Audit of HbA1c Analysis and Reporting in the Presence of Variant Haemoglobins in Scotland 2020

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The Association for Clinical Biochemistry & Laboratory Medicine
Scotland Audit Group

10 responses were received (71%) with 1 response covering 3 sites.

Q1 All responding labs performed HbA1c analysis on site:
1 stated Blood Sciences,
6 Biochemistry
2 Haematology
1 not stated.

Q2 4 respondents used the Menarini 8180, 5 the Tosoh G8 or G11 as their primary method and 1 respondent used the Sebia Capillarys 3 TERA.

Q3 3 respondents had an alternate HbA1c method available on-site or within the trust, however 1 of these used it only as a POCT analyser and not as an alternative for the primary method.

Q4 5 labs administer secondary care clinics, of which
2 use Menarini analysers and are staffed by BMS
1 uses a Tosoh analyser operated by lab staff
1 uses a Tosoh analyser operated by nursing staff and
1 uses the DCA operated by nursing staff.
1 lab also administers primary care clinics which are nurse-led and use the DCA Vantage.

Q5 All labs using more than 1 method apply the same reference interval to both:

Ref intervals:	27-47		1 lab
	<41	42-47 = High Risk	1 lab
	20-41		1 lab
	20-42		5 labs
	31-44		1 lab
	No ref int		1 lab

Q6 All 10 primary methods flag variants,
1 secondary method (DCA used by 2 labs) does not.

8 labs report that variants are indicated by analysers alerts,
5 by analyser alerts and scrutiny of chromatograms and
1 by scrutiny only.

Chromatograms are checked by BMS staff across the board with most labs specifying Band 6 and above. One lab states bands 4-8.

4 labs have no means of flagging known patients and rely on checking previous reports.

4 flag patients in the LIMS and 2 in the analyser software.

Does analyser indicated variant type? Yes 7 No 3

Q7 Half of labs keep both paper and electronic records of patients with variants,
4 keep only electronically and
1 keeps only a paper record.

Q8 8 labs will automatically perform an alternative analysis, either HbA1c by an unaffected method or fructosamine if sample available (or recommend fructosamine if sample not available).

1 lab stated fructosamine would only be analysed if requested by a diabetologist.

2 labs automatically perform Hb electrophoresis, 3 recommend boronate affinity 3 recommend fructosamine and 1 automatically performs immunoassay.

Q9 Hb electrophoresis is recommended by 9 of 10 labs, but most (7) will not automatically add it.

Q10 The majority of labs report variants in the results and/or comments field in their LIMS. 1 laboratory which automatically refers to Haem in own dept for Hb electrophoresis states that result is reported from Haematology. 2 labs contact the requesting clinician by letter, e-mail or phone in addition to reporting in LIMS and 1 refers variants to Haem consultant.

Q11

Result	HbA1c Reported		Comments
	Yes	No	
New finding of high HbF below manufacturer cut-off	10	0	1. Hb variant detected. Please interpret absolute HbA1c values with caution; results may be useful for monitoring trend. Suggest send EDTA sample to haematology for haemoglobin electrophoresis. Serum fructosamine may be more accurate for assessment of glycaemic control.*Or if a serum tube is available*: Hb variant detected. Please interpret absolute HbA1c values with caution; results may be useful for monitoring trend. Suggest send EDTA sample to haematology for haemoglobin electrophoresis. Serum sample sent for fructosamine for assessment of glycaemic control. 2. A small peak of HbF was detected (approx X%) but this did not interfere with HbA1c analysis. Suggest send EDTA sample to haematology for haemoglobin electrophoresis. 3. Increased level of HbF present. HbA1c result should not be used for diagnosis/exclusion of diabetes – check glucose level. HbA1c can be used to monitor diabetes. However usual target values may not apply. Consider discussion with haematologist/haemoglobin electrophoresis. • 7 labs would make no comment.
New finding of high HbF above manufacturer cut-off	5	5	1 Hb variant detected. Please interpret absolute HbA1c values with caution; results may be useful for monitoring trend. Suggest send EDTA sample to haematology for haemoglobin electrophoresis. Serum fructosamine may be more accurate for assessment of glycaemic control.*Or if a serum tube is available*: Hb variant detected. Please interpret absolute HbA1c values with caution; results may be useful for monitoring trend. Suggest send EDTA sample to haematology for haemoglobin electrophoresis. Serum sample sent for fructosamine for assessment of glycaemic control. 2 High HbF which may interfere with HbA1c measurement. Interpret result with caution. 3 High levels of HbF detected. HbA1c result not reliable for diagnosis or management by WHO guidelines but may be used to follow trend in glycaemic control. For diagnosis suggest testing by fasting glucose. 4 Possible haemoglobin variant detected. Please send fresh EDTA sample for haemoglobinopathy screen.

