Hemoglobin A_{1C} on the DCA Vantage ®+ Analyzer

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Reviewed	Date	Reviewed	Date

Revisions:

5/2020- moved DCA testing from RIH to TMH	

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PRINCIPLE:

This assay is a quantitative method for measuring the percent concentration of hemoglobin A1c (A1c) in blood and is based on a latex immuno-agglutination inhibition methodology. Results are available in 6 minutes.

The A1c test result, also known as glycated hemoglobin or glycohemoglobin, reflects the patient average blood glucose level for the previous 2 to 3 months. Thus, this test assists doctors and patients in evaluating how well patients are managing their diabetes. Good long-term management is important in reducing the risk of diabetes complications that include blindness, kidney disease, nerve damage, stroke and heart disease.

SPECIMEN:

Patient Preparation:
None

Sample Type:
1µL of venous whole blood.

Acceptable anticoagulants:	EDTA (anticoagulant of choice) Heparin Fluoride/oxalate Citrate
Unacceptable anticoagulants:	all other anticoagulants not listed above.

Handling Conditions:
Once the glass capillary is filled with a 1µL blood sample, analysis must begin within five (5) minutes. If the blood remains in the glass capillary for more than five (5) minutes before beginning the test, reject the sample for testing and refill another capillary. EDTA, heparin and citrate preserved whole blood may be stored at -70° to 5°C (-94° to 41°F) for two weeks, or up to 25°C (77°F) for one week.

- Do not refreeze previously frozen blood samples.
- Do not store blood samples in a self-defrosting freezer.
- Allow the blood to reach room temperature naturally.
- Mix the blood sample thoroughly before use.

EQUIPMENT AND MATERIALS:

Equipment:
DCA Vantage Analyzer

Materials:
DCA HbA1c Control Kit
DCA HbA1c Reagent Cartridges
Capillary Holders
Calibration Card

Calibration Log
Daily Control Log
Lint-free tissue

Reagent Preparation:

Allow the reagent cartridge to warm to room temperature naturally:

1. Remove the cartridge from refrigerated storage.
2. For an unopened foil pouch, allow ten (10) minutes for temperature equilibration.
3. For a cartridge removed from the foil pouch immediately on removal from the refrigerator, allow five (5) minutes. Failure to allow a reagent cartridge to reach room temperature may cause condensation to form in the optical window inside the cartridge. This causes erroneous results or an error message.
4. After opening the foil pouch, the cartridge must be used within one (1) hour.
5. Do not refrigerate the reagent cartridge after the foil pouch has been opened.

Opening the foil pouch of reagent cartridge:

6. Tear down from the corner notch on the pouch until the entire *long* side of the pouch is open.
7. Do not use scissors to cut open the foil pouch. Scissors can damage the reagent cartridge, the flexible pull-tab on the cartridge or the sack of desiccant.
8. Avoid touching the optical window at the base of the cartridge.
9. Discard the reagent cartridge if:
    - the cartridge is damaged
    - the flexible cartridge pull-tab is loose or missing
    - the desiccant is missing
    - loose desiccant particles are found inside the foil pouch.

Control Preparation:

Both of the A1C Normal and Abnormal controls for DCA are lyophilized and must be reconstituted prior to use. The following directions for reconstitution should be followed:

1. Remove the control bottle from the refrigerator just prior to reconstitution. Write the "opened" expiration date (3 months from date of reconstitution) on the control vial.
2. Gently tap the bottom of the control bottle on the counter to collect as much material as possible on the bottom of the bottle.
3. Carefully remove the cap from the control bottle.
4. Holding the Reconstitution Fluid dropper bottle vertically, add six (6) drops of fluid to the control bottle. **Note:** Discard the first drop to ensure constant volume of the drops thereafter.
5. Carefully replace the cap, not the eyedropper, and swirl the control bottle several times. Let the control stand at room temperature for 15 minutes.
6. After 15 minutes, coat all surfaces by rotating and inverting the bottle. Continue mixing until the solution is homogenous and all lyophilized material is reconstituted.
7. Remove and discard the cap. Replace the cap with an Eyedropper Cap Assembly. Use reconstituted controls within 30 minutes or refrigerate to store for later use.

