AACC Better health through laboratory medicine.

PEARLS OF LABORATORY MEDICINE

An Update on Hemoglobin A1c analysis and potential interferences

Sydney Webb Strickland

Clinical Chemistry Fellow
University of Virginia

DOI: 10.15428/CCTC.2018.290759

CCTC Clinical Chemistry Trainee Council

What is Hemoglobin A1c?

- Glycation is the non-enzymatic addition of a sugar to amino groups of proteins.
  - High A1c% = Increased Time + High glucose

- Diagnostic Criteria
  - ≥6.5% = diabetes
  - 5.7-6.4% = prediabetes
  - ≤ 5.6% = optimal

- Measured every 3 months
  - RBC life-cycle 120 days

Hemoglobin A1c Assays

- Ion Exchange HPLC (31% of US Laboratories)
- Capillary Electrophoresis (1%)
- Boronate Affinity Chromatography (2%)
- Immunoassay (26%)
- Point of Care (POC) (40%)

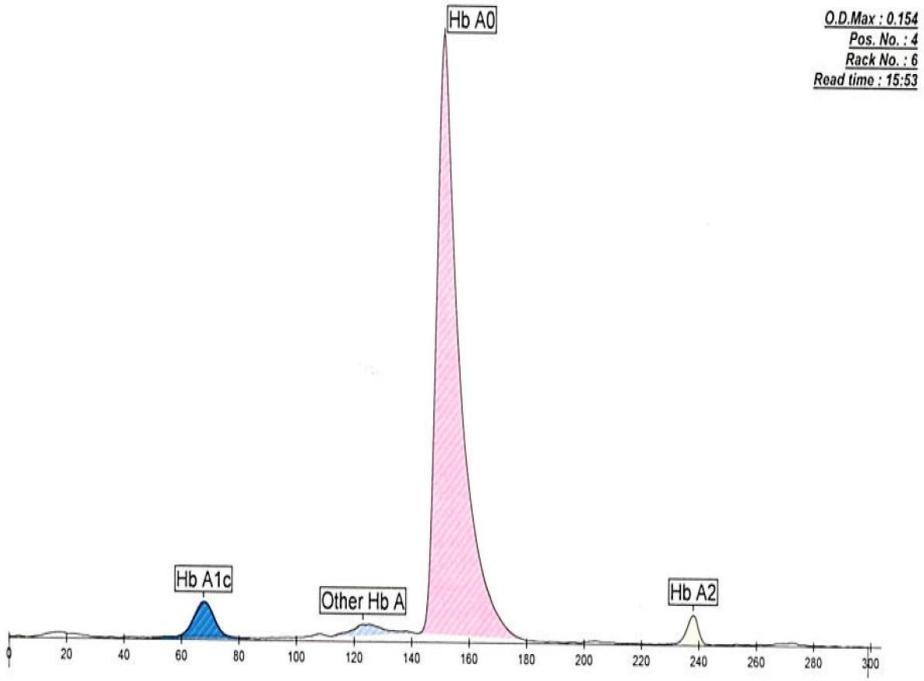
Hemoglobin A1c Assays: Chromatography

- **CE**
  - Separates by:
    - Size and charge
  - A1c calculated:
    - $\frac{\text{A1c}}{\text{A1c + HbA}}$
- **IE-HPLC**
  - Separates by:
    - Charge
  - A1c calculated:
    - $\frac{\text{A1c}}{\text{Total HbA*}}$

**Total HbA can include: Hb A, Hb A1a, Hb A1b, Hb F (this varies based on manufacturer)*

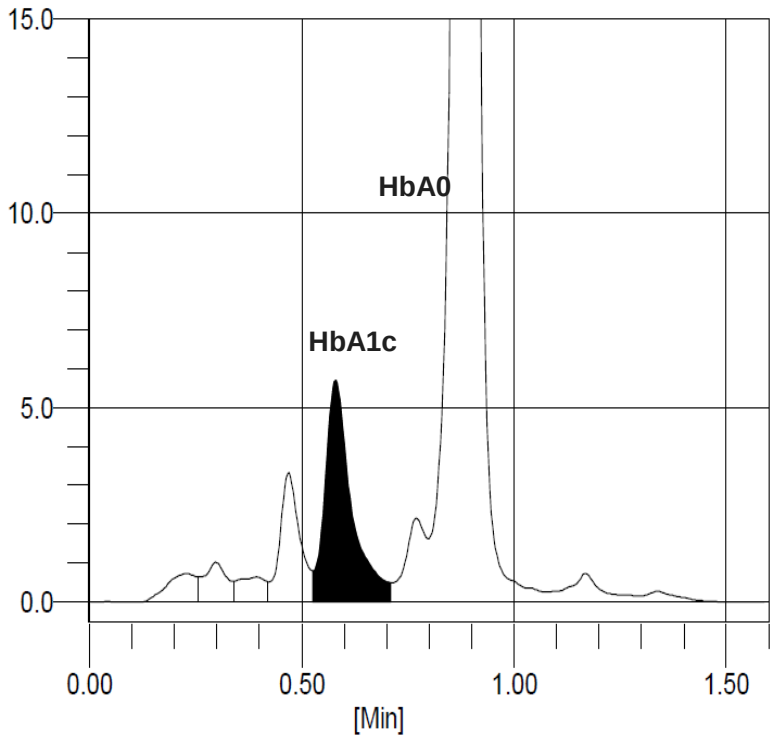
Chromatography Methods: Diabetic patient