			5 HbA1c cannot be measured due to high concentration of HbF. HbA1c result should not be used for diagnosis/exclusion of diabetes – check glucose level. If HbA1c requested for monitoring diabetes please send serum sample for fructosamine. • 2 labs would leave it to duty biochemist to make free text comment. • 2 labs would make no comment.
New finding of non-interfering variant	9	1	1 Hb variant detected. Please interpret absolute HbA1c values with caution; results may be useful for monitoring trend. Suggest send EDTA sample to haematology for haemoglobin electrophoresis. Serum fructosamine may be more accurate for assessment of glycaemic control.*Or if a serum tube is available*: Hb variant detected. Please interpret absolute HbA1c values with caution; results may be useful for monitoring trend. Suggest send EDTA sample to haematology for haemoglobin electrophoresis. Serum sample sent for fructosamine for assessment of glycaemic control. 2 Possible haemoglobin variant detected which does not interfere with HbA1c measurement. If new finding suggest send sample to haematology to confirm. 3 Abnormal separation on routine HbA1c method. Please consider referral for genetic variant counselling. 4 Abnormal chromatography on HbA1c analysis consistent with Haemoglobin variant. Result not reliable for diagnosis/screening by WHO guideline but may be used to follow trend in glycaemic control. For diagnosis suggest testing by fasting glucose. 5 Possible haemoglobin variant detected. Please send fresh EDTA sample for haemoglobinopathy screen. 6 Variant Hb detected. To investigate further please send EDTA sample to Haematology with a request for Haemoglobin Electrophoresis 7 Hb variant detected but HbA1c result still valid. Suggest send EDTA sample to haematology for haemoglobin electrophoresis 8 Haemoglobin variant present. HbA1c result should not be used for diagnosis/exclusion of diabetes – check glucose level. HbA1c can be used to monitor diabetes. However, usual target values may not apply. • 2 labs would make no comment.
New finding of interfering variant with reportable HbA1c	6	4	1 Hb variant detected. Please interpret absolute HbA1c values with caution; results may be useful for monitoring trend. Suggest send EDTA sample to haematology for haemoglobin electrophoresis. Serum fructosamine may be more accurate for assessment of glycaemic control.*Or if a serum tube is available*: Hb variant detected. Please interpret absolute HbA1c

			values with caution; results may be useful for monitoring trend. Suggest send EDTA sample to haematology for haemoglobin electrophoresis. Serum sample sent for fructosamine for assessment of glycaemic control. 2 HbA1c cannot be quantified due to abnormal separation of Hb peaks. Glycaemic status should be assessed by other means. Causes include the presence of a haemoglobin variants. Consider sending sample to haematology for variant detection if indicated. 3 HbA1c will be measured by alternate method, this result to follow. Abnormal separation on routine HbA1c method. Please consider referral for genetic variant counselling. 4 Abnormal chromatography on HbA1c analysis consistent with Haemoglobin variant. Result not reliable for diagnosis/screening by WHO guideline but may be used to follow trend in glycaemic control. For diagnosis suggest testing by fasting glucose. 5 Possible haemoglobin variant detected. Please send fresh EDTA sample for haemoglobinopathy screen. 6 Variant Hb detected. To investigate further please send EDTA sample to Haematology with a request for Haemoglobin Electrophoresis AND Hb variant present. Please interpret HbA1c results with caution. 7 Hb variant detected. Please interpret absolute HbA1c values with caution, results may be useful for monitoring trend. Suggest send EDTA sample to haematology for haemoglobin electrophoresis. Serum fructosamine may be more accurate for assessment of glycaemic control (if serum gel sample available then this will be sent away otherwise clinical team recommended to send gel sample). 8 Haemoglobin variant detected. HbA1c cannot be measured. HbA1c result should not be used for diagnosis/exclusion of diabetes – check glucose level. If HbA1c requested for monitoring diabetes please send serum sample for fructosamine. Consider discussion with haematologist/haemoglobin electrophoresis. • 2 labs would make no comment.
New finding of interfering variant with non-reportable HbA1c	0	10	1 Unable to obtain a valid HbA1c result, due to variant haemoglobin. Suggest send EDTA sample to haematology for haemoglobin electrophoresis. Serum fructosamine may be more accurate for assessment of glycaemic control. *Or if a serum tube is available:* Unable to obtain a valid HbA1c result, due to variant haemoglobin. Suggest send EDTA sample to haematology for haemoglobin electrophoresis. Serum sample sent for fructosamine for assessment of glycaemic control.

