Glycated Haemoglobin in Diagnosis and Monitoring of Diabetes Mellitus

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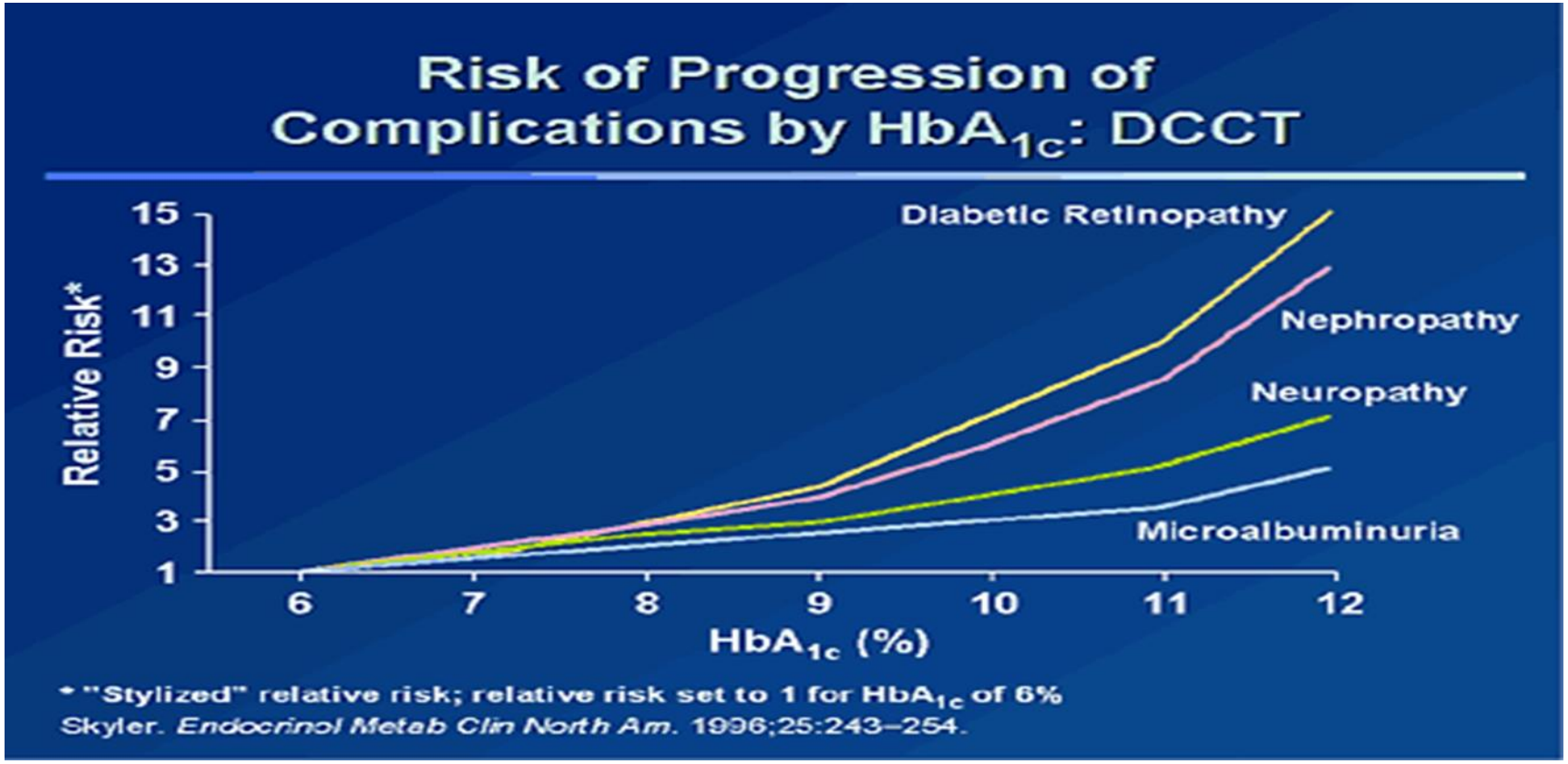
Learning Objectives

- The Use of HbA1c
- HbA1c measurement techniques
- Samples not suitable for reporting
- Alternative Monitoring Strategies
- The effects of disease states on HbA1c
- How we manage variant samples at Imperial College Healthcare NHS Trust

HbA1c - a brief History

- HbA1c value average glucose concentration over the previous 2 - 3 months
- Use of % (DCCT) HbA1c has now been superseded by mmol/mol (SI unit)
- 2011 WHO - glycated Haemglobin as a Diagnostic test for **Type 2 Diabetes**
- DCCT trial showed that a 10% reduction of total HbA1c correlated with a 45% lower risk of retinopathy (microvascular complication)
- UKPDS showed for every % drop in HbA1c there was a 35% drop in risk in microvscular complications
- Both studies showed that better significant outcomes with treating micro vascular complications vs macrovascular initially, however risk of MI had significantly reduced in the UKPDS trial in a ten year follow-up

HbA1c - a brief History



HbA1c – The diagnostic tool

- Better pre-analytical stability
- Lack of diurnal variation
- Lack of biological variation

	HbA1c	Fasting Glucose	2h OGTT
Within-subject biological variation	3.6%	5.7%	16.3%

Selvin E *et al Arch Internal Med 2007; 167: 1545-1551*

- Method standardisation (method should be calibrated against IFCC reference material) for method a method to be used for diagnosis we need the most accurate and precise methodology

HbA1c is accepted for the diagnosis of type 2 diabetes in the UK

Indications for use

DO NOT USE to diagnose

type 1 diabetes
childhood,
pregnancy,
renal failure,
haemoglobinopathy trait,
anaemia,
HIV,
abnormal red cell turnover, or any recent drug treatment likely to affect glycaemia or red-cell turnover.

Type 2 diabetes diagnosis WHO: ≥48 mmol/mol with second indicator (either symptomatic or laboratory).

Type 2 diabetes mellitus NICE CG66 treatment target 48 - 59 mmol/mol.

The effects of disease states on HbA1c

Effects	Raised HbA1c	Lower HbA1c	Variable HbA1c
Red Cell Life span	Increased red cell survival: Previous splenectomy Iron deficiency anaemia Erythropoietin B12 treatment Iron treatment Erythropoiesis reduction	Red cell destruction: Splenomegaly Rheumatoid arthritis Haemolysis Drugs such as ribavirin or antiretrovirals	Haemoglobinopathies Hb F MetHb
Glycation	Alcoholism Chronic renal failure	Aspirin Vitamin C and E Haemoglobinopathies	Genetic heterogeneity
Analytical interference	Hyperbilirubinaemia High dose aspirin Opiates Carbamylated Hb	Hypertriglyceridaemia	Haemoglobinopathies

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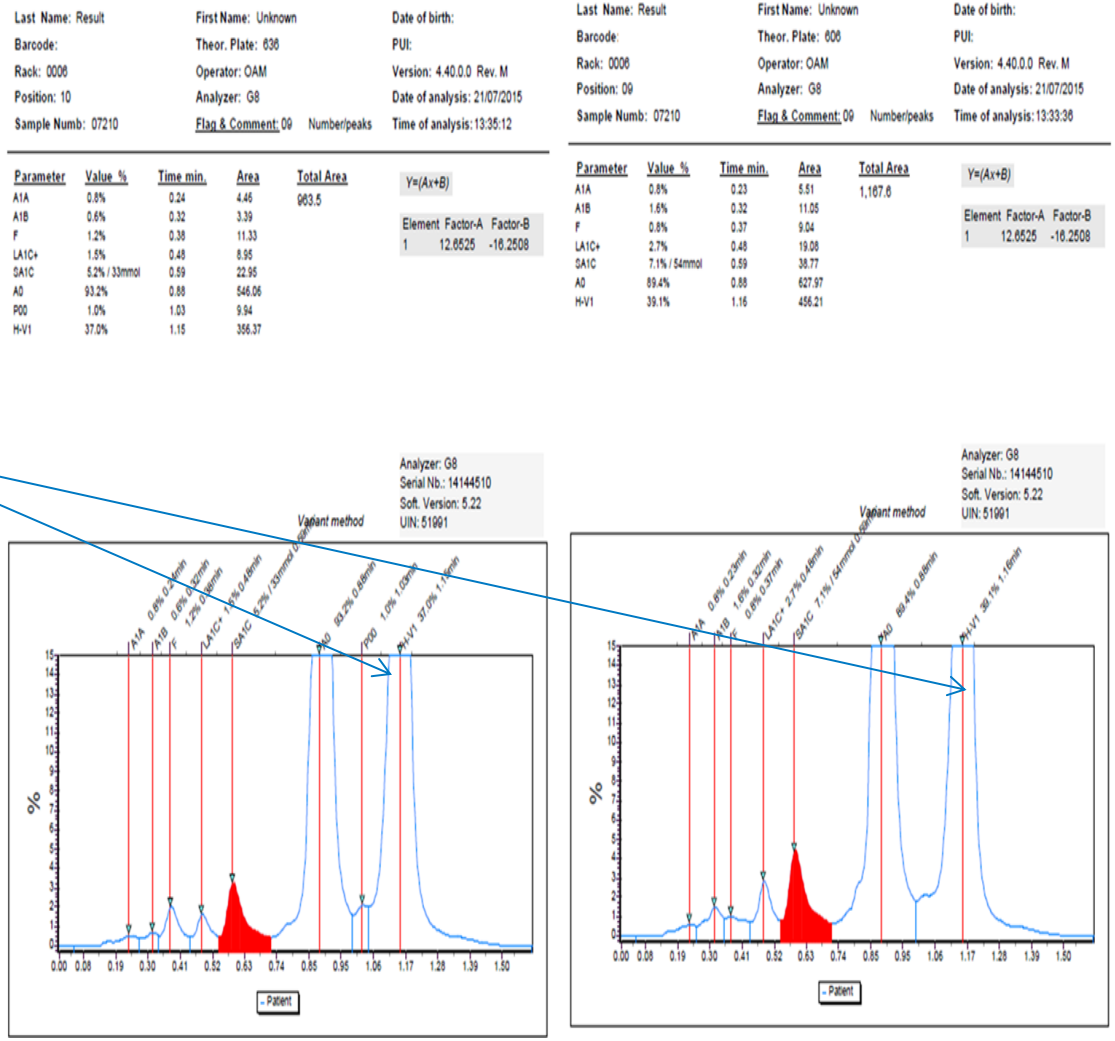
GB-2

