Hemoglobin A1c

CHAPTER 10: HEMOGLOBIN A1c BY DCA 2000
Procedure: Hemoglobin A1c by Bayer DCA 2000

PRINCIPLE
A reagent containing an antibody specific for HbA1c coated on latex beads reacts with a synthetic agglutinator containing HbA1c antigen, resulting in the aggregation of the beads and increasing the turbidity of the reaction mixture. HbA1c in a blood sample is quantified by measuring the inhibition of the aggregation resulting from competition for the antibody by the HbA1c in the sample. The total hemoglobin is determined colorimetrically and the results are expressed as percent HbA1c. The glycosylated fraction of hemoglobin (HbA1c) reflects the glucose level in the blood and is a measure of long term glucose control.

CLINICAL SIGNIFICANCE
HbA1c is formed in two steps by the nonenzymatic reaction of glucose with the N-terminal amino group of the β-chain of normal adult Hb (HbA). The first step is reversible and yields labile HbA1c. This slowly rearranges in the second reaction step to yield stable HbA1c. In erythrocytes the relative amount of HbA converted to stable HbA1c increases with the average concentration of glucose in the blood. The conversion to stable HbA1c is limited by the erythrocyte's life span of approximately 100 to 120 days. As a result, HbA1c reflects the average blood glucose level during the preceding two to three months. HbA1c is thus suitable to monitor long-term blood glucose control in individuals with diabetes mellitus. More recent glucose levels have a greater influence on the HbA1c level.

The risk of diabetic complications, such as diabetic nephropathy and retinopathy increases with poor metabolic control. In accordance with its function as an indicator for the mean blood glucose level, HbA1c predicts the development of diabetic complications.

SPECIMEN REQUIREMENTS
Patient Preparation:
- No pre-visit preparation is necessary.
- The physician requests that a blood sample be obtained for testing while the patient is present.
- The blood sampling and testing is done in the clinic during the patient’s visit.
- Only one patient sample is obtained at a time.

Specimen Type:
- Whole blood. A 1 µL blood sample from a finger stick is obtained at the time of the determination.

Handling Conditions:
- When the sample is obtained, the patient’s name is written on a form and the form accompanies the sample to the testing instrument. The determination is performed immediately.
- After the capillary is filled with sample, the analysis must begin within 5 minutes.

REAGENTS
Equipment: DCA 2000 Hemoglobin HbA1c system.

Materials:
Bayer reagent kit (5035B) containing:
- reagent cartridges containing:
  - HbA1c antibody-coated latex beads in glycine buffer
  - poly aspartate agglutinator with bound HbA1c hapten
  - buffer solution
  - potassium ferricyanide
- capillary holders
- calibration card.
Hemoglobin A1c Control Kit (5068) containing:

- 2 bottles of Normal control
- 2 bottles of Abnormal control
- Reconstitution fluid
- Eyedropper cap assemblies
- Control card

Reagent Preparation:

- The reagent cartridges are complete and require no additional preparation.
- Refrigerated cartridges should be warmed to room temperature for 10 minutes before use.
- When a cartridge package is opened, the cartridge must be used within one hour.

Reagent Storage Requirements:

- Store reagent cartridges in original box at 2-6 °C in refrigerator. The refrigerator’s temperature log must be properly maintained.
- Record the date received on each box.
- When opened, record the date opened on each box.
- If stored refrigerated, reagents can be used until the expiration date.
- Unopened cartridges may be kept at room temperature for up to 3 months, but not beyond the printed expiration date:
  - Record on the outside of the box, the date the carton was placed at room temperature and the expiration date (three months or the printed expiration date, whichever is sooner).
  - If the temperature indicator is red, do not use the kit.
- Use before expiration date.

CALIBRATION

Instrument calibration:

- The instrument is calibrated by the manufacturer. It self-adjusts on power-up and with each assay.
- For each lot of reagents, calibration parameters are encoded onto calibration card. The lot’s calibration card must be read by the instrument before it can use that lot. (See “Calibration procedure” below.)
- For each shipment, check the lot numbers, and perform the calibration procedure if there is a new lot number of reagent.
- If no calibration curve is in the instrument for a particular lot number, the instrument prompts the user to scan the calibration card.

Calibration procedure:

- Pass the calibration card through the bar code scanner on the instrument. A beep sounds to signal a successful scan. Reading of the calibration card by the scanner works best if the calibration card is positioned to slide up against the read window on the right side of the scanning slot.

PROFICIENCY TESTING

The RWJMS Point of Care Testing program subscribes to the College of American Pathologists CAP survey GH. Three challenges are sent per year, two samples per challenge. When proficiency testing samples arrive, run them promptly, as you would patient samples, and forward the results to the RWJMS Point of Care office. Do not submit results directly to the CAP.

QUALITY CONTROL

When to run Quality Control samples:

- Based on the number of patients to be tested daily, and the manufacturer’s guidelines for performing quality control, both Quality Control samples will be run when each box of reagent is opened. This schedule will satisfy all other requirements for performing quality control.
Quality control material:
- Controls must be reconstituted before use.
- Un-reconstituted controls can be used until the expiration date on the vial.
- Reconstituted controls can be used for up to 3 months (but not beyond the expiration date on the vial).
- Controls may remain out for no more than 30 minutes at room temp. Always put them back in the refrigerator.