			2 HbA1c cannot be quantified due to abnormal separation of Hb peaks. Glycaemic status should be assessed by other means. Causes include the presence of a haemoglobin variant. Consider sending sample to haematology for variant detection if indicated. HbA1c will be measured by alternate method, this result to follow. 3 Abnormal separation on routine HbA1c method. Please consider referral for genetic variant counselling. 4 Possible haemoglobin variant detected. Please send fresh EDTA sample for haemoglobinopathy screen. Sample has been sent to King's College London for analysis by boronate affinity 5 Unable to obtain valid HbA1c result due to variant haemoglobin. Suggest serum fructosamine analysis to monitor glycaemic control. 6 Unable to obtain valid HbA1c result due to variant haemoglobin. Suggest EDTA sample to haematology for haemoglobin electrophoresis. Serum fructosamine may be more accurate for assessment of glycaemic control (if serum gel sample available then this will be sent away otherwise clinical team recommended to sent gel sample). 7 One lab only comments on HbE or other unspecified unreportable variant stating sample is unsuitable and a variant has been detected. This lab uses the DCA as an alternative. 8 Haemoglobin variant detected. HbA1c cannot be measured. HbA1c result should not be used for diagnosis/exclusion of diabetes – check glucose level. If HbA1c requested for monitoring diabetes please send serum sample for fructosamine. Consider discussion with haematologist/haemoglobin electrophoresis. • 2 labs would make no comment.
Known high HbF below cut-off	7	1	1 Variant Hb detected, but HbA1c result still valid. Suggest send EDTA sample to haematology for haemoglobin electrophoresis. 2 Increased level of HbF present. HbA1c result should not be used for diagnosis/exclusion of diabetes – check glucose level. HbA1c can be used to monitor diabetes. However usual target values may not apply. Consider discussion with haematologist/haemoglobin electrophoresis – comment used if Hb turnover affected, otherwise no comment. • 8 labs would make no comment.
Known high HbF above cut-off	8	1	1. Hb variant detected. Please interpret absolute HbA1c values with caution; results may be useful for monitoring trend. Suggest send EDTA sample to haematology for haemoglobin electrophoresis. Serum fructosamine may be more accurate for assessment of glycaemic control. *Or if a serum tube is available*: Hb variant detected. Please interpret absolute HbA1c

			values with caution; results may be useful for monitoring trend. Suggest send EDTA sample to haematology for haemoglobin electrophoresis. Serum sample sent for fructosamine for assessment of glycaemic control. 2. High HbF which may interfere with HbA1c measurement. Interpret result with caution. 3. High levels of HbF detected. HbA1c result not reliable for diagnosis or management by WHO guidelines but may be used to follow trend in glycaemic control. For diagnosis suggest testing by fasting glucose 4. Unable to obtain valid HbA1c result due to variant haemoglobin. Suggest serum fructosamine analysis to monitor glycaemic control. 5. Free text comment if desired. 6. 1 lab would not comment unless Hb turnover was affected in which case "Increased level of HbF present. HbA1c result should not be used for diagnosis/exclusion of diabetes – check glucose level. HbA1c can be used to monitor diabetes. However usual target values may not apply" is used. 7. HbA1c cannot be measured due to high concentration of HbF. HbA1c result should not be used for diagnosis/exclusion of diabetes – check glucose level. If HbA1c requested for monitoring diabetes please send serum sample for fructosamine. • 4 labs would make no comment.
Known non-interfering variant	7	2	1. Hb variant detected. Please interpret absolute HbA1c values with caution; results may be useful for monitoring trend. Suggest send EDTA sample to haematology for haemoglobin electrophoresis. Serum fructosamine may be more accurate for assessment of glycaemic control. *Or if a serum tube is available*: Hb variant detected. Please interpret absolute HbA1c values with caution; results may be useful for monitoring trend. Suggest send EDTA sample to haematology for haemoglobin electrophoresis. Serum sample sent for fructosamine for assessment of glycaemic control. 2. Possible haemoglobin variant detected which does not interfere with HbA1c measurement. If new finding suggest send sample to haematology to confirm. 3. Abnormal chromatography on HbA1c analysis consistent with Haemoglobin variant. Result not reliable for diagnosis/screening by WHO guideline but may be used to follow trend in glycaemic control. For diagnosis suggest testing by fasting glucose. 4. Hb variant detected but HbA1c result still valid. Suggest send EDTA sample to haematology for haemoglobin electrophoresis.