Chromatograms with Presumptive Variants

H-V0 presumptive D trait
~0.98-1.06 mins

H-V1 presumptive S trait
~1.13-1.22

H-V2 presumptive C trait
~1.26-1.35



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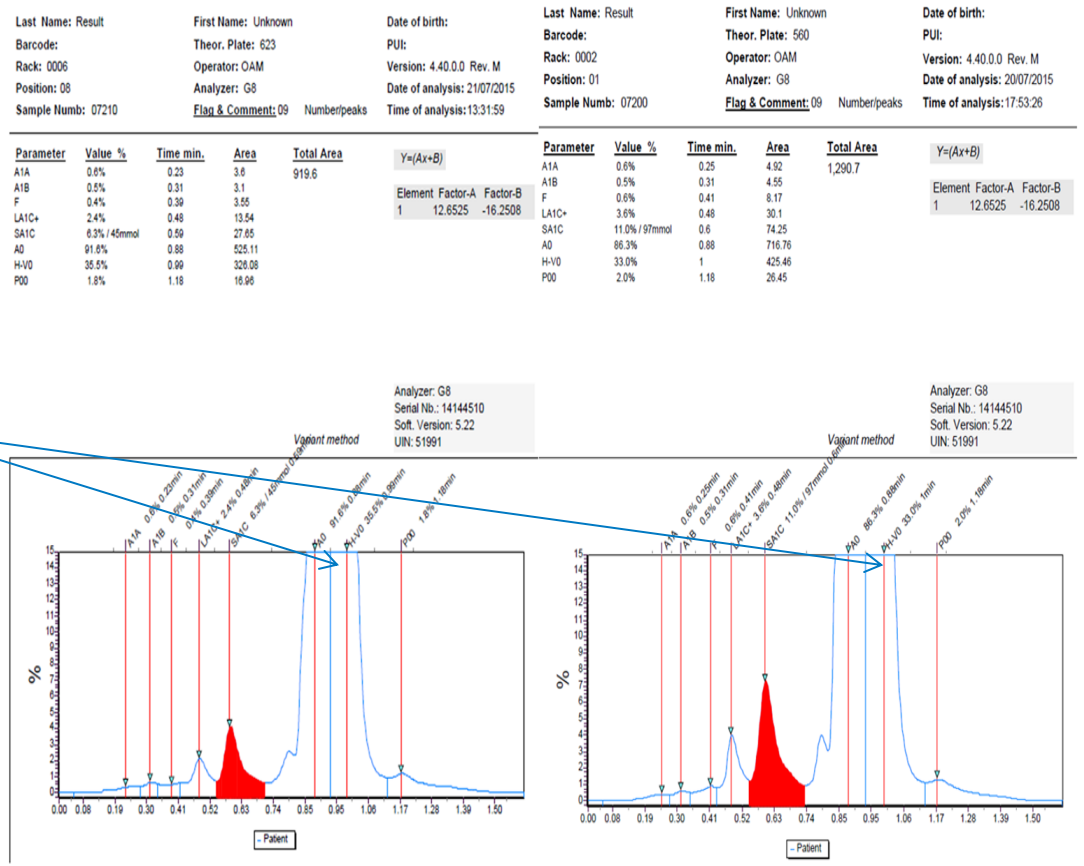


