

HbA1c: what the GP needs to know

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Learning Objectives

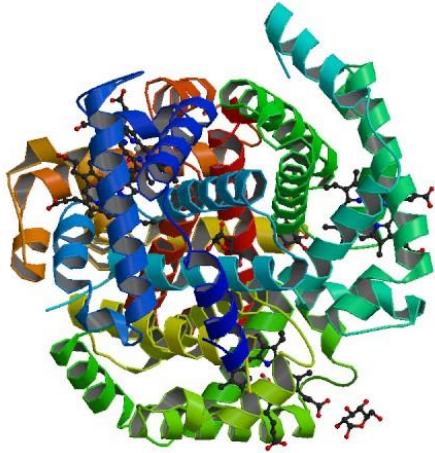
- Review
 - biochemistry of HbA1c
 - clinical role of HbA1c measurement
 - common interferences in HbA1c measurement
 - haemoglobin variants and how they may affect HbA1c
 - when HbA1c measurement is invalid
 - alternative measures of glycaemia
 - cases

Glycated Haemoglobin

- Hb A (2 α & 2 β) 97%
- Hb F (2 α & 2 γ)
- Hb A2 (2 α & 2 δ)

Allen 1958	{	Hb A _{1a1}	Fructose 1,6 diphosphate	~0.2%
		Hb A _{1a2}	Glucose - 6 – phosphate	~0.2%
McDonald 1978	{	Hb A _{1b}	Pyruvic acid	
		Hb A _{1c}	Glucose	~ 5%

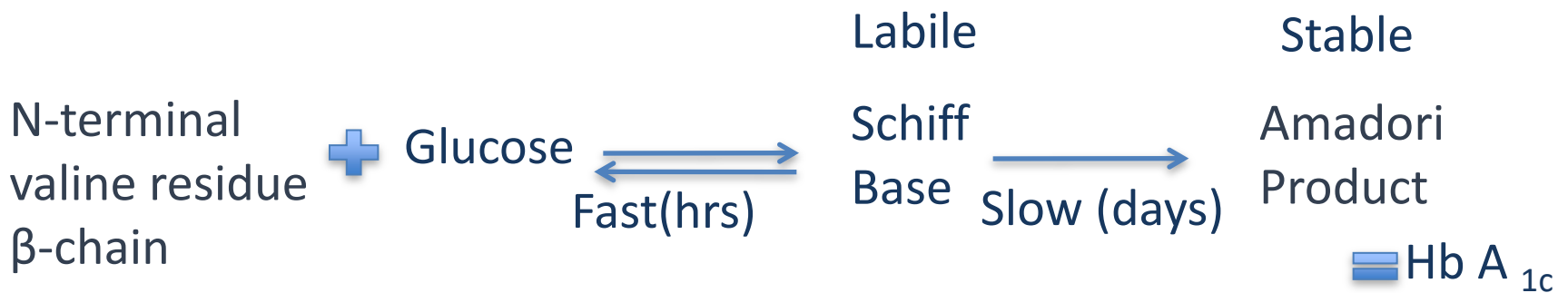
Note: The Hb A_{1c} row is highlighted with a green border in the original image.



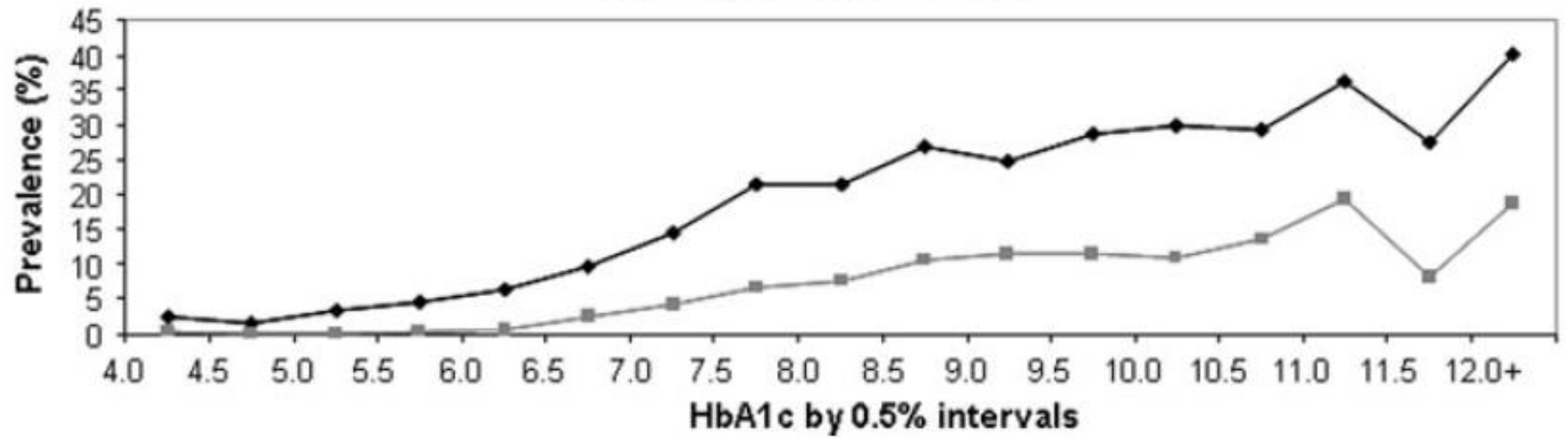
HbA_{1c}

- Reflect last 3 months of glycaemia
- Biased to the 30 days preceding measurement