			5. Haemoglobin variant detected. HbA1c result should not be used for diagnosis/exclusion of diabetes – check glucose level HbA1c can be used to monitor diabetes. However usual target values may not apply. If Hb turnover is not affected then no comment. • 5 labs would make no comment.
Known interfering variant with reportable HbA1c	4	5	1. 1 Unable to obtain a valid HbA1c result, due to variant haemoglobin. Suggest send EDTA sample to haematology for haemoglobin electrophoresis. Serum fructosamine may be more accurate for assessment of glycaemic control. *If a serum tube is available:* Unable to obtain a valid HbA1c result, due to variant haemoglobin. Suggest send EDTA sample to haematology for haemoglobin electrophoresis. Serum sample sent for fructosamine for assessment of glycaemic control. 2. HbA1c cannot be quantified due to abnormal separation of Hb peaks. Glycaemic status should be assessed by other means. Causes include the presence of a haemoglobin variants. Consider sending sample to haematology for variant detection if indicated. HbA1c will be measured by alternate method, this result to follow. 3. Abnormal chromatography on HbA1c analysis consistent with Haemoglobin variant. Result not reliable for diagnosis/screening by WHO guideline but may be used to follow trend in glycaemic control. For diagnosis suggest testing by fasting glucose. 4. Hb variant present. Please interpret HbA1c results with caution. 5. Hb variant detected. Please interpret absolute HbA1c values with caution, results may be useful for monitoring trend. Suggest send EDTA sample to haematology for haemoglobin electrophoresis Serum fructosamine may be more accurate for assessment of glycaemic control (if serum gel sample available then this will be sent away otherwise clinical team recommended to sent gel sample). 6. Known haemoglobin variant present. HbA1c cannot be measured. HbA1c result should not be used for diagnosis/exclusion of diabetes – check glucose level.HbA1c can be used to monitor diabetes. However, usual target values may not apply. • 4 labs would make no comment.
Known interfering variant with non-reportable HbA1c	0	8	1. 1 Unable to obtain a valid HbA1c result, due to variant haemoglobin. Suggest send EDTA sample to haematology for haemoglobin electrophoresis. Serum fructosamine may be more accurate for assessment of glycaemic control. *If a serum tube is available:* Unable to obtain a valid HbA1c result, due to variant haemoglobin. Suggest send EDTA sample to haematology for haemoglobin electrophoresis. Serum sample sent for fructosamine for assessment of glycaemic control.

			2. HbA1c cannot be quantified due to abnormal separation of Hb peaks. Glycaemic status should be assessed by other means. Causes include the presence of a haemoglobin variants. Consider sending sample to haematology for variant detection if indicated. HbA1c will be measured by alternate method, this result to follow. 3. This sample has been sent to King's College London for analysis by boronate affinity due to presence of Haemoglobin X 4. Unable to obtain valid HbA1c result due to variant haemoglobin. Suggest serum fructosamine analysis to monitor glycaemic control. 5. Unable to obtain valid HbA1c result due to variant haemoglobin. Suggest EDTA sample to haematology for haemoglobin electrophoresis. Serum fructosamine may be more accurate for assessment of glycaemic control (if serum gel sample available then this will be sent away otherwise clinical team recommended to sent gel sample). 6. 1 lab only comments on HbE or other unspecified unreportable HbA1c by both Tosoh and DCA analyser stating unsuitable and variant detected. 7. Known haemoglobin variant present. HbA1c cannot be measured. HbA1c result should not be used for diagnosis/exclusion of diabetes – check glucose level If HbA1c requested for monitoring diabetes please send serum sample for fructosamine. • 3 labs would make no comment.