Last Name: Result
Barcode:
Rack: 0006
Position: 08
Sample Numb: 07210
First Name: Unknown
Theor. Plate: 623
Operator: OAM
Analyzer: G8
Flag & Comment: 09 Number/peaks
Date of birth:
PUI:
Version: 4.40.0.0 Rev. M
Date of analysis: 21/07/2015
Time of analysis: 13:31:59

Parameter	Value %	Time min.	Area	Total Area
A1A	0.6%	0.23	3.6	919.6
A1B	0.5%	0.31	3.1	
F	0.4%	0.39	3.55	
LA1C+	2.4%	0.48	13.54	
SA1C	6.3% / 45mmol	0.59	27.65	
A0	91.6%	0.88	525.11	
H-V0	35.5%	0.99	326.08	
P00	1.8%	1.18	16.96	

Y=(Ax+B)

Element	Factor-A	Factor-B
1	12.6525	-16.2508

Last Name: Result
Barcode:
Rack: 0002
Position: 01
Sample Numb: 07200
First Name: Unknown
Theor. Plate: 560
Operator: OAM
Analyzer: G8
Flag & Comment: 09 Number/peaks
Date of birth:
PUI:
Version: 4.40.0.0 Rev. M
Date of analysis: 20/07/2015
Time of analysis: 17:53:26

Parameter	Value %	Time min.	Area	Total Area
A1A	0.6%	0.25	4.92	1,290.7
A1B	0.5%	0.31	4.55	
F	0.6%	0.41	8.17	
LA1C+	3.6%	0.48	30.1	
SA1C	11.0% / 97mmol	0.6	74.25	
A0	86.3%	0.88	716.76	
H-V0	33.0%	1	425.46	
P00	2.0%	1.18	26.45	

Y=(Ax+B)

Element	Factor-A	Factor-B
1	12.6525	-16.2508

- HbA1c results are reported with comments below
- Sample with variant traits detected should only be used for monitoring due to their effect on analytical accuracy of results

Samples on first presentation

‘This sample shows a haemoglobin variant. Please send for Hb electrophoresis in order to type variant, if appropriate. **Result should only be used for individual diabetes monitoring and not for diagnosis**