Performance Parameters:

Refer to the reagent and control package inserts for specific performance characteristics.

Storage Requirements:

<u>DCA Vantage A1C Analyzer Instrument Operating Conditions:</u>

Ambient Operating Temperature Range 15° to 32°C (59° to 90°F) Hemoglobin A_{1C}

Operating Relative Humidity Range	10% to 90% RH (non-condensing)

Reagent Cartridge Storage:

- Upon receipt of the kit containing reagent cartridges and the calibration card, check the temperature indicator. If the indicator has turned red, the reagent cartridges should not be used. To obtain a replacement kit, refer to the instructions given on the lid of the carton.
- Store reagent cartridges as directed in package insert (2° to 8°C). Cartridges can be used until the last day of the expiration month shown on the cartridge.
- Use Life: Cartridges can be used until the last day of the expiration month shown on the cartridge. Reagent cartridges can be kept for up to three (3) months at room temperature anytime before the expiration date. Record on the carton, the date the carton was placed at room temperature.
- Capillary holders may be stored refrigerated or at room temperature 15° to 30° C (59° F to 86° F). Unused capillary holders may be saved and used with any lot of reagent cartridges.

Control Storage:

- Store *lyophilized* DCA A1c Normal and Abnormal controls at 2° to 8° C (36° to 46° F) or -20°C (-4°F). Controls can be used until the last day of the expiration month shown on the bottle. Do not use if moisture is present in the vial prior to reconstitution. This is an indication of deterioration of the control material.
- *Reconstituted* DCA Hemoglobin A1c Normal and Abnormal Control solution should not be frozen. Leave the bottle cap on when not in use. Controls may remain at room temperature for a period of 30 minutes when testing, and should be stored refrigerated at all other times. Store the controls refrigerated in an upright position and tightly capped. Discard any reconstituted control solution appearing turbid or obviously contaminated. The reconstituted control is stable for three (3) months from the date of reconstitution when stored refrigerated.

CALIBRATION:

- Calibrate at least every six months and each time a new lot number of reagent is used.
- Values for reagent calibration parameters are encoded onto the calibration card provided with each lot of reagent cartridges.
- Prior to use of reagent cartridges, the calibration bar code must be scanned. The instrument stores two calibrations for the DCA A1c assay. Each calibration is for a different lot number. Before the sample can be analyzed, the reagent cartridge bar code (containing lot number and test name) is scanned. This accesses the appropriate calibration parameter values (calibration curve) for the particular lot number of reagent in use.
- If the calibration curve has not been scanned into the instrument for the particular lot number of cartridges in use, the DCA Vantage software prompts the user to scan the calibration card.

Standard Preparation:

The only material required is the Calibration Card provided in DCA A1C Reagent Kit.

Calibration Procedure:

1. Locate the dot (on the instrument) next to the bar code track.
2. Locate the bar code on the Calibration Card.
3. Hold the card so that the bar code faces right.
4. Insert the card into the bar code track (above dot). Hold card gently against the right side of track.
5. Quickly slide the card down past the dot.
   - A beep sounds to signal a successful scan.
   - If no beep sounds, repeat the scanning procedure. If a beep repeatedly fails to sound, refer to Troubleshooting, Section 6 of the Operating Manual.
6. To view the calibration status of the DCA Vantage Analyzer:
   - Press the MENU key,
   - Press the MENU/NEXT key until [VIEW CALIBRATION STATUS] is displayed.
   - Press ENTER to view the most recent calibration status.
   - Press NEXT to recall a second stored calibration.
   - To exit the menu, press ESCAPE twice or scan a bar code.

QUALITY CONTROL:

Controls will be run every day that patient testing is performed. All control results must be within the acceptable range before any patient sample is tested and the result reported. If the control result is not acceptable (out-of-range), carefully troubleshoot the system, correct any problems identified and re-assay the controls. When control results are within range, patient samples may be tested and reported. Record all troubleshooting efforts on the Maintenance Log Sheet. Retain a permanent record of control results.