- Glycated NOT glycosylated (enzymatic)
- Therefore linear relationship
- Irreversible reaction



DETECT-2 Study



The role of HbA1c

Monitoring of any type of diabetes

→ Every 3 months

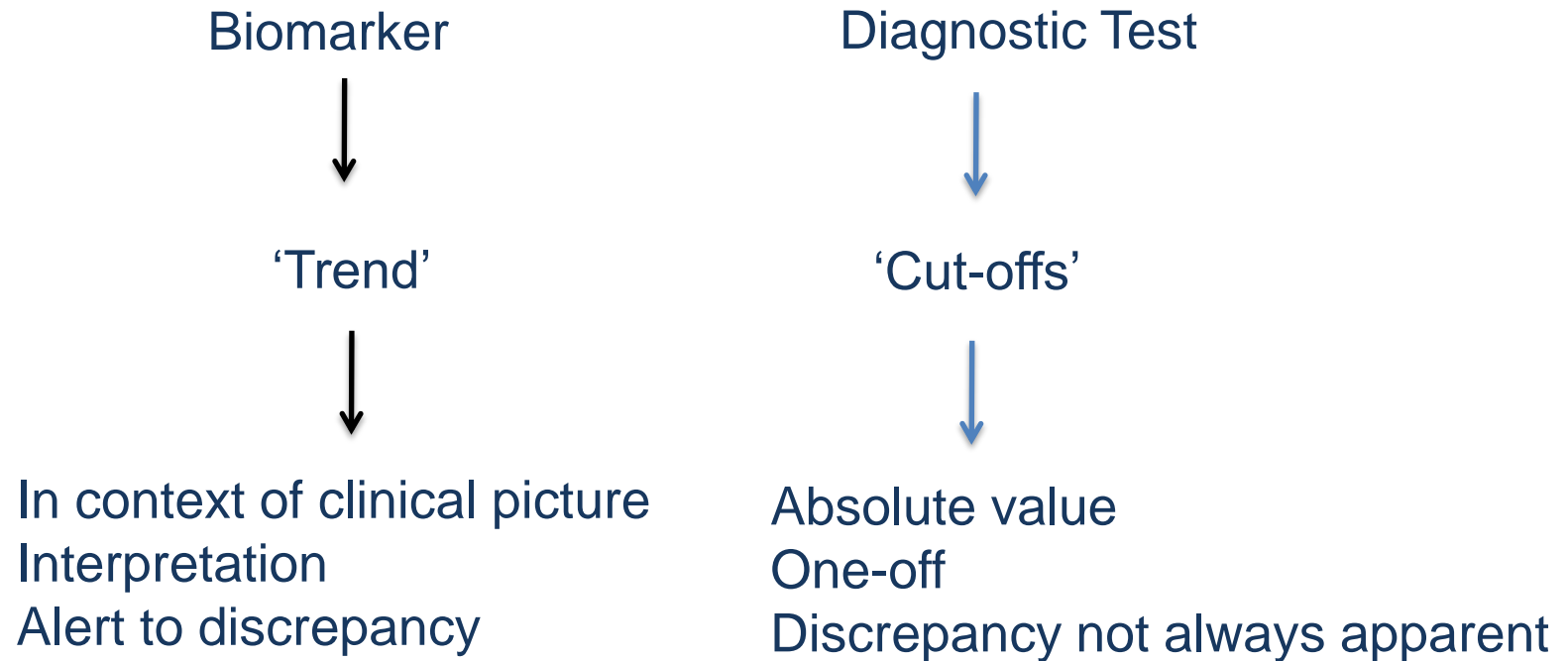
Diagnosis of type 2 diabetes

→ ≥ 48 mmol/mol + symptoms
or
 ≥ 48 mmol/mol on 2 occasions

Identification of non-diabetic hyperglycaemia

→ 42-47 mmol/mol

Changing role of HbA1c



What interferes with HbA1c?

- Anything that affects red cell turnover

1. Erythropoiesis

Increased HbA1c: iron, vitamin B12 deficiency, decreased erythropoiesis.

Decreased HbA1c: administration of erythropoietin, iron, vitamin B12, reticulocytosis, chronic liver disease.

2. Altered Haemoglobin

Genetic or chemical alterations in haemoglobin: haemoglobinopathies, HbF, methaemoglobin, may increase or decrease HbA1c.

3. Glycation

Increased HbA1c: alcoholism, chronic renal failure, decreased intra-erythrocyte pH.

Decreased HbA1c: aspirin, vitamin C and E, certain haemoglobinopathies, increased intra-erythrocyte pH.

Variable HbA1c: genetic determinants.