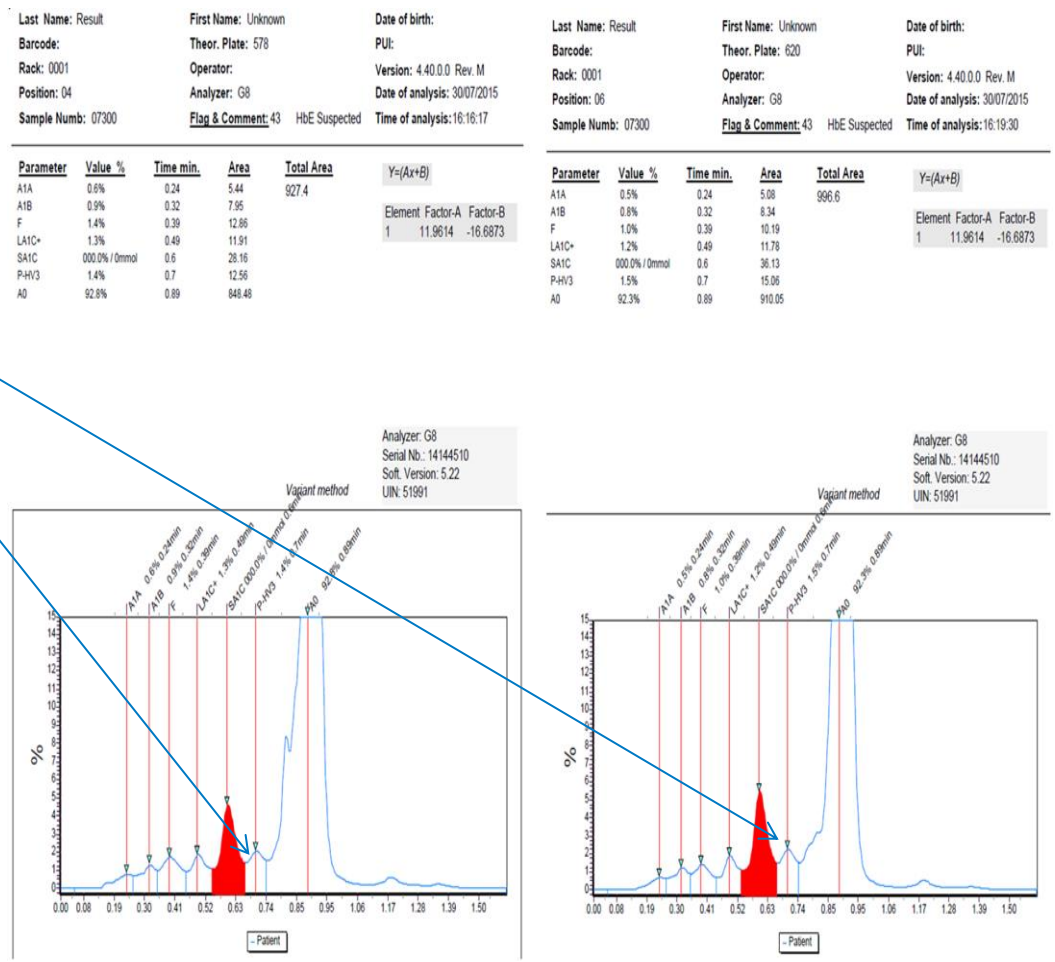
Samples with known variant

Known Hb variant – please see previous report

Samples we can't do by our method

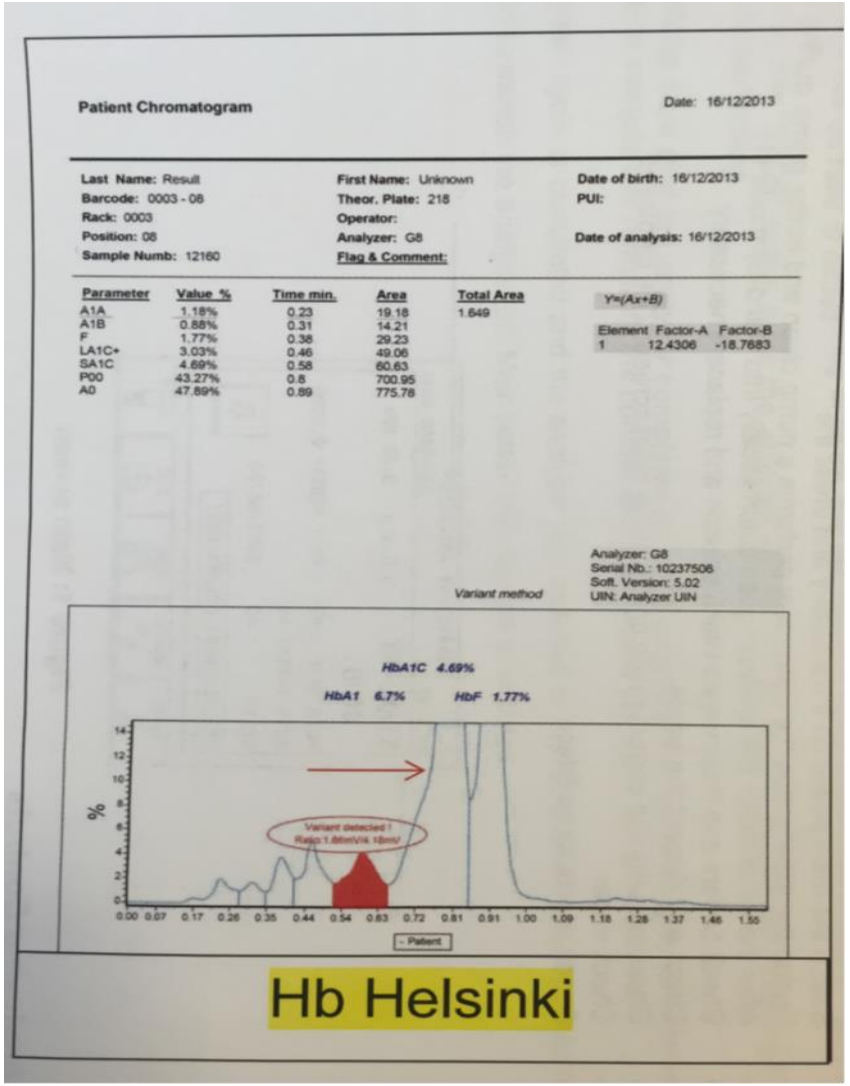
HbE variants

- Sample has been sent for alternative method as our method is affected by the particular variant. The total glycohaemoglobin result can be used for monitoring purposes only and not for **diagnosis of diabetes**.



Samples we can't do by our method

- Other "Fast" variants also cause a problem in HbA1c reporting by our method.
- The Lab will deal with these on a case by case basis.
- These samples will normally be sent away
- But we may contact you to discuss the use of HbA1c (diagnostic or monitoring)
- Known ones will always be sent away



The Implications of Hb variants in HbA1c diagnosis

- Whilst the analytical implications of haemoglobin variants have been well characterised, their effect on red blood cell survival or glycation has not always been
  Well understood. – why HbA1c cannot be used for diagnosis