Troubleshooting Steps

Check the following:

1. Reagent cartridges (expiration date, appearance, calibration, etc.)
2. Controls (expiration date, appearance, handling, etc.)
3. Instrument
4. Environmental conditions
5. Analyst technique
6. Refer to the Operating Manual; Section 6 “Error Codes and Troubleshooting”

Quality Control Procedure- Stepwise*:*

Collect aliquots of reconstituted control for testing as follows:

1. Prepare the controls according to the manufacturer’s instructions.
2. From a DCA A1c Reagent Kit, obtain a capillary holder and remove it from the plastic package.
3. Unscrew the control bottle eyedropper cap assembly. Avoid introducing air bubbles into the sample. While applying only slight pressure to the bulb, insert the tip of the

eyedropper into the control solution (tilt bottle as necessary). Release pressure on bulb to aspirate a very small amount of control solution.

4. Hold the glass capillary tube to the control solution collected in the eyedropper and completely fill the 1 μL tube. Touch *only* the tip of the tube to the control solution. If an air bubble(s) is present in the filled tube, discard the capillary holder and refill a new one.

   Note: Use caution to prevent the control solution from coming in contact with the plastic part of the capillary holder. If control solution comes in contact with the plastic of the capillary holder, discard the capillary holder.
5. Avoid touching the eyedropper to any other surfaces. Squeeze any excess control solution out of the eyedropper back into the control solution bottle. Carefully replace and screw the eyedropper cap assembly back onto control bottle.
6. Using a lint-free tissue, carefully wipe any control solution off the sides of the glass capillary tube. Use caution not to touch the tissue to the open end of the tube. Contact with the open end could result in loss of sample. If sample loss is obvious, discard the capillary holder and refill a new one.
7. Carefully insert the capillary holder into a DCA A1C Reagent Cartridge until the holder gently snaps into place. See the DCA Vantage Analyzer Operating Manual for instructions on placing the capillary holder in the reagent cartridge.

SCAN/LOAD/PULL:

8. Locate the dot (on the instrument) next to the bar code track.
9. Locate the bar code on the Reagent Cartridge.
10. Hold the cartridge so that the bar code faces right.
11. Insert the cartridge into the bar code track (above dot). Hold the cartridge gently against the right side of the bar code track.
12. Slide the cartridge down past the dot.A beep sounds to signal a successful scan. If no beep sounds, repeat the procedure. If a beep repeatedly fails to sound, refer to the Troubleshooting Section (6) of the Operating Manual.
13. At the display prompt, [LOAD CARTRIDGE or CONTROL, PULL-TAB, CLOSE DOOR], open the cartridge compartment door.
14. Hold the cartridge so that the bar code faces RIGHT.
15. Insert the cartridge into the compartment until a subtle snap is heard/felt.
16. Using a smooth, slow, continuous motion, pull the flexible pull-tab completely out of the reagent cartridge. Close the door and dispose of the pull-tab. Five (5) seconds after the door is closed, a beep sounds and the assay begins.

    Note: If you accidentally close the door before you pull the flexible tab, you have five (5) seconds to re-open the door; the display returns to "LOAD CARTRIDGE." You may now pull the tab or correct the existing problem.
17. The DCA prompts for certified operator identification.

AFTER THE TEST IS COMPLETE:

18. Record the displayed A1c control result on the log before removing the reagent cartridge.
19. Remove the reagent cartridge from the DCA
20. Open the cartridge compartment door.

21. Locate the button on the right side of the cartridge compartment. Push and hold it down with your right hand. With your left hand, gently push the plastic tab on the cartridge to the right; this action releases (unlocks) cartridge.
22. Pull the reagent cartridge out of the compartment. If the door is opened (within 15 minutes after assay completion), the test result is displayed for only 30 seconds. If the

door is not opened, the test result will remain displayed for 15 minutes. At 15 minutes, an audible tone (error buzz) sounds and the display changes to "READY: REMOVE TEST"

23. Closely examine the capillary tube tip. It should be clear of any control material. If any control material remains in the tip, repeat the control.