4. Erythrocyte destruction

Increased HbA1c: increased erythrocyte life span: Splenectomy.

Decreased A1c: decreased erythrocyte life span: haemoglobinopathies, splenomegaly, rheumatoid arthritis or drugs such as antiretrovirals, ribavirin and dapsone.

Interferences

- *In vivo*
 - The HbA1c level is affected **in the body**, leading to a higher or lower level that does not accurately reflect true glycaemia
- *In vitro*
 - The HbA1c level is affected **during the measurement process**, which leads to a higher or lower level that does not accurately reflect true glycaemia

Hb Variants

- >1200 haemoglobin variants
- Frequency
 - Common
 - HbS, HbC, HbD
 - Rare
 - Hb Camperdown, Hb Woolwich, Hb Sherwood Forest etc.
- Affect red cell turnover
 - Some affect
 - HbS or Hb C etc
 - Silent
 - No **known** affect on red cell turnover
 - (Most not studied)

Type of variants

- Hb XX or Hb XZ:
 - homozygote of compound heterozygote
 - No HbA present
- HbAX
 - Heterozygous
 - HbA present + HbX
- HbA + other problem

- The patient does not make HbA

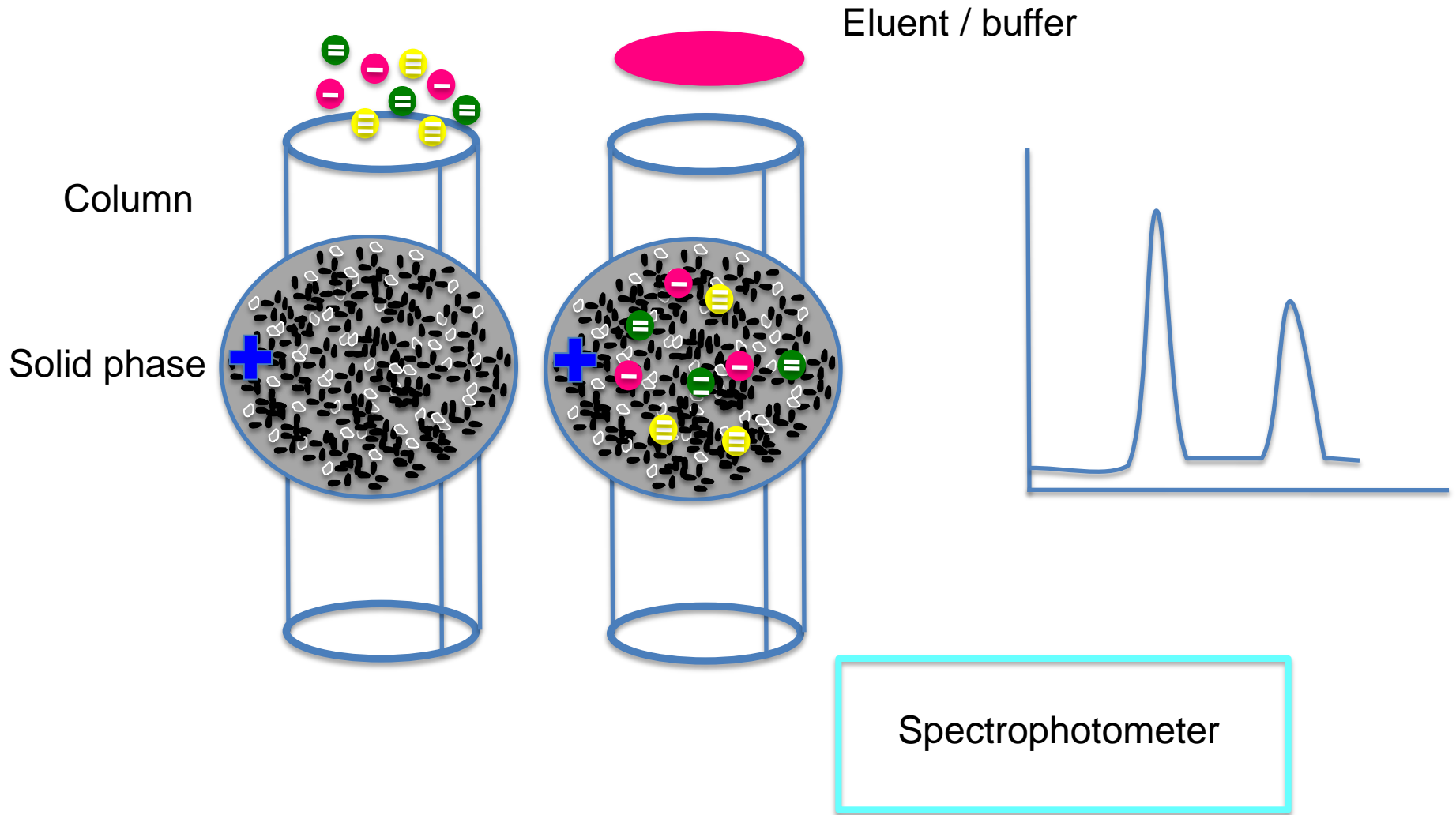
- If a person is a homozygote for a particular Hb variant e.g. Hb SS
 - They will not make HbA1c
 - They will theoretically make HbS1c
- If a person is a compound heterozygote for a particular Hb variant e.g. Hb SC
 - They will not make HbA1c
 - They will theoretically make HbS1c and HbC1c

- Requesting an HbA1c in a homozygote or compound heterozygote at NWLP:
 - would not be able to provide a result, as no HbA1c is generated
 - There is no value in measuring the glycated variant
 - But, if you've worked somewhere else you may have received a result in the past
 - Why the discrepancy?
 - Who is right?