2 laboratories have no standard comments for HbA1c analysis.

Discussion

HbA1c Analysis and Reporting

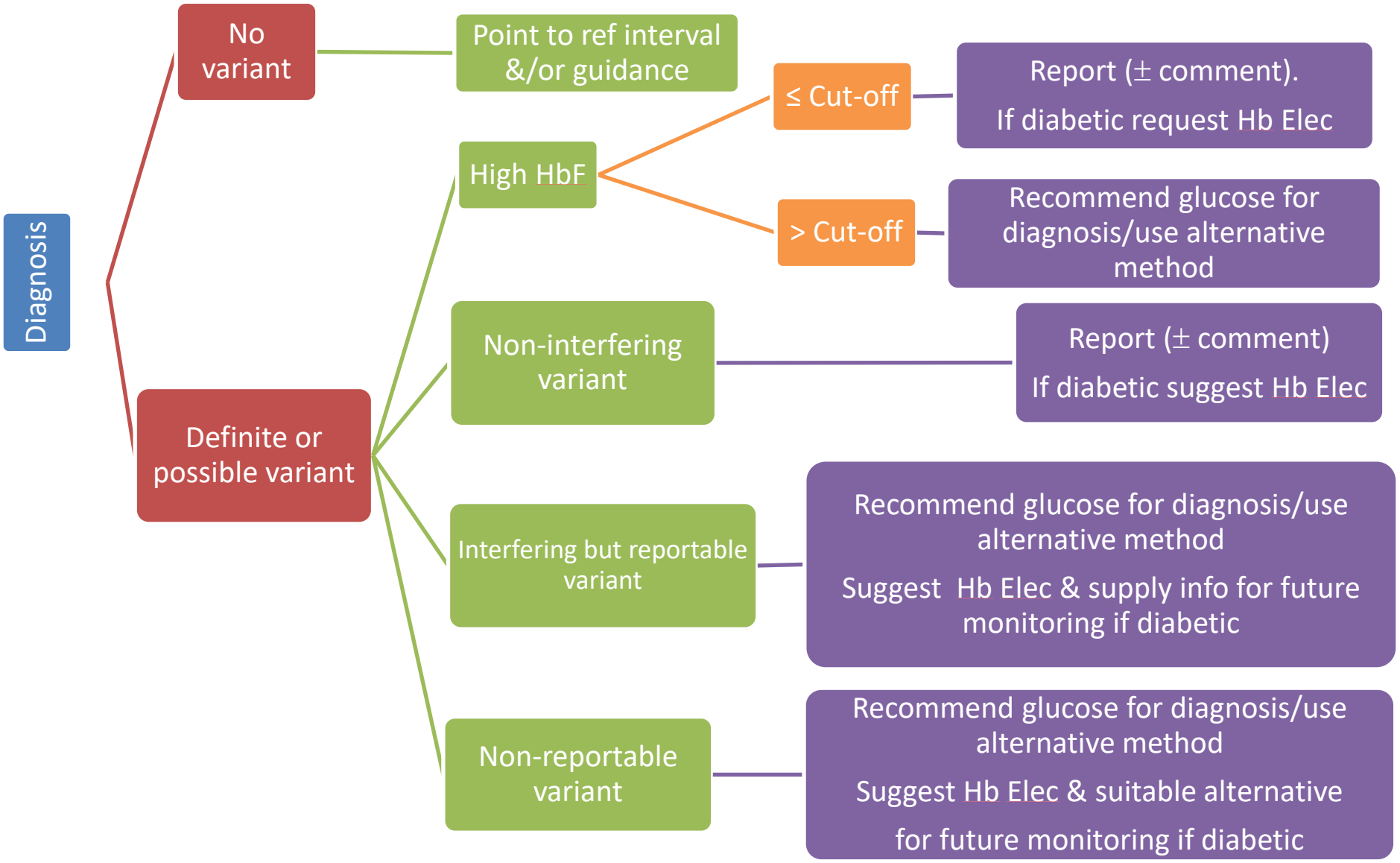
- A limited range of methodologies are being used across the country. Most labs have established in-house or external methodologies used to get a valid HbA1c result in samples with significant variants. The variant type is not generally reported.
- The use of reference intervals for reporting HbA1c is complicated and for this reason some laboratories choose not to supply a reference interval. Most, however, include the “normal” (non-diabetic) range.