TEST PROCEDURE:

1. Set the DCA Vantage power switch to ON. Allow time for the instrument to warm up (1 to 8 minutes). [READY: SCAN BAR CODE] is displayed and a beep is heard when the instrument is ready.
2. Mix the sample well (invert several times or use an aliquot mixer) to prevent separation of red blood cells and plasma.
3. Remove the stopper from the blood collection tube. It is desirable for a small amount of blood to remain on the inside of the rubber stopper.
4. Hold the capillary holder at an angle.
5. Touch *only* the tip of the capillary to the blood sample on the stopper.

   Note: Any blood touching the plastic of the capillary holder can cause an invalid A1c result or possibly an error message. If blood contacts the plastic part of the capillary holder, discard the capillary holder and start over.
6. Using a lint-free tissue, carefully wipe the outside of the glass capillary. Avoid allowing the tissue to touch the open end of the glass capillary. If the sample loss is obvious, discard the capillary holder, and then repeat the procedure using a new capillary holder. Inspect the glass the capillary for the presence of bubble(s). If any are present, discard the capillary holder and use a new one.
7. Carefully insert the capillary holder into the reagent cartridge until the holder gently snaps into place.

SCAN/LOAD/PULL:

8. Locate the dot (on the instrument) next to the bar code track.
9. Locate the bar code on the Reagent Cartridge.
10. Hold the cartridge so that the bar code faces right.
11. Insert the cartridge into the bar code track (above dot). Hold the cartridge gently against the right side of the bar code track.
12. Slide the cartridge down past the dot.
13. A beep sounds to signal a successful scan. If no beep sounds, repeat the procedure. If a beep repeatedly fails to sound, refer to the Troubleshooting Section (6) of the Operating Manual.
14. At the display prompt, [LOAD CARTRIDGE or CONTROL, PULL-TAB, CLOSE DOOR], open the cartridge compartment door.
15. Hold the cartridge so that the bar code faces RIGHT.
16. Insert the cartridge into the compartment until a subtle snap is heard/felt.
17. Using a smooth, slow, continuous motion, pull the flexible pull-tab completely out of the reagent cartridge. Close the door and dispose of the pull-tab. Five (5) seconds after the door is closed, a beep sounds and the assay begins.

    Note: If you accidentally close the door before you pull the flexible tab, you have five (5) seconds to re-open the door; the display returns to "LOAD CARTRIDGE." You may now pull the tab or correct the existing problem.
18. The DCA prompts for Patient Identification to be entered using two identifiers: name and Medical record number or account number.
19. The DCA prompts for certified operator identification.

AFTER THE TEST IS COMPLETE:

20. Record the displayed A1c result before removing the reagent cartridge.
21. Remove the reagent cartridge from the DCA Vantage
22. Open the cartridge compartment door.
23. Locate the button on the right side of the cartridge compartment.
24. Push and hold it down with your right hand.
25. With your left hand, gently push the plastic tab on the cartridge to the right; this action releases (unlocks) cartridge.
26. Pull the reagent cartridge out of the compartment.
27. If the door is opened (within 15 minutes after assay completion), the test result is displayed for only 30 seconds. If the door is not opened, the test result will remain displayed for 15 minutes. At 15 minutes, an audible tone (error buzz) sounds and the display changes to "READY: REMOVE TEST"

    Note: If the displayed test result was not recorded, use the MENU to recall up to 16 test results (refer to Section 3, Operating Manual).

28. ***Closely examine the capillary tube tip. It should be clear of any blood. If any blood remains in the tip, the result is invalid and you must repeat the test.***

PROCEDURE NOTES:

- Refer to the DCA Vantage Analyzer Operating Manual for:
  - *Instrument Set-up*
  - *Cleaning*
  - *Troubleshooting Errors*
- When the instrument is not in use, the power may be turned OFF without loss of stored results. However, when the power is subsequently restored, a warm up period of 1-8 minutes is required.
- When the instrument is not in use for more than 5 minutes, the display will change to a block- shaped moving cursor. If the screen saver is on, press any key to return to a normal display before performing any other steps.
- The current time is displayed using a "blinking" colon. When the colon does not blink, the time displayed is the time the assay began.

LIMITATIONS OF THE PROCEDURE:

- The DCA Vantage A1c assay gives accurate and precise results over a range of total hemoglobin of 7 to 24 g/dL. Most patients have hemoglobin concentrations within these values. However, patients with severe anemias may have hemoglobin concentrations lower than 7 g/dL, and patients with polycythemia may have hemoglobin concentrations above 24g/dL. Patient specimens should be assayed by a test employing a different assay principle if their total hemoglobin concentrations are outside of this acceptable range.
- Samples containing high amounts of Hemoglobin F (>10%) may yield lower than expected Hemoglobin A1c results with this test. Hemoglobin A1c results for such patients should not be compared to published normal or abnormal values.
- Highly lipemic blood samples stored for long periods of time and/or refrozen should not be assayed using this method. Refer to the package insert for details on limitations for use.

- For error codes and warning messages, refer to Chapter 6 of the DCA Vantage Operating Manual.
- **Effect of Hemoglobin Variants:** The antibody in the DCA HbA1c assay is specific for the first few amino acid residues of the glycated amino-terminus of the β-chain of Hemoglobin A. Any glycated hemoglobin molecule having this same structure will be measured in the assay. Most glycated hemoglobin variants are immunoreactive in the DCA 2000 HbA1c assay (e.g., HbS1c, HbC1c, HbE1c). The point mutations in these molecules occur at the 6 position of the β-chain (HbS and HbC) and at the 26 position of the β-chain (HbE). Thus, the point mutations in these variants do not affect the binding of the antibody used in the DCA 2000 HbA1c assay. The DCA 2000 will report %HbA1c values that will reflect the glycemic control of patients with these hemoglobinopathies.
- **Effect of Pre-HbA1c (Labile Fraction):** The labile fraction (Schiff base attachment of glucose to HbA, or pre-HbA1c) does not affect the assay result because the antibody is specific for the stable ketoamine.
- **Effect of Carbamylated Hemoglobin:** Carbamylated hemoglobin (elevated in patients with uremia) does not affect the assay result because the antibody is specific for the sugar moiety of HbA1c.

CALCULATIONS:

The displayed test result requires no further calculation

REPORTING RESULTS:

- The DCA Vantage A1c reportable range is: 2.5% to 14.0%
- A result displayed as "<" indicates a concentration below the lower limit (2.5%). Result should be reported as <2.5% (result in SOFT as <2.5 (space bar) @L)
- A result displayed as ">" indicates a concentration above the upper limit (14.0%). Result should be reported as >14.0% (result in SOFT as <14.0 (space bar) @L)
- Quality controls must be confirmed before resulting patient testing. All results are footnoted using the canned comment @VARI which reads "Suspected hemoglobin variant present. Test performed by the Laboratory on the DCA Vantage analyzer."

Reference Ranges:

4.3 – 5.6% for all ages

- **Reporting Format:** DCA results will be entered into the LIS system in Result Entry using the canned comment @VARI which reads "Suspected hemoglobin variant present. Test performed by the Laboratory on the DCA Vantage analyzer."
- The verified LIS instant report should be printed, reviewed and filed alphabetically in the appropriate binder.

REFERENCES:

1. NCCLS Document GP2-A2, Vol. 4, No. 2, NCCLS, Wayne, PA.
2. DCA Vantage Analyzer Operating Manual. Siemens Medical Solutions Diagnostics, Tarrytown, NY 10591. 2007
3. Package insert: DCA Hemoglobin A_{1C} Reagent Kit. Siemens Medical Solutions Diagnostics, Tarrytown, NY 10591. Revised 05/06.
4. Package insert: DCA Hemoglobin A_{1C} Normal and Abnormal Control Kit. Siemens Medical Solutions Diagnostics, Tarrytown, NY 10591. Revised 6/03.