Our method

- Tosoh G8
- Anion exchange HPLC
- Very specifically only identifies HbA1c

Anion exchange chromatography



Other methods

- Some methods
 - Don't identify the presence of Hb variants
 - Don't specifically measure HbA1c, but instead identify any Hb with glucose attached (glycohaemoglobin)
 - Marketed as *'not susceptible to interference from Hb variants'*

Which is better?

- Our expert opinion
 - It's better to know about potential Hb variants that can affect red cell turnover than to fly blind
 - We **do not** advocate measuring glycohaemoglobin in these situations as the result is meaningless
 - It is unknown if glycated variants have the same relationship with microvascular complications
 - A falsely high or low result may result in erroneous management decisions

- Patient is a heterozygote for a particular Hb variant:

Key question

- In most cases of heterozygotes for a particular variant, we can issue an HbA1c result.
- However, is this HbA1c an accurate measure of glycaemia?
 - Is there abnormal red cell turnover?
 - Is there a discrepancy between HbA1c and blood glucose monitoring?

- There may be inaccuracies in HbA1c levels in the presence of a variant
 - Therefore NOT to be used for diagnosis as cannot be confident of absolute values
 - Assuming variant stable, can be used for monitoring of diabetes

- Hb A + Other

- High HbF
 - may signify abnormal red cell turnover
 - we flag and do not report HbA1c in these situations
- Other abnormal peaks detected
 - we may corroborate the result using an alternative method

Comments that accompany results

- Standard variant comment
 - *“This sample shows a haemoglobin variant. Please send for Hb electrophoresis if appropriate. Result should only be used for diabetes monitoring and not diagnosis”*
- Homozygous cases
 - “Patient exhibits a haemoglobin variant and does not make HbA, therefore measurement of HbA1c is invalid”

Comment

No variant comment

No HbA

Variant comment

Concerns that variant may be interfering with HbA1c in vitro

Clinical question

Is there a discrepancy between HBGM & result?

Does the patient need haematological investigations?

Clear separation of variant from HbA1c peak

Is there evidence of a red cell turnover issue?

HbA1c by alternative method

Action

Can be used for diagnosis and monitoring

Need different modality to measure glycaemic control

Yes

No

Can only be used for monitoring if variant stable

Alternatives to HbA1c

- Monitoring
 - Home blood glucose monitoring
 - 7-point profile gives a good indication of control in most individuals
 - Continuous glucose monitoring
 - in more complex cases, e.g. insulin treated, referral to a Diabetologist for CGM may be warranted (ICHNT team happy to receive referrals)

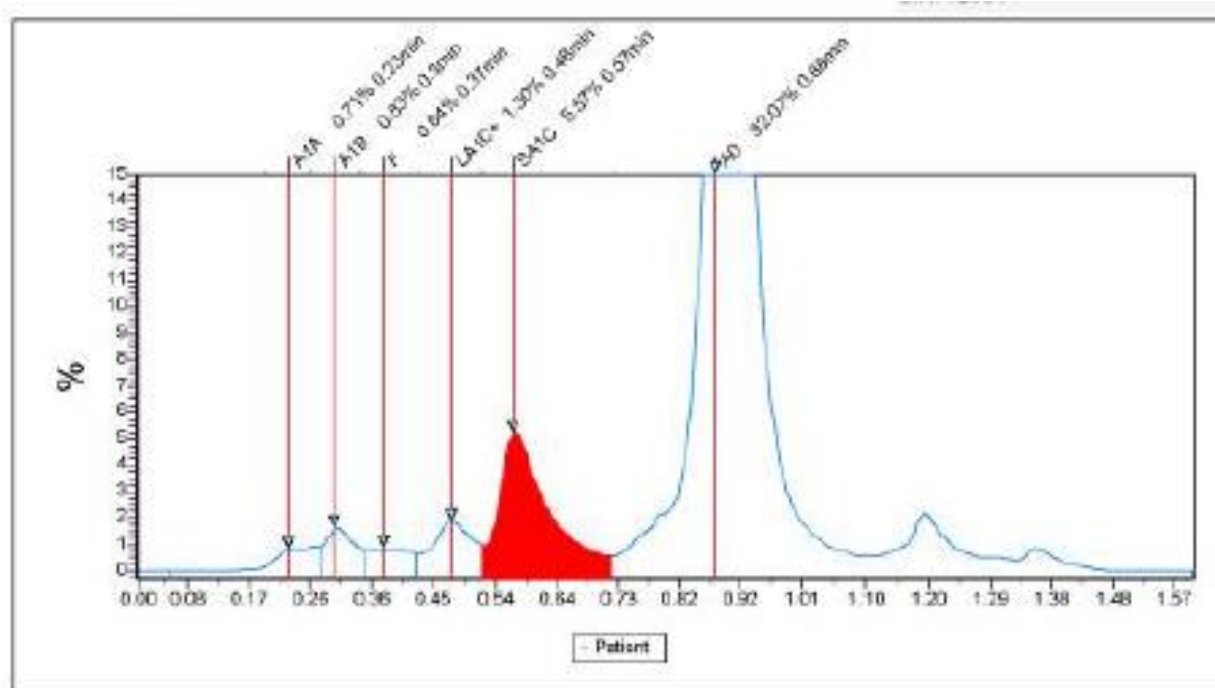
- We do not advocate measurement of fructosamine
 - it is not validated as a measure of glycaemia i.e. we do not know what the target should be
 - it is affected by CKD and proteinuria
 - assays are imprecise

Alternatives to HbA1c

- Diagnosis
 - 2 hour- OGTT
 - fasting glucose

Cases

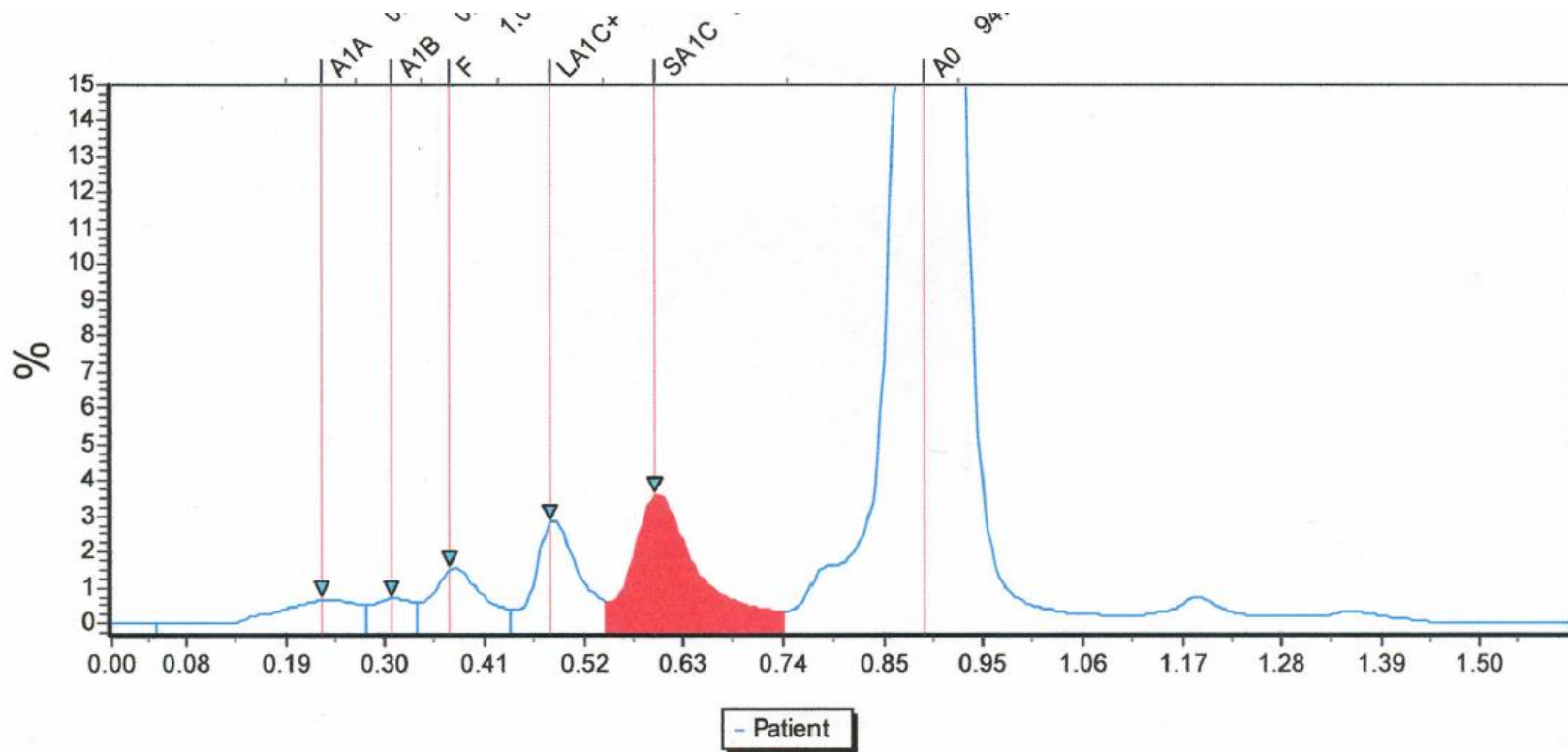
- A normal chromatogram



56 year old man T2DM

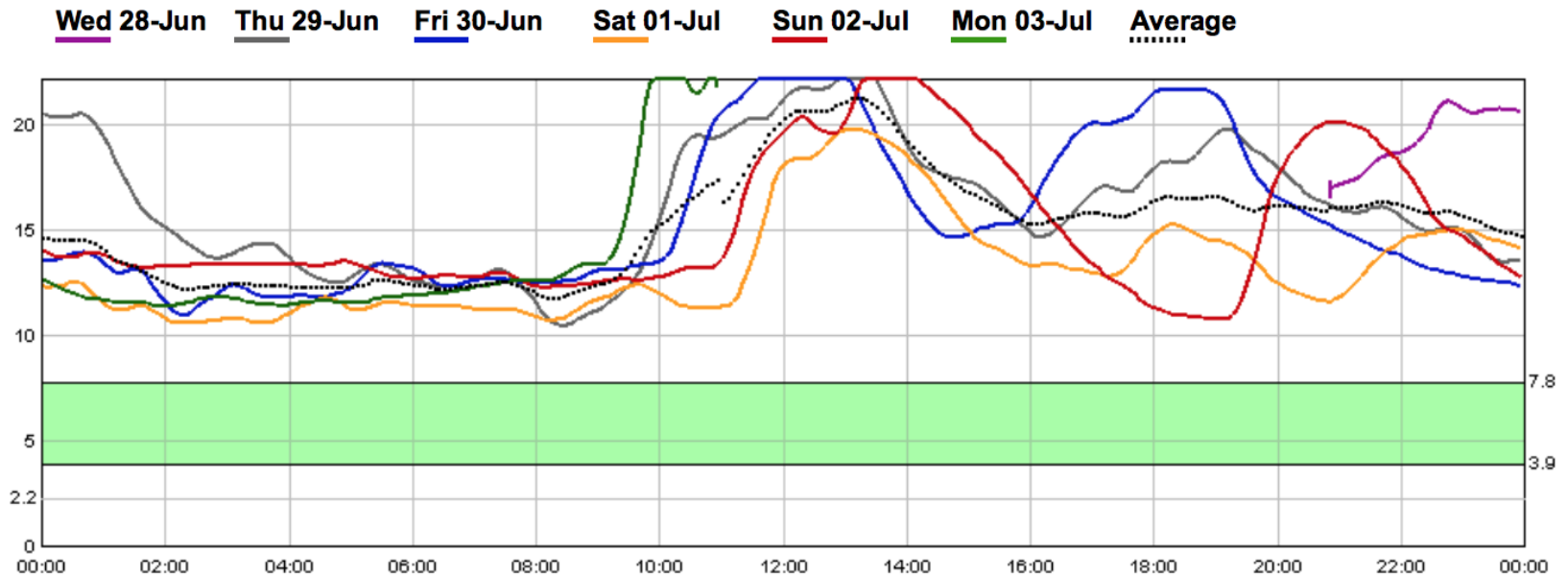
- HbA1c 29 mmol/mol
- HBGM: 10-12 mmol/L
- Previous HbA1c 52 mmol/mol

Normal chromatogram, but small HbA1c peak



Referred to CX diabetes by GP

Sensor Data (mmol/L)



