vol. 310, No 6, Feb 9, 1994)

| $HbA_{1C}$ % | Mean BG mg % |
|---|---|
| 5 | 80.5 |
| 7 | 147.1 |
| 9 | 213.7 |
| 11 | 280.3 |

# IMPLICATIONS

- TIGHT CORRELATION BETWEEN HBA1C AND AG ALLOWS US TO TRANSLATE HBA1C INTO AN ESTIMATED AVERAGE GLUCOSE (eAG)
- eAG WILL APPLY TO THE MAJORITY OF PATIENTS WITH DIABETES
  - BARRING "TRADITIONAL" CONDITIONS INTERFERING WITH THE ASSAY OR THE RELATIONSHIP BETWEEN GLYCEMIA AND HBA1C

# eAG: the advantages

1. Simplicity: for patients/ docs/ no decimals

2. Logical: vascular complications are linked to hyperglycemia and not “hyper-glycated HbAemia”

3. Common currency : No dissonance between SMBG and the A1C

Home PD. HbA1c: the case for using eAG. Diabetic Medicine 2008; 25: 895-98

# eAG: the disadvantages !

1. The relationship between A1C and mean glucose can vary (age, inter-individual)
2. Overlapping values. Also: Pregnant women/children no data
3. HbA1c and not eAG has been used in the landmark DCCT/ UKPDS Study

Kilpatrick ES. eAG: fit for purpose ? Diabetic Medicine 2008;899-901

# WHAT CLINICIANS CAN DO?

- CHOOSE WHICH TERM – A1C OR AVERAGE GLUCOSE – TO USE WITH EACH PATIENT (SOME MAY ALREADY BE USED TO A1C)
- IN VERBAL COMMUNICATIONS, NO NEED TO SAY "ESTIMATED"
- USE UPDATED TABLE, CALCULATOR OR OTHER TOOLS TO CONVERT A1C TO AVERAGE GLUCOSE
- "LOBBY" YOUR LAB TO REPORT BOTH NUMBERS

*Thank you*

# CONCLUSIONS

- STANDARIZATION OF Hb A1C WAS ACCOMPISHED BY THE NGSP FOR SOME COUNTRIES ,BUT WHAT ABOUT OTHER COUNTRIE?
- THE NEED IN OUR REGION TO IMPLEMENT THE NGSP STANDARDS
- eAG SHOUD REPLACE THE TRADITIONAL Hb A1c WITH MANY ADAVANTAGES & SOME DISADVANTAGES
- PRACTIONERS & CHEMISTS SHOUD COORDINATE TO TRANSLATE THE HbA1C TO eVG

# THE NEW IFCC GLYCATED HEMOGLOBIN METHOD & STANDARDS (1995)

1-IFCC RECOMMENDS THE NAME OF THE ASSAY REFLECT WHAT IS ACTUALLY BEING MEASURED: N-[1DEOXYLFRUCTOSE-1-YL] HEMOGLOBIN BETA CHAIN OR DOF HEMOGLOBIN

2-RESULTS USING THE OLD AND NEW METHODS CORRELATE FAIRLY CLOSELY, BUT THE NEW IFCC REFERENCE NUMBERS ARE ≈1.3-1.9% LOWER THAN CURRENT VALUES

Interpretation of HbA1c relies on RBC having a normal lifespan Conditions with shortened RBC survival or higher fraction of young RBC have reduced HbA1c Higher HbA1c where older population of RBC exists

**Ion-exchange chromatography** Measures HBA1 – total glycated haemoglobins (A1a + 1b + 1c)

**HPLC** Both HbA1c and HbA1 can be reported, **Electrophoresis** can measure HbA1c but less specific .

**Isoelectrophoresis** HbA1c adequately resolved from HbA1a1, HbA1b and S and F.

**Immunoassay** antibodies raised against the Amadori product of glucose (ketoamine linkage) plus the first 4-8 amino acids at the N-terminal of the beta chain by inhibition of latex agglutination. **Specific for HbA1c**

**Affinity chromatography** uses

m-aminophenylboronic acid bound to agarose or glass fibre matrix to react with cis-diol groups of glucose bound to haemoaglobin.

**Measures HbA1**

**Diabetes Control and Complications Trial (DCCT) 1993 multicenter randomized trial**

HbA1c measurement systems have been standardized through a process of alignment with the original DCCT method. This has been undertaken by the **US National Glycohemoglobin Standardisation Program (NGSP) .**

**UK Consensus Statement**

Glycemic control is best measured by HbA1c

The method should be a DCCT –aligned HBA1c method

The assay should have acceptable within assay precision <3% and between assay imprecision <5%

## CMC METHOD BIORAD VARIANT HbA1c PROGRAM

Utilizes the principles of ion-exchange HPLC , without interference from labile A1c, lipaemia or temperature fluctuations.