- Haemoglobinopathies approximately 7% of the world population is a carrier.
- There are over 1586 recognised variants

Patients where Glycated Haemoglobin cannot be used

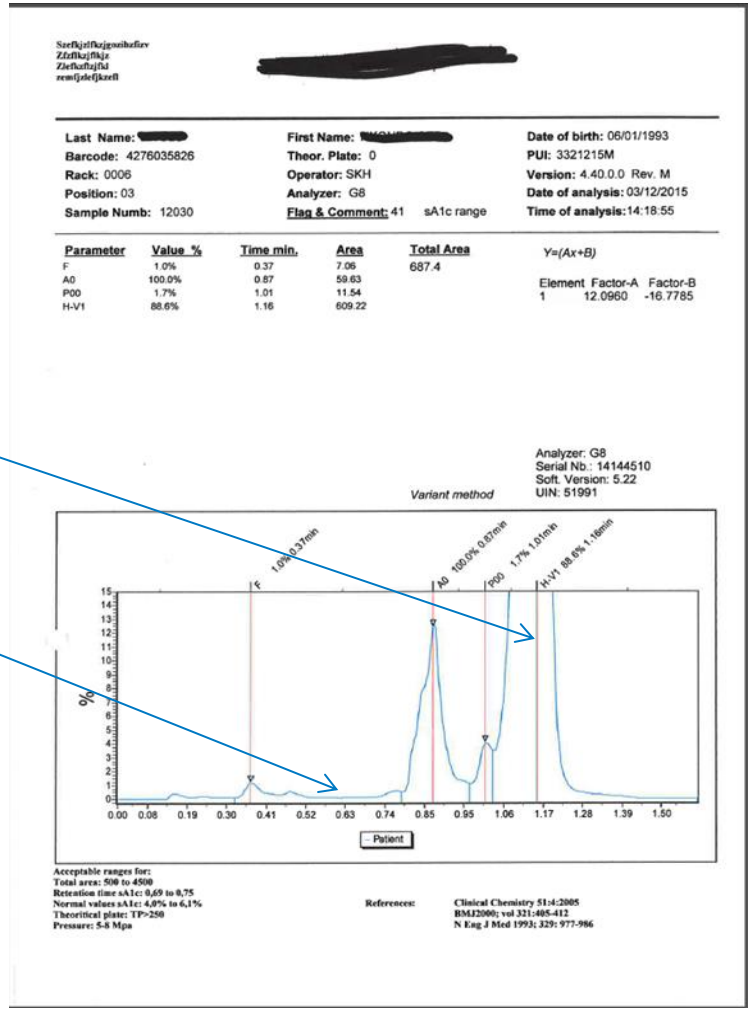
- Patient exhibits a haemoglobin variant and does not make HbA, therefore measurement of HbA1c is invalid. If required please contact the duty biochemist on 0203 313 0348 to discuss alternatives of assessing glycaemic control

- *No HbA1c produced*
- *You may consider sending a sample for Hb electrophoresis for formal diagnosis, if appropriate.*
- *A total glycohaemoglobin is not valid in these cases as no HbA1c is being made and measures of other glycated species will be affected by abnormal red cell turnover. Also reference range is aligned to A1C and not other glycated species HbS or E.*
- *use venous glucose or OGTT for diagnosis of diabetes in these cases. For monitoring they should refer the patient to a specialist diabetes team for further input*

