Sheffield Teaching Hospitals
NHS Foundation Trust

A comparative study of the impact of Haemoglobin variants on HbA1c measurement. ARKRAY HA-8190V & Sebia Capillarys 3

Mr J. Eyre. Haematology Dept, Royal Hallamshire Hospital, Sheffield Teaching Hospitals NHS Foundation Trust
Mr R. Green. Clinical Applications Specialist, Arkray UK Limited

Introduction:

Glycated haemoglobin (HbA1c) is used as a marker for long term glycaemic control in diabetic patients as well as in the diagnosis of new diabetes cases.
Running between 1983 and 1993 the Diabetes Control and Complications trial (DCCT)[1] established the link between well controlled HbA1c levels and the reduced incidence of complications associated with diabetes, and marked a new era in diabetes management.

The increasing importance of HbA1c measurement in diagnosis and monitoring of diabetes, and pre-diabetes, means that awareness of associated factors that can affect laboratory HbA1c measurement is essential.

HbA1c can be measured using a variety of methods, each of which can be influenced by several factors leading to erroneous results. Haemoglobin variants may affect HbA1c values by influencing the binding of glucose to Hb or affecting HPLC/CE peak measurements.

HPLC and CE methods for HbA1c quantification separate Hb fractions based on charge differences and are known to be susceptible to interference from Hb variants. Over 1300 Hb variants have been identified to date[2] with several known to co-elute with HbA0. Failure to resolve the variant Hb components from HbA1c and/or HbA0 may cause problems with peak integration and lead to anomalous HbA1c results. Inaccurate HbA1c values can be observed when Hb variants, or their glycated derivatives, cannot be separated from HbA or HbA1c. The presence of relatively common Hb variants is often easily recognized when using HPLC and CE methods by the presence of an additional peak in the chromatogram. However, further studies are required to identify the Hb variant.

Sheffield Teaching Hospitals NHS Trust uses Sebia Capillarys 3 (CE) analysers to process it's workload of approximately 247,000 HbA1c tests per year. This study aims to assess the performance of the Arkray HA8190V (HPLC) against this established service, in particular with regard to the effect, if any, of the more common haemoglobin variants encountered during routine HbA1c testing, on the test result.

The Arkray HA8190V operates in 2 modes: Fast (24 Seconds) and Variant (58 Seconds) modes. This study will focus on Variant mode, as Fast mode is only used for previously known patients with no Hb variants.

Arkray HA-8190V

Initial comparison and regression analysis shows the two technologies correlate well with each other (r=0.998) in the absence of haemoglobin variants

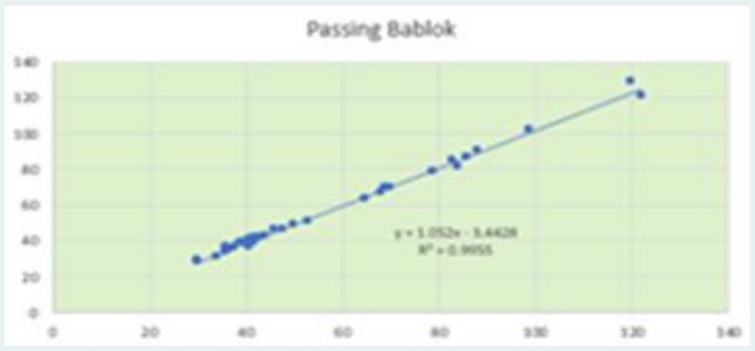

Fig 3 Passing Bablok Sebia Capllarys 3 Vs Arkray HA-8190V

In the presence of common Haemoglobin variants, the comparison of HbA1c results between the 2 instruments were equally well correlated

Variant	Correlation (r)
Haemoglobin C	0.961
Haemglobin D	0.992
Haemoglobin E	0.975
Haemoglobin S	0.961

Fig 2. Correlation of Sebia Capillarys 3 Vs Arkray HA-8190V in the presence of major variants

HbC, HbD, HbE and HbS account for 99% of the approximately 1300 known Hb variants so far discovered. [3]

Abnormalities of haemoglobin synthesis are usually inherited but may also arise as a secondary manifestation of another disease, usually a haematological neoplasia.
Acquired haemoglobin disorders may be seen in any population and are not restricted to any particular family origin or ethnicity.

To examine this the comparison extended to more unusual Haemoglobin variants; Haemoglobin Valletta and Haemoglobin Athens Georgia.

In the presence of these variants, the Sebia Capillarys 3 was unable to separate the fractions to generate an HbA1c result. These samples required examination by an alternative method.

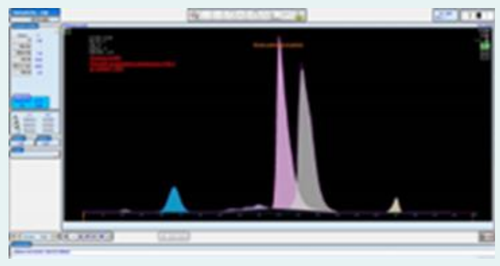

Fig 4. Hb Athens Georgia Capillarys 3 without HbA1c peak

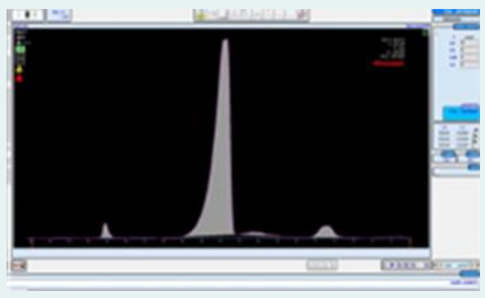

Fig 5. Hb Valletta Capillarys 3 without HbA1c peak

The Arkray HA-8190V was able to separate the peaks and generate an HbA1c result comparable to the laboratory alternative

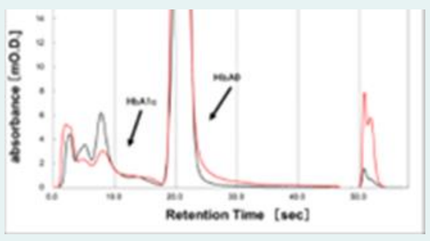

Fig 6. Valletta HA-8190V with HbA1c peak

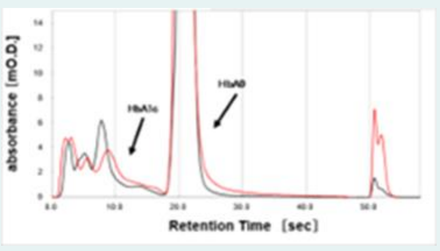

Fig 7. Athens Georgia HA-8190V with HbA1c peak

References:

1. Diabetes Control and Complications Trial (DCCT): results of feasibility study. The DCCT Research Group. American Diabetes Association. Diabetes Care. Vol 10, Issue 1. Jan/Feb 1987

2. HbVar (psu.edu)

3. Patrinos GP, Giardine B, Riemer C, Miller W, Chui DH, Anagnou NP, Wajcman H, Hardison RC: Improvements in the HbVar database of human hemoglobin variants and thalassemia mutations for population and sequence variation studies. Nucleic Acids Res 2004, 32(Database issue):D537-54

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Conclusion:

The 2 methods produced statistically similar results under normal circumstances across the clinical range. However, in the samples with rare Hemoglobin variants (Athens GA, Valletta) the Sebia Capillarys 3 was unable to generate HbA1c results. These samples needed to be retested on a secondary instrument. The Arkray HA-8190V was able to generate HbA1c results in these circumstances which correlated well with the laboratory's secondary method.

In comparison to the Sebia Capillarys 3 the Arkray HA-8190V is a fast compact bench top analyser with demonstrated ability to produce valid HbA1c results in the presence of rare Haemoglobin variants.