**Certification/traceability of reference material**

Certified by the NGSP as having documented traceability to the DCCT reference method. The haemoglobin A1c calibrators provided in the kit are **traceable to the Kyoto 2002 Calibrator set prepared by the IFCC working group on standardization of HbA1c. The specimens were prepared in the Netherlands at a hospital with ISO 9001:2000 certificate.**

Sample EDTA whole blood stable 1 week at 4°C

HbA1c half life 35 days

A 1% increase in %HbA1c is equivalent to a rise in average blood glucose of 35 mg/dL.

Clin Chem 2009; 55: 1612-14

International Expert Committee says HbA1c should be the diagnostic test for diabetes.

The value of ≥ 6.5% decision point

6.0-6.4% indicate individuals at high risk of developing diabetes

# Estimation of $HbA_{1c}$

- There are many methods of estimation
- HPLC (High Performance Liquid Chromatography) – Gold standard.
- Immuno-turbimetric meth. – $HbA_{1c}Ab$
- Affinity chromatography
- Electrophoretic methods
- Method based on chemical reactions.

# Methods for determining glycated haemoglobins

those based on **charge differences:**

ion-exchange chromatography,

HPLC, electrophoresis,

and isoelectric focusing

and those based on **structural differences**

affinity chromatography and immunoassay.

**Chemical methods** a third option rarely used.

# Reference values of $HbA_{1c}$

1. Less than 6% - Normal
2. 6 to 7.5% - Good control of DM
3. 7.6 to 9% - Unsatisfactory control
4. More than 9% - Very poor control

Values depend on the method of estimation

They vary from lab to lab.

Note if all GHb is measured instead of $HbA_{1c}$

WBG 12-15% less than plasma glucose. Loss of glucose **approx 5-7% per hour (5-10 mg/dL)**
Fasting blood glucose (FBG) should be 10 hour fast not 16 hrs
EDTA/Fluoride specimen is stable for 7 days is a closed tube at $4^0$C or 24 hours at 15-$25^0$C.
CSF should be analysed within 2 hours.
Hexokinase and GOD/POD methods are not suitable for urine.

**Clin Chem 2005; 51:1573-1576**
Harmonisation of POCT devices with laboratory use a factor of 1.11 to convert POCT values in whole blood to plasma values

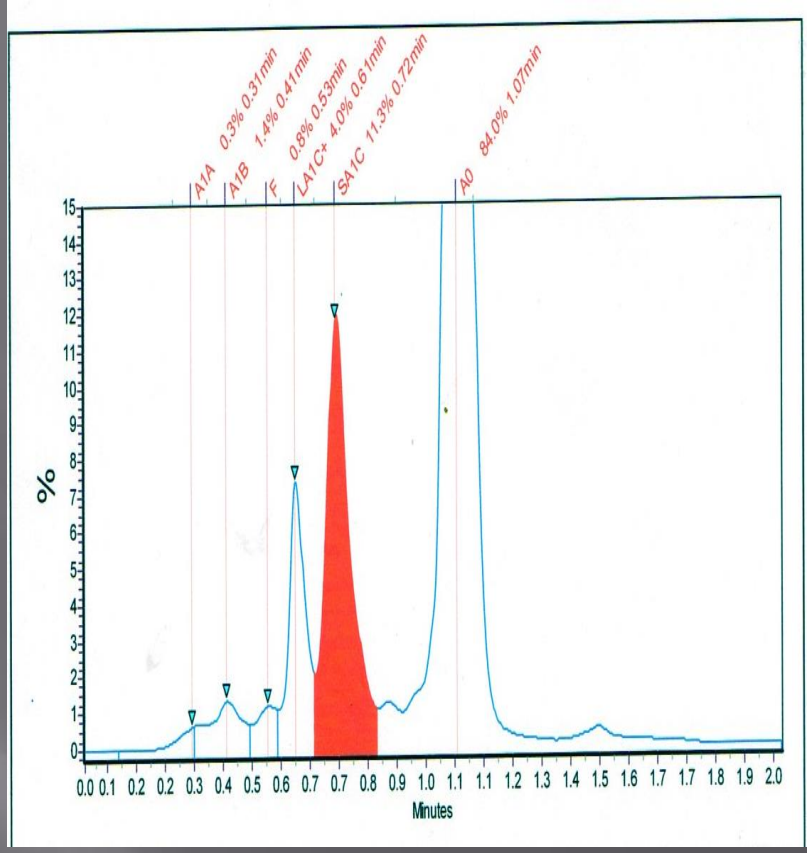
# Standardisation of HbA1c Assays.