Patients where Glycated Haemoglobin cannot be used

Presumptive SS

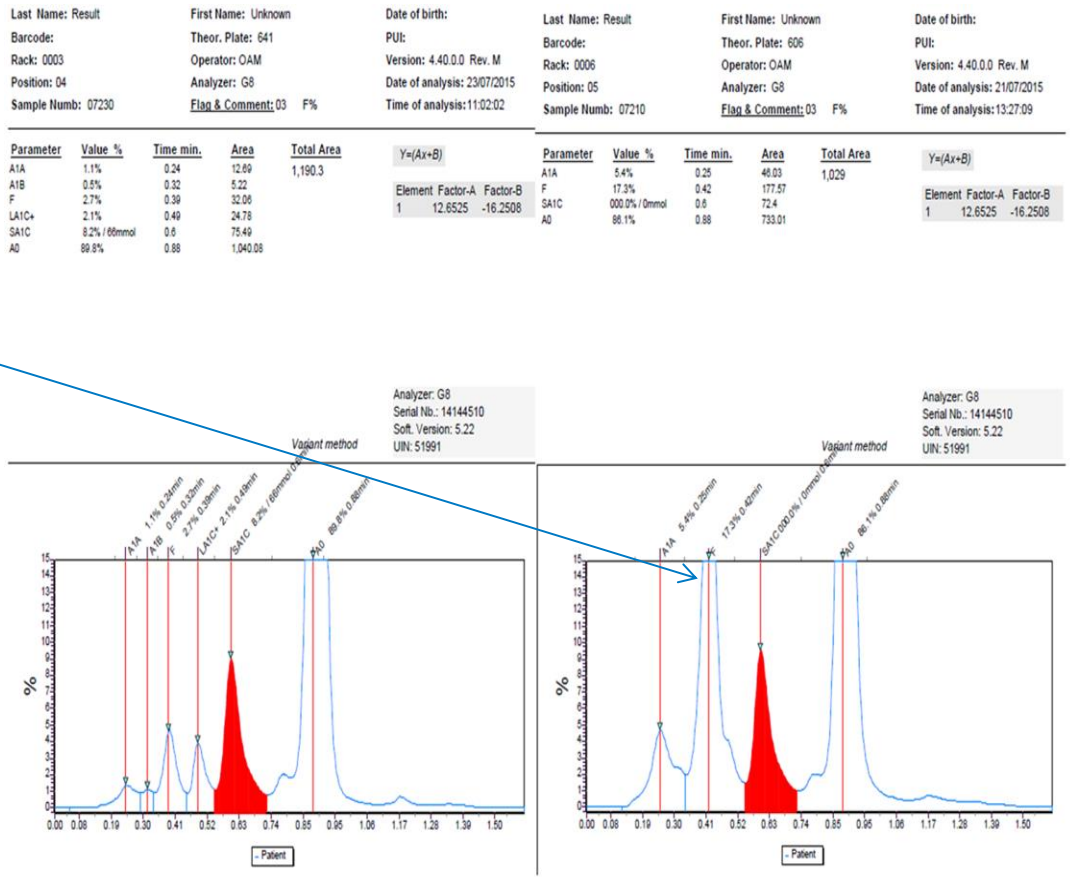
no HbA1c present



Patients where Glycated Haemoglobin cannot be used

HbF >15%

HbF > 15% This patient has a raised fetal haemoglobin level, which may signify abnormal red cell turnover. HbA1c measurement is invalid. If required please contact the duty biochemist on 0203 313 0348 to discuss alternatives of assessing glycaemic control.



