Hemoglobin A1c: New Factors, New Perspectives in the Monitoring of Diabetes

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At: California Association of Medical Laboratory Technology’s Annual Meeting and Exhibits

Hemoglobin A1c is used as a key test in the diagnosis of diabetes and as a metric for long-term tracking of glycemic state in known patients. It is a good analyte due to hemoglobin being a robust protein that remains in normal circulation for an average of 120 days and whose slow conversion to HbA1c in the presence of glucose (by glycation at the N-terminal β-chain) enables its use as a reflection of average glycemic control in patients.

The measurement of HbA1c has proceeded in the laboratory through the development of four independent techniques and has gradually moved towards greater refinement in these tests’ developments to improve patient outcomes.

1) Immunoassay- driven by antibody recognition of the glycated N-terminal of Hb β-chains
   a. Unable to recognize the presence of Hb Variants
   b. May or may not differentiate between glycated Hb Variants

2) Bornoate Affinity-driven by column affinity of glycated fractions of hemoglobin, including HbA1c
   a. Unable to recognize the presence of Hb Variants
   b. Assumes the relationship between GHb and HbA1c is constant

3) Ion-exchange Chromatography- driven by charge differences in glycated hemoglobins
   a. Able to recognize the presence of most common Hb Variants
   b. Separates individual glycated fractions to specifically isolate HbA1c
   c. Increased resolution brings with it complex formulations, possibilities for co-migration/interferences from elevated uncommon fractions (Hb F, Labile, Carbamylated)

4) Capillary Electrophoresis-driven by separation of proteins in electrical field (size, charge)
   a. Able to recognize the presence of most common Hb Variants
   b. Increased resolution, but engineered to isolate HbA1c and Hb A from most potential interferences by elevated uncommon fractions/variants.
   c. Employs the IFCC definition of HbA1c% calculation

Improvement in the detection and measuring of HbA1c% by each of these assays has led to gradual tightening of regulations surrounding these measurements. Over the years CAP has tightened the “pass” requirements on their HbA1c surveys to +/- 6% and examination of current CAP results reveal that some testing techniques/manufacturers have had difficulty in adapting to these increasingly stringent demands.

The use of HbA1c and Hemoglobin as a monitor for diabetic state brings with it the inherent peculiarities of the hemoglobin molecule which may prove to be an obstacle to best patient care.
Hemoglobinopathies are the most common single gene disorder in humans. Hemoglobin variants are genetic mutations in the structure of hemoglobin chains that lead to altered hemoglobin structure and may have altered formation rates and glycation rates at the key N-terminal of the β chains where HbA1c is formed. Thalassemias are genetic mutations which result in reduced production of the constituent chains of hemoglobin, resulting in overall reduced hemoglobin production.

Some HbA1c measurement techniques are incapable of distinguishing between normal and variant hemoglobins, and may give erroneous or misleading results about a patient’s glycemic control as a result. There are even recorded cases of patients who have no normal Hemoglobin A being resulted as having reportable HbA1c values. (HbA1c cannot form in the absence of Hb A.)

Hemoglobin variants and thalassemias have additional impact upon a patient’s HbA1c value beyond analytical interference with some assay techniques. Hemoglobin variants and thalassemias have been shown to significantly impact red blood cell lifespan, reducing overall RBC time in the circulation. As a patient’s HbA1c level is achieved by accumulation of HbA1c through the slow reaction of glucose with HbA, a significant shortening of RBC lifespan in the circulation will artificially suppress HbA1c accumulation. Being aware that a diabetic patient has a Hemoglobin variant or thalassemia is key to proper interpretation of HbA1c results and providing best patient care.

In light of these factors, the American Diabetes Association have made changes to their Standards of Care (Diabetes Care, The Journal of Clinical and Applied Research and Education: January 2018, Volume 41 Supplement 1) “Additional information and recommendations have been added to help ensure appropriate use of the A1C test to diagnose diabetes and for monitoring glycemic control in people with diabetes. The ADA stresses that the A1C test can give skewed results in people with certain genetic traits that alter the molecules in their red blood cells. Moreover, the ADA emphasizes that health care providers need to be aware of these limitations, to use the correct type of A1C test, and to consider alternate diagnostic tests (fasting plasma glucose test or oral glucose tolerance test) if there is disagreement between A1C and blood glucose levels.”

HbA1c remains a key test in the diagnosis and long term monitoring of patient glycemic control. However with the tightening standards and improved awareness of the impact of genetic factors on HbA1c measurement, it is advisable that labs considering a change in their methodology take into consideration:

1) Performance standards of the current methodologies,
2) The ability to identify hemoglobin variants and detect interferences caused by them
3) The ability to flag samples from patients who may have thalassemias,
4) The ability to integrate this detection/flagging into a workflow that does not unduly burden laboratory staff