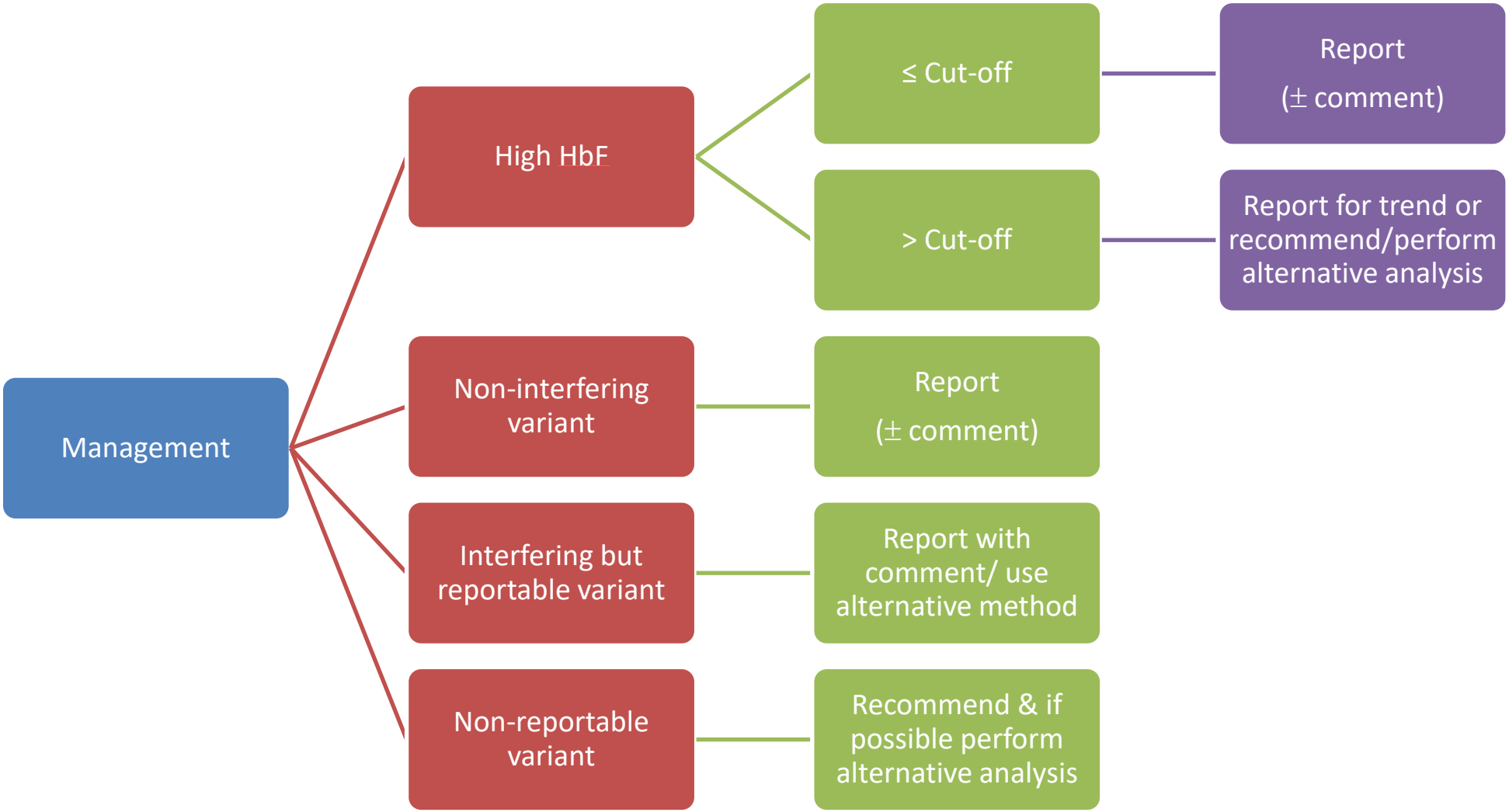
Variant Reporting

- While some labs offer no comments, a range of comments are in use, some of which don’t quite make sense in all the contexts in which they are applied. Labs may be able to make use of this survey to hone their practice.
- A few labs are communicating directly with clinicians when a variant is discovered to try to ensure appropriate on-going monitoring.