Reconstituting controls:
1. Remove the appropriate control vial from the refrigerator.
2. Gently tap the bottom of the bottle so the contents fall to the bottom of the bottle, then remove the cap.
3. With the reconstitution fluid vial vertical, discard a drop of fluid, then dispense SIX drops into the control bottle.
4. Replace the cap (not the dropper).
5. Swirl the bottle several times, then allow the bottle to stand for 15 minutes.
6. After 15 minutes, swirl, rotate and invert the bottle so that all material on the inside is reconstituted.
7. Discard the cap and replace it with an eyedropper cap.
8. Date the control vial with the date it was reconstituted, and the date that it expires (3 months, or the printed expiration date, whichever is sooner).

Running control samples:
1. Scan the control card. Make sure that the appropriate side of the control card is facing the read window.
2. After the control card is read, press the enter key.
3. The instrument will ask you to specify Control 1 or Control 2. Use Control 1 for the normal control, and Control 1 for the abnormal.
4. Press the enter key again.
5. Aspirate a small amount of control solution into the eyedropper in the control bottle.
6. Fill a capillary with control from the eyedropper. Be careful not to get any control solution on the plastic part of the capillary holder. If the control solution gets on the plastic part, discard and use a new capillary and holder.
7. Wipe and control solution off of the outside of the capillary tube.
8. Continue to run the test as you would run a patient sample (see Patient Testing Procedure below).

Control results:
- Record control results in the testing log.
- The instrument will alert the user if the control results are not within range. If this occurs, repeat the control assay using freshly reconstituted control solutions.
  - Do not use the instrument for patient testing if the control readings are inaccurate.
  - Contact the RWJMS POCT office, and contact the supplier for a new instrument if quality control sample fails twice.

TESTING PROCEDURE:

1. Check the temperature indicator on the box before taking a cartridge out.
2. Refrigerated cartridges should be warmed to room temperature for 10 minutes before use.
3. When a cartridge package is opened, the cartridge must be used within one hour.
   - Check the cartridge and do not use it:
     - if the cartridge is damaged,
     - if the flexible cartridge pull-tab is loose or missing, or
     - if the desiccant is missing or loose desiccant particles are found inside the foil pouch.
4. Room temperature must be between 59° and 90°F. Do not test if temperature exceeds this range. Room temperature must be recorded on the test log for each test that is done.
5. Allow the instrument enough time to warm up at the beginning of the day.
6. Pass cartridge through reader. A beep sound indicates a successful scan.
7. Fill capillary holder with blood (1 microliter from finger stick). Wipe away the first drop of blood before collecting the specimen. Wipe outside of holder. Testing must begin within 5 minutes of collecting the specimen.

8. If blood contacts the plastic outside of the holder, discard the holder and use another one.

9. Insert holder into the reagent cartridge, with the rounded side of the holder to the outside.

10. Hold the cartridge with the foil to the left, and insert cartridge into instrument until it snaps into place.

11. Remove the tab and foil from the cartridge.

12. Close the door on the instrument. Reaction is complete in 6 minutes.

13. Read percent HbA1c before removing the cartridge.
   - The range of the instrument is 2.5% to 14.0%.
   - The result is displayed as percent HbA1c.
   - Results proceeded by a < sign indicates a level below the range and a > indicates a level above the range, and should be recorded as such.

14. Record the result in the testing log, and in the patient’s chart. All results are reported to the physician immediately.

15. Remove the cartridge by pushing down on the gray tab while sliding the cartridge to the right, toward the gray tab—then lift the cartridge out of the instrument and discard it in a biohazard container.

CALCULATIONS
   None. The result is displayed as percent HbA1c.

REPORTING RESULTS

Reference Range: 4.2% to 6.5% HbA1c, determined by manufacturer.

MAINTENANCE

Daily:
   Clean any spills as they occur.

Weekly:
   Clean the Bar code window weekly.
   - Use a lint-free tissue dampened with water or alcohol wipe down the exterior and the bar code window.
   - At all times avoid dripping liquid into the analyzer.
   - Document this maintenance in the maintenance log.

Quarterly:
   The filter must be changed every three months.
   - Twist off the gray cap to the right of the machine, carefully remove the white filter paper.
   - Replace with clean filter.
   - Be sure to line the filter paper up so the cap will twist on correctly.
   - Document this maintenance in the maintenance log.

PROCEDURE NOTES:
If the instrument fails calibration or if control values are outside range, a new instrument can be obtained within 48 hours. During this time, the physician may request that an HbA1c assay be determined by an outside laboratory. Record on the back of the testing log, the date, the problem, the name of the problem as indicated on the display and the date that the new instrument is received, and the name of the person handling the problem.

LIMITATIONS OF THE PROCEDURE:
• The results are accurate over a range of total hemoglobin of 7 to 24 g/dl. Samples from patients with hemoglobin outside this range cannot be used.
• This test does not detect glycosylated hemoglobin F. Samples with greater than 10% hemoglobin F cannot be used because the HbA1c will be falsely lowered by missing the glycosylated hemoglobin F.
• Because of shortened red cell survival, results from patients with hemolytic anemia, polycythemia and homozygous HbS and HbC will not accurately reflect long term glycemic control.

REFERENCES:

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