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Barcode:
Rack: 0003
Position: 04
Sample Numb: 07230
First Name: Unknown
Theor. Plate: 641
Operator: OAM
Analyzer: G8
Flag & Comment: 03 F%
Date of birth:
PUI:
Version: 4.40.0.0 Rev. M
Date of analysis: 23/07/2015
Time of analysis: 11:02:02

Parameter	Value %	Time min.	Area	Total Area
A1A	1.1%	0.24	12.69	1,190.3
A1B	0.5%	0.32	5.22	
F	2.7%	0.39	32.06	
LA1C+	2.1%	0.49	24.78	
SA1C	8.2% / 66mmol	0.6	75.49	
A0	89.8%	0.88	1,040.08	

Y=(Ax+B)

Element	Factor-A	Factor-B
1	12.6525	-16.2508

Last Name: Result
Barcode:
Rack: 0006
Position: 05
Sample Numb: 07210
First Name: Unknown
Theor. Plate: 606
Operator: OAM
Analyzer: G8
Flag & Comment: 03 F%
Date of birth:
PUI:
Version: 4.40.0.0 Rev. M
Date of analysis: 21/07/2015
Time of analysis: 13:27:09

Parameter	Value %	Time min.	Area	Total Area
A1A	5.4%	0.25	46.03	1,029
F	17.3%	0.42	177.57	
SA1C	000.0% / 0mmol	0.6	72.4	
A0	86.1%	0.88	733.01	

Y=(Ax+B)

Element	Factor-A	Factor-B
1	12.6525	-16.2508

Analyzer: G8
Serial Nb.: 14144510
Soft. Version: 5.22
UIN: 51991
Variant method
A1A 1.1% 0.24min
A1B 0.5% 0.32min
F 2.7% 0.39min
LA1C+ 2.1% 0.49min
SA1C 8.2% / 66mmol 0.6min
A0 89.8% 0.88min
%
Patient

Analyzer: G8
Serial Nb.: 14144510
Soft. Version: 5.22
UIN: 51991
Variant method
A1A 5.4% 0.25min
F 17.3% 0.42min
SA1C 000.0% / 0mmol 0.6min
A0 86.1% 0.88min
%
Patient

J Diabetes Sci Technol. 2009 May; 3(3): 446–451.

Patients where Glycated Haemoglobin cannot be used

- HbF >15%
- If HbF> 15% this may signify abnormal red cell turnover.
- Advise discussion with haematology regarding further investigations.
- HbA1c testing is invalid due to the likely underlying abnormal red cell turnover, which may falsely increase or decrease HbA1c.
- Use venous glucose or OGTT for diagnosis of diabetes in these cases.
- For monitoring they should refer the patient to a specialist diabetes team for further input.
- J Diabetes Sci Technol. 2009 May; 3(3): 446–451. Boronate affinity not suitable for raised HbF

What are the alternatives if HbA1c is not available?

- *When we considering other glycation measures, please note that we do not recommend fructosamine or glycated albumin as alternative measures.*
- *lack of evidence base*
- *method standardization*
- *interferences and difficulty in results interpretation*

What are the alternatives HbA1c is not available?

Symptomatic	Asymptomatic
Single *fasting glucose ≥7 mmol/L	Two fasting glucose ≥7mmol/L
Single random glucose ≥11.1 mmol/L	Two random glucose ≥11.1 mmol/L
	~~Two HbA1c measurements ≥6.5% (≥ 48mmol/mol) (need to be at least 6 to 8 weeks apart)~~
	~~One HbA1c ≥ 6.5 % (≥48 mmol/mol)~~ and a concurrent measurement of fasting glucose ≥7 mmol/L or one random glucose ≥11.1 mmol/L
Impaired Fasting Glucose (IFG)	Fasting glucose of 6.1 – 6.9 mmol/L
Pre-Diabeties	~~HbA1c 6 – 6.4 % (42 – 47 mmol/mol)~~
Impaired Glucose Tolerance (IGT)	Fasting Glucose <7.0 mmol/L **AND** a 2 hr Plasma glucose (after 75g Oral glucose load) 7.8 – 11 mmol/L ***

In Summary:

There are 3 main reporting pathways we use at Imperial
1. Normal - Diagnosis and monitoring
2. Hb varrients – monitoring only
3. HbA1c not sutible in Compound heterozygous, variant homozygous, HbF >15%

Affinity Chromatography measures total glycohaemglobin and should only be used for monitoring in our case (patients with Fast varients and HbE trait)

Retrospective monitoring by Fructosamine and glycated albumin is not recommended.

In Summary:

Our Method for measuring HbA1c is rapid, aligned to DCCT and the IFCC – please be assured by the accuracy of your results to make a diagnosis

Our method gives you the assurance to use your HbA1c result with confidence knowing we have accounted for and possible variants

We process over 156’000 HbA1c samples a year of where extremely few patients cannot get a HbA1c result due to a variant

We have the expertise you need to look at alternatives for monitoring these patients

Thank you to The Specialist diabetes team for there assistance with this Process