- Raised reticulocytes
- Anaemia

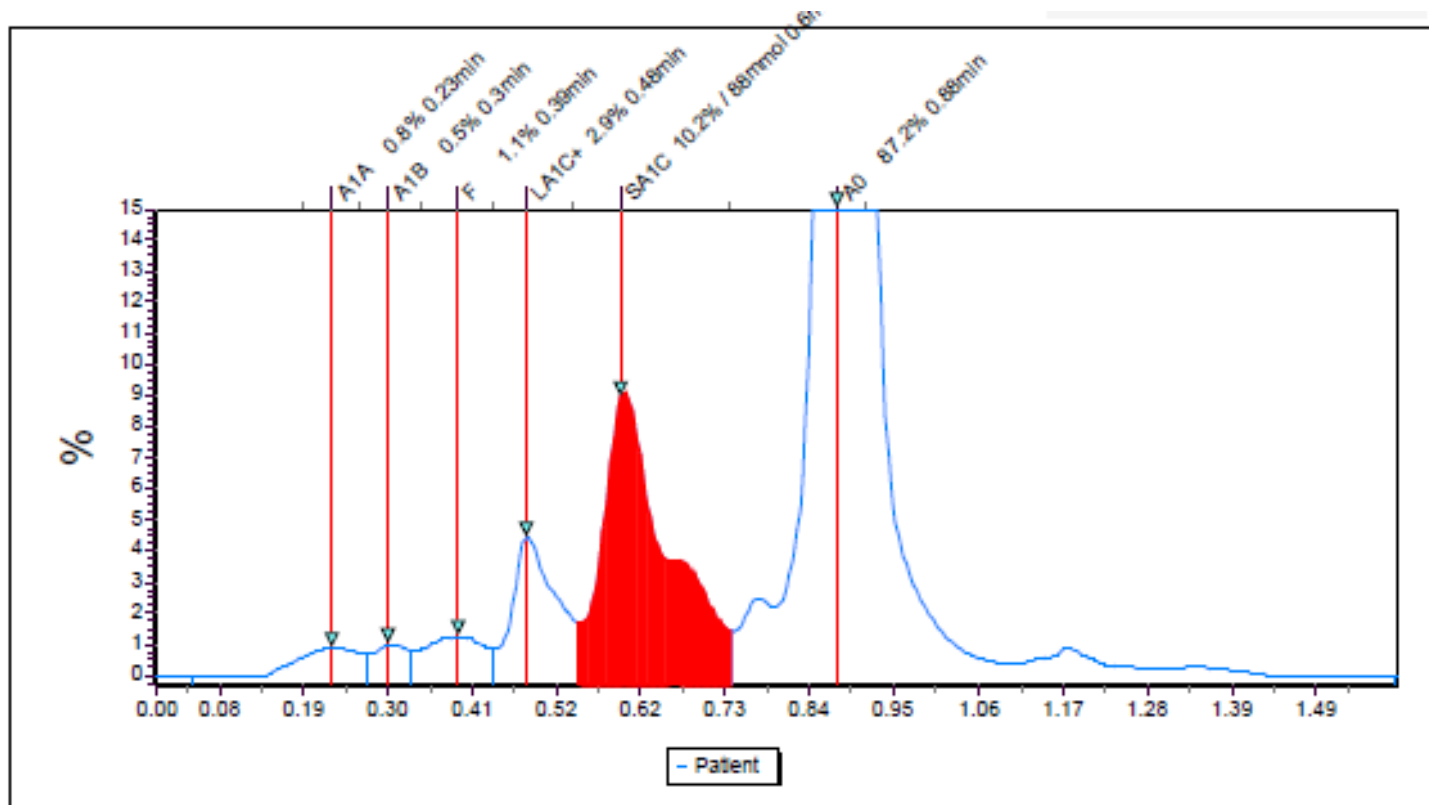
- Haemolysing on dapsone

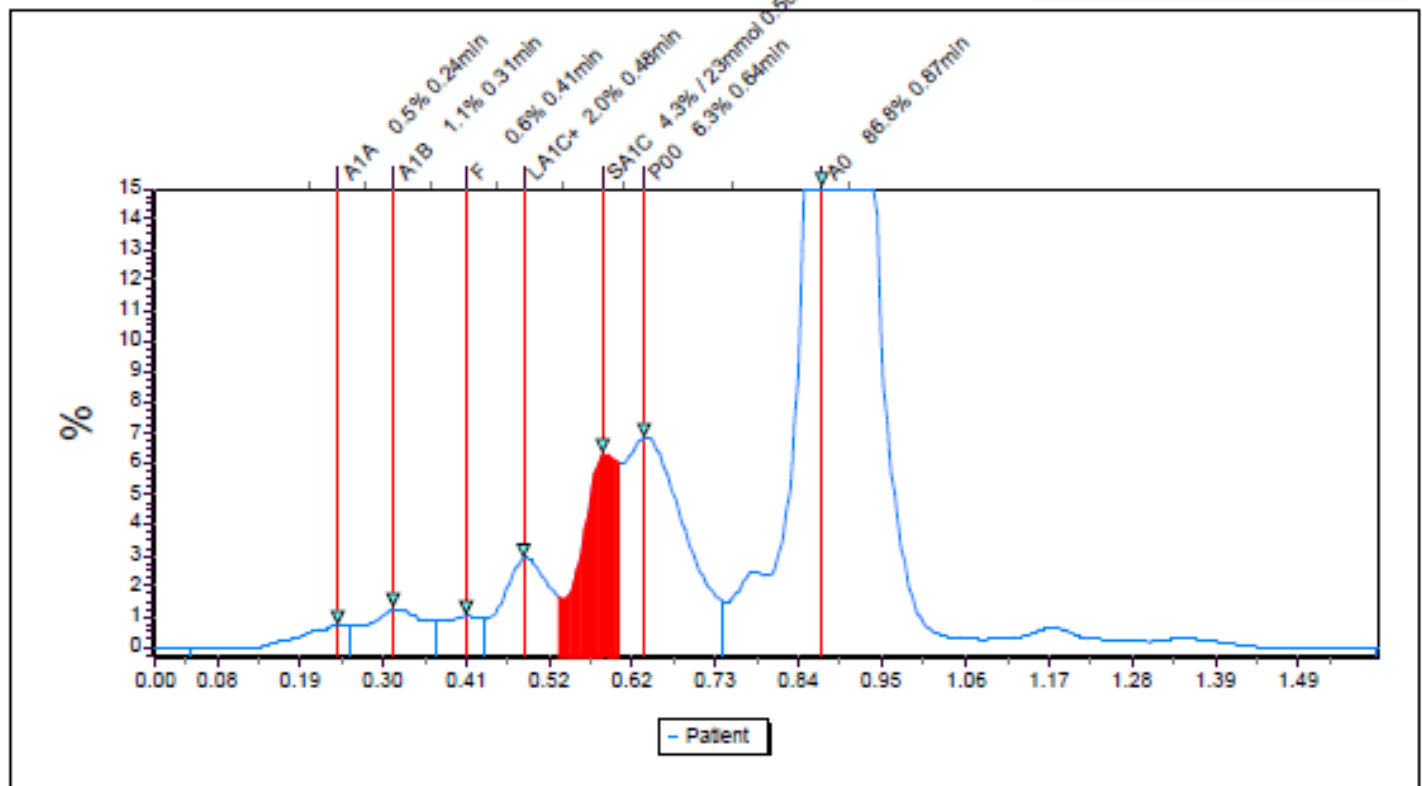
Haemolysis

- A cause of low HbA1c
 - Drugs
 - Haemolytic anaemia
 - G6PD deficiency
- We will flag low HbA1c's <20 mmol/mol

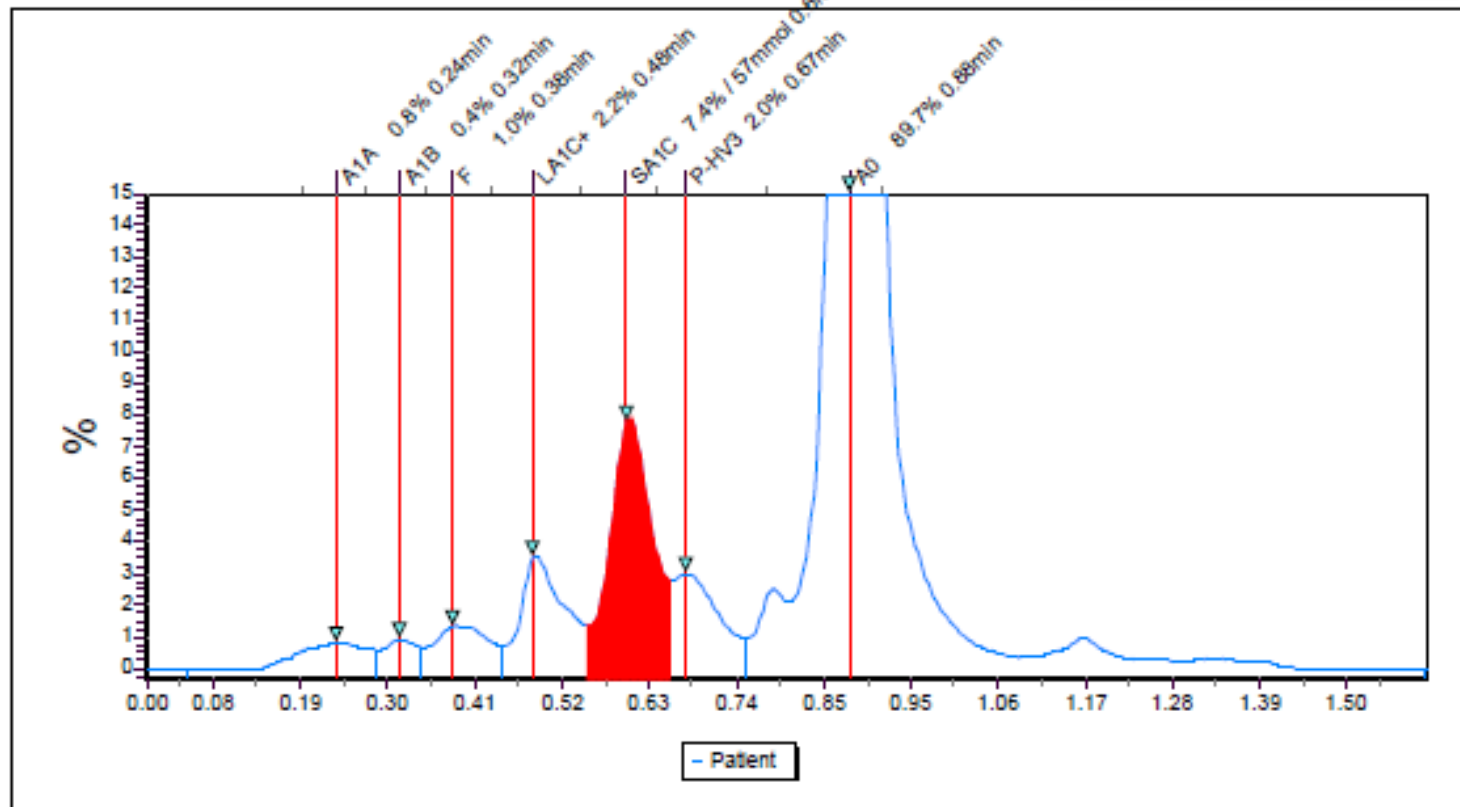
- Why do we sometimes report HbA1c, but subsequently add a variant comment?

HbA1c peak isn't always clear





Acceptable ranges for:



Acceptable range for:

Conclusion

- HbA1c is surrogate marker of glycaemia
- Affected by numerous factors
- Variants may or may not impact on result
- Cannot use HbA1c for diagnosis in the presence of a variant
- Query discordant HbA1c results

How to contact us

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 - Professor Tricia Tan
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