CE



A1c% = 6.6

HPLC



A1c% = 6.8

Other Hemoglobin A1c Assays

- **Boronate Affinity Chromatography**
  - Quantified by:
    - Boronate binds cis-diol groups of glucose to separate glycated from non-glycated
  - A1c calculated:
    - $\frac{\text{Glycated Hb}}{\text{Total Hb}}$
- **Immunoassay**
  - Quantified by:
    - Antibody specific for the glycation epitope of A1c (turbidimetry)
    - Spectrophotometric measurement of Hb
  - A1c calculated:
    - $\frac{\text{Hb A1c}}{\text{Total Hb}}$

Other Hemoglobin A1c Assays

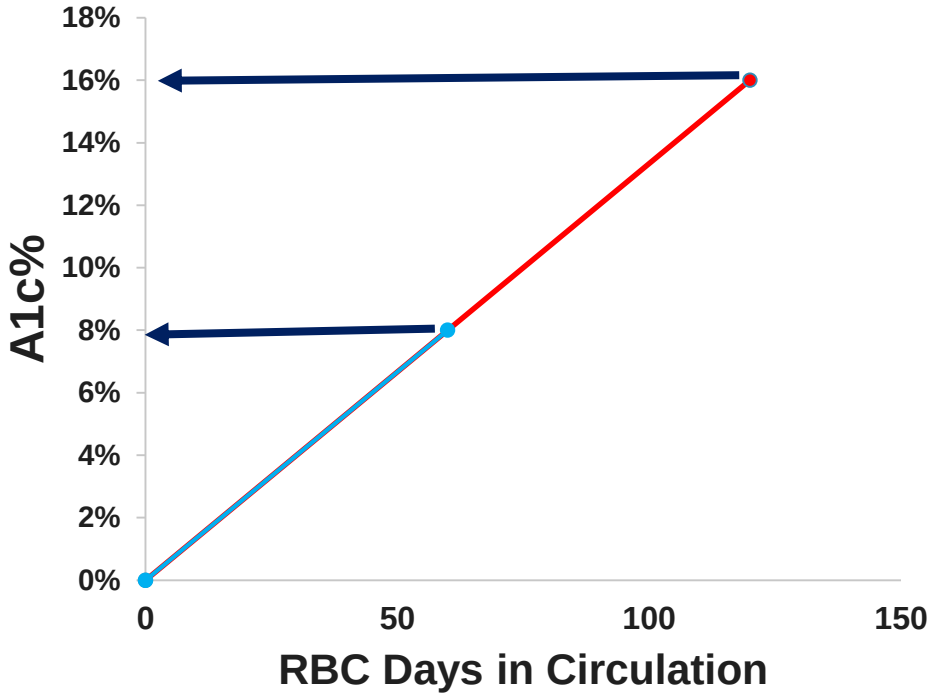
- **Enzymatic**
  - Quantified by:
    - Reaction of fructosyl peptide oxidase with the β-chain peptide produced by fructosyl peptide protease
      - ***Results in formation of H_2O_2 and a color change***
        - » Directly proportional to the HbA1c concentration
    - Spectrophotometric measurement of Hb (Hb → Met Hb during pre-treatment)
  - A1c calculated:
    - $\frac{\text{Hb A1c}}{\text{Total Hb}}$

Other Hemoglobin A1c Assays

- Point of Care (POC) Hb A1c
  - Mostly immunoassays
    - One Boronate method seeking FDA approval
  - Current guidelines
    - Diagnosis **cannot** be made using a POC device
  - Good quality assurance programs (QAP) are needed
    - Capable of meeting quality goals when QC is used
  - Cannot detect Hb variants

What can change Hb A1c?