Recommendations

- The following flow-charts and table suggest how the reporting of HbA1c in the presence of variants may be managed, based on the audit returns received.
- The use of reference intervals for reporting HbA1c is complicated by the use of HbA1c in diagnosis and monitoring. The reference interval may refer to the “normal” i.e. non-diabetic state, to well, moderately or poorly controlled diabetes or may be used to trigger treatment or indicate high-risk (pre-diabetes) state if used diagnostically. It may be worth considering substituting or augmenting a reference intervals with clear indicators of when they should be applied, perhaps by providing links to local or national guidance.
- Labs who find that HbA1c is persistently requested despite repeated reporting of interfering variants may wish to consider more direct communication to clinicians e.g. by letter.


Diagnosis
No variant
Point to ref interval &/or guidance
Definite or possible variant
High HbF
≤ Cut-off
Report (± comment).
If diabetic request Hb Elec
> Cut-off
Recommend glucose for diagnosis/use alternative method
Non-interfering variant
Report (± comment)
If diabetic suggest Hb Elec
Interfering but reportable variant
Recommend glucose for diagnosis/use alternative method
Suggest Hb Elec & supply info for future monitoring if diabetic
Non-reportable variant
Recommend glucose for diagnosis/use alternative method
Suggest Hb Elec & suitable alternative
for future monitoring if diabetic


Management
High HbF
≤ Cut-off
Report
(± comment)
> Cut-off
Report for trend or recommend/perform alternative analysis
Non-interfering variant
Report
(± comment)
Interfering but reportable variant
Report with comment/ use alternative method
Non-reportable variant
Recommend & if possible perform alternative analysis

Suggested Comments

	New Finding	Pre-existing
Non-interfering HbF or other variant	• Haemoglobin variant detected which does NOT interfere with HbA1c analysis by our current method. OR • Small peak of HbF detected which does NOT interfere with HbA1c analysis by our current method. • Suggest send EDTA sample to haematology for haemoglobin electrophoresis.	• Haemoglobin variant detected which does NOT interfere with HbA1c analysis by our current method. OR • Small peak of HbF detected which does NOT interfere with HbA1c analysis by our current method.
Interfering HbF (If alternative method not automatically available)	• High HbF detected which interferes with HbA1c analysis by our current method. POSSIBLY WITH* • HbA1c result not reliable for diagnosis or management by WHO guidelines but may be used to follow trend in glycaemic control. • Suggest send EDTA sample to haematology for haemoglobin electrophoresis. • For diagnosis of diabetes mellitus suggest fasting glucose.	• High HbF detected which interferes with HbA1c analysis by our current method. POSSIBLY WITH* • HbA1c result not reliable for diagnosis or management by WHO guidelines but may be used to follow trend in glycaemic control. *(DEPENDING ON MANUFACTURER'S RECOMMENDATION)

	New Finding	Pre-existing
Interfering but reportable variant (If alternative method not automatically available	• Haemoglobin variant detected which interferes with HbA1c analysis by our current method. HbA1c result not reliable for diagnosis or management by WHO guidelines but may be used to follow trend in glycaemic control. • Suggest send EDTA sample to haematology for haemoglobin electrophoresis. • For diagnosis of diabetes mellitus suggest fasting glucose.	• Haemoglobin variant detected which interferes with HbA1c analysis by our current method. HbA1c result not reliable for diagnosis or management by WHO guidelines but may be used to follow trend in glycaemic control.
Non-reportable variant (If alternative method not automatically available)	• Unable to obtain a valid HbA1c result with current method due to variant haemoglobin. • Suggest send EDTA sample to haematology for haemoglobin electrophoresis. • For diagnosis of diabetes mellitus suggest fasting glucose. • Use (alternative method) for assessment of glycaemic control if diabetes is confirmed.	• Unable to obtain a valid HbA1c result with current method due to variant haemoglobin. • Please request (alternative test) in future for assessment of glycaemic control.