HbA1c measured by ion-exchange chromatography as the glucose changes the charge on the haemoglobin molecule.

Originally calculated as a percentage of the HBA peak.

After the DCCT trial and the importance of meeting a set target was established a standardised approach was used to align methods to the DCCT method.

Not a true standard, so a reference standard was produced and a method assigned by isotope dilution mass spectrometry. Enables to report values in molar terms.



# Relationship between HbA1c and average finger blood glucose

| | | | | | |
|---|---|---|---|---|---|
| HbA1c of | 9% | = | 260 | mg/dl | (14.4 mmol/l) |
| HbA1c of | 8% | = | 220 | “ | (12.2 “ ) |
| HbA1c of | 7% | = | 180 | “ | (10.0 “ ) |
| HbA1c of | 6% | = | 140 | “ | (7.7 “ ) |
| HbA1c of | 5% | = | 100 | “ | (5.5 “ ) |

pretest preparation such as a diet or fasting, and is stable when collected in the appropriate specimen tube. HbA1c has recently been endorsed as a diagnostic test for diabetes by the World Health Organization, the International Diabetes Federation and the American Diabetes Association.14,15 The Australian Diabetes Society established an expert committee in 2011, including

**The test**

Analysis of venous HbA1c in UK laboratories participating in national quality assurance schemes currently fulfils WHO requirements. HbA1c should usually be measured on a laboratory venous blood sample. Point-of-care HbA1c should not be used for diagnosis unless the healthcare staff have been appropriately trained and the HbA1c method used can demonstrate an internal quality control and external quality assessment performance that matches that of a laboratory method. Confirm a point-of-care diabetes diagnosis with laboratory venous HbA1c

# How true ?

Once there was a tiger which boasted that it can run faster than any one.
One day he chased a rabbit and failed to catch it.
"All right" said the tiger; "of course I failed on my boast.
But, remember the rabbit was running for its life and I, for my dinner."
Now, decide who is the rabbit and who is the tiger - among we and our patient !

# Glycated Hb - GHb

Different types of Glycation products are formed from the $HbA_0$ depending on the carbohydrate moiety – namely

- $HbA_{1a1}$ - Fr 1,6 diphos –N-term. valine
- $HbA_{1a2}$ - Gl 6 phos –N-terminal valine
- $HbA_{1b}$ - Other CHO – N-term. valine
- $HbA_{1c}$ - Glucose –N-terminal valine

# Conditions which preclude HbA1c testing

Some Haemoglobin traits HbAS, Hb AC, Hb AE, Hb AD interfere with some methods but alternative methods  are available.

# Factors affecting $HbA_{1c}$

- Acute hyperglycemia
- Severe aneamia
- Gestational diabetes
- Life span of the RBC
- Abnormal Hb like S-Hb, Hb C
- Serum opalescence - ↑ TG
- On the method of estimation

Correlates with risk of developing microvascular complications

A reduced red blood cell survival time will lower the HbA1c level and may lead to a false negative result. Red blood cell survival time is reduced in any haemolytic anaemia, and it can also be reduced in chronic renal failure, severe liver disease and anaemia of chronic disease. Vitamin B12 and folic acid deficiencies may shorten red blood cell survival time. A common clinical situation that shortens red blood cell survival time occurs when patients undergo regular phlebotomy for medical indications (eg, haemochromatosis) or because they are regular blood donors. Iron deficiency may also have an impact on red blood cell survival and the HbA1c level. The congenital variants of the haemoglobin molecule (haemoglobinopathies), which may be relatively common in certain ethnic communities (eg, African, Mediterranean) in Australia, affect glycation

**WHO Recommendation 20111**

HbA1c can be used as a diagnostic test for diabetes providing that stringent quality assurance tests are in place and assays are standardised to criteria aligned to the international reference values, and there are no conditions present which preclude its accurate measurement.

An HbA1c of **48 mmol/mol (6.5%) is recommended as the cut point for diagnosing diabetes. A value of less than 48 mmol/mol (6.5%) does not exclude diabetes diagnosed using glucose tests.**

# 1-LACK OF STANDARDIZATION IN HEMOGLOBIN $A_1C$ ASSAYS

1978 – ASSAYS COMMERCIALLY AVAILABLE

CURRENTLY > 30 GLYCOHEMOGLOBIN ASSAY METHODS

- IMMUNOASSAYS
- ION-EXCHANGE HPLC
- BORONATE AFFINITY HPLC

WITH CONSIDERABLE DIVERGENCE EXISTING BETWEEN HBA1C RESULTS.