- RBC survival (shorter life = decreased A1c)
- Iron deficiency anemia and Vitamin B12 deficiency (increased A1c)
- Hemoglobin variants (varies)
  - Point mutations in Hb A (i.e. Hb C, D, E, F, and S)



Hemoglobin Variants

- Point mutations in Hemoglobin genes (α,β,δ, etc.)
  - Can be clinically silent or clinically significant
  - Common variants: Hb C, D, E, F, and S
  - >1000 identified to date
    - Some rare variants: Hb J-Baltimore, La Desirade, G-Philadelphia

- Phenotypes:
  - Anemia
  - Changes in oxygen binding affinity
  - Structural stability alterations
  - Shorten/Lengthen RBC half-lives

What to do if the A1c is not reliable

- Alternative analytes:
  - Fructosamine
    - Assesses glycemic control over the past **3 weeks**
    - Measures primarily glycated albumin
    - Clinical assays sensitive to interferences and preanalytical variables
  - 1,5-anhydroglucitol (GlycoMark)
    - Assesses glycemic control over **2-14 days**
    - Decreased levels → Increase glucose (>180 mg/dL)
      - Excreted in urine when glucose is high
    - Can also be decreased in patients with CKD stage 4/5

What to do if the A1c is not reliable

- Alternative analytes:
  - Glycated Albumin
    - Albumin is 10x more sensitive to glycation than Hb
    - Assesses glycemic control over **2-3 weeks**
      - Albumin half-life = 2 weeks
    - Correlates well with Hb A1c values (Japanese Study Group)

Conclusions

- Five main methods for measuring A1c
  - Capillary Electrophoresis
  - IE-HPLC
  - Boronate HPLC
  - Immunoassays
  - Enzymatic

- No Consensus for how to calculate A1c
  - But harmonization has helped

- Useful tool for clinicians to monitor glycemic control in diabetic patients
  - Linked to negative outcomes of diabetes
    - Example: Retinopathy

References

1. Strickland SW, Campbell ST, Little RR, et al. Recognition of rare hemoglobin variants by hemoglobin A1c measurement procedures. Clin Chim Acta 2018; 476:67-74.
2. Trivelli LA, Ranney HM, Lai HT. Hemoglobin components in patients with diabetes mellitus. N Engl J Med 1971; 284(7):353-7.
3. The International Expert Committee. International Expert Committee Report on the Role of the A1c Assay in the Diagnosis of Diabetes. Diabetes Care 2009; 32(7):1327-34.
4. College of American Pathologists. CAP: Proficiency Testing. 2017; http://www.cap.org/web/home/lab/proficiency-testing?_adf.ctrl-state=1696i96evc_4&_afrLoop=385519710531163#!
5. Lacy ME, Wellenius GA, Sumner AE, et al. Association of Sickle Cell Trait with Hemoglobin A1c in African Americans. JAMA 2017; 317(5):507-15.
6. Leslie D, Weykamp C, Mosca A, et al. Diabetes: Learning Guide series. Abbott Diagnositics. 2018; https://www.corelaboratory.abbott/sal/learningGuide/ADD-00061643_Diabetes_Learning_Guide.pdf
7. Medes, N, Ribeiro RT, and Serrano F. Beyond self-monitored plasma glucose and Hb A1c: the role of non-traditional glycaemic markers in gestational diabetes mellitus (2018) J Obstet Gynaecol. 2018; Apr 5:1-8.
8. Furusyo N, Koga T, Ai M, et al. Utility of glycated albumin for the diagnosis of diabetes mellitus in a Japanese population study: results from the Kyushu and Okinawa Population Study (KOPS). Diabetologia. 2011; 54(12):3028-36.
9. Danese E, Montagnana M, Nouvenne A, et al. Advantages and Pitfalls of Fructosamine and Glycated Albumin in the Diagnosis and Treatment of Diabetes. J Diabetes Sci Technol 2015; 9(2):169-176.
10. Solvik UO, Roraas T, Christensen NG, et al. Diagnosing diabetes mellitus: performance of hemoglobin A1c point-of-care instruments in general practice offices. Clin Chem. 2013; 59(12):1790-801.

Disclosures/Potential Conflicts of Interest

Upon Pearl submission, the presenter completed the Clinical Chemistry disclosure form. Disclosures and/or potential conflicts of interest:

- **Employment or Leadership**: No disclosures
- **Consultant or Advisory Role:** No disclosures
- **Stock Ownership:** No disclosures
- **Honoraria:** No disclosures
- **Research Funding:** No disclosures
- **Expert Testimony:** No disclosures
- **Patents:** No disclosures

Thank you for participating in this *Clinical Chemistry* Trainee Council Pearl of Laboratory Medicine.

Find our upcoming Pearls and other Trainee Council information at www.traineecouncil.org

Download the free *Clinical Chemistry* app on iTunes today for additional content!

Follow us: