Pediatric Short Stay – H. Plaza

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ACTION	*DATE*	*INITIAL*
Put in Use	12/6/08	MAS
Reviewed	1/10/15	MAS
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Supersedes:

TITLE: DCA Vantage – Hemoglobin A1c (HbA1c)

PRINCIPLE

For the measurement of total hemoglobin, potassium ferricyanide is used to oxidize hemoglobin in the sample to methemoglobin. The methemoglobin then complexes with thiocyanate to form thiocyanmethemoglobin, the colored species that is measured. The extent of color development at 531 nm is proportional to the concentration of total hemoglobin in the sample.

For the measurement of specific HbA_{1c}, an inhibition of latex agglutination assay is used. An agglutinator (synthetic polymer containing multiple copies of the immunoreactive portion of HbA_{1c}) causes agglutination of latex coated with HbA_{1c} specific mouse monoclonal antibody. This agglutination reaction causes increased scattering of light, which is measured as an increase in absorbance at 531 nm. HbA_{1c} in whole blood specimens competes for the limited number of antibody-latex binding sites causing an inhibition of agglutination and a decreased scattering of light. The decreased scattering is measured as a decrease in absorbance at 531 nm. The HbA_{1c} concentration is then quantified using a calibration curve of absorbance versus HbA_{1c} concentration. The percent HbA_{1c} in the sample is then calculated as follows:

$\%\ HbA_{1c} = ([HbA_{1c}] / [\text{Total Hemoglobin}]) \times 100$

Both the concentration of hemoglobin A_{1c} specifically and the concentration of total hemoglobin are measured, and the ratio reported as percent hemoglobin A_{1c}.

All measurements and calculations are performed automatically by the DCA Analyzer, and the screen displays percent HbA_{1c} at the end of the assay.

The DCA Vantage is a semi-automated, bench top system. It is designed to quantitatively measure the percent of Hemoglobin A_{1c} in blood.

The DCA Vantage system is intended for professional use in a physician's office or hospital laboratory. Tests performed using the DCA Vantage system is intended for *in vitro* diagnostic use. As with all diagnostic tests, do not base a definitive diagnosis on the results of a single test. A physician should make a diagnosis after all clinical and laboratory findings are evaluated.

The system is a spectrophotometer that analyzes the intensity of the light transmitted through the cartridge optical window and reports the results in clinically meaningful units. No calculations are required by the user. When an operator swipes a calibration card, the barcode reader reads the card and the system automatically performs the calibration.

CLINICAL APPLICATION AND USE

This assay provides a convenient, quantitative method for *in vitro* diagnostic use to measure the percent concentration of hemoglobin A_{1c} in blood. The measurement of hemoglobin A_{1c} concentration is recommended for monitoring the long-term care of persons with diabetes.

Hemoglobin A_{1c} is formed by the non-enzymatic glycation of the N-terminus of the β-chain of hemoglobin Ao. The level of hemoglobin A_{1c} is proportional to the level of glucose in the blood over a period of approximately two months. Thus, hemoglobin A_{1c} is accepted as an indicator of the mean daily blood glucose concentration over the preceding two months. Studies have shown that the clinical values obtained through regular measurement of hemoglobin A_{1c} leads to changes in diabetes treatment and improvement of metabolic control as indicated by a lowering of hemoglobin A_{1c} values.

The Diabetes Control and Complications Trial (DCCT) showed the importance of improved glycemic control in reducing the risk and progression of the complications of diabetes. Glycemic control was determined by the measurement of hemoglobin A_{1c}. The American Diabetes Association (ADA) recommends measurement of hemoglobin A_{1c} levels two to four times per year, less frequently in patients with stable control.

SPECIMEN COLLECTION AND HANDLING

Important: After the glass capillary is filled with sample, analysis must begin within **5 minutes.**

BIOHAZARD
All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear facial protection, gloves, and protective clothing.

- Collect blood in the provided glass capillary (within plastic capillary holder).
- The glass capillary holds 1 µL of whole blood.
- The blood sample may be obtained by fingerstick or venipuncture.
- Acceptable anticoagulants are EDTA, heparin, flouride/oxalate, and citrate.

Specimen Storage and Stability

- Preserved whole blood may be stored at -70°C to 5°C (-94°F to 41°F) for two weeks, or up to 25°C (77°F) for one week.
- Do not refreeze previously frozen blood samples or store in a self-defrosting freezer.
- Allow blood sample to reach room temperature. Mix blood sample thoroughly before use.

REAGENT

Storage and Stability

Upon receipt of the kit, check the temperature indicator located on the front of the carton. If the indicator has turned red, do not use the reagent cartridges. Note time and date received, and for assistance in obtaining a replacement kit, refer to instructions given on the carton.

- Store reagent cartridges refrigerated at 2°-8°C (36°-46°F).
- Capillary holders may be stored refrigerated or at room temperature (15°-30°C [59°-86°F]).

- Reagent cartridges can be kept for up to **three months at room temperature** anytime before the expiration date.
- Record on the carton, the date the carton was placed at room temperature.

IMPORTANT: Do not use reagent cartridges after the last day of the expiration month.

Ingredients

DCA HbA_{1c} Reagent Cartridges contain the following ingredients:

Reagent	*Volume (Amount)*	*Ingredients*
Antibody Latex	10 µL dried in each reagent cartridge	HbA_{1c}-specific mouse monoclonal antibody adsorbed onto latex particles. 2.5% w/v antibody-latex in 10 mM glycine buffer; 16% w/v nonreactive ingredients.
Agglutinator	10 µL dried in each reagent cartridge	0.005% w/v poly (aspartic acid) polymer covalently attached to the HbA_{1c} hapten in 20 mM sodium citrate buffer containing 0.1% w/v bovine serum albumin and 1% w/v nonreactive ingredients.
Buffer Solution	10 mL dried in each cartridge	8.1% w/v lithium thiocyanate, 0.01% digitonin in 200 mM glycine buffer (0.6 mL in each cartridge). Oxidant: 1.5% w/v potassium ferricyanide in water with 21% w/v nonreactive ingredients.

REAGENT SPECIAL PREPARATION AND HANDLING

Reagent Cartridges

To open the foil pouch, tear down from the corner notch (until the entire long side of the pouch is open).

Discard the reagent cartridge if any of the following conditions exist:

- The cartridge is damaged.
- The flexible pull-tab is loose or missing.
- The desiccant bag is missing or open.
- Loose desiccant particles are found inside the foil package.
- If the foil package is open for more than 60 minutes.

Upon removal from refrigerated storage, allow the reagent cartridge to warm up at room temperature for 10 minutes in the unopened foil pouch, or five minutes if removed from the foil pouch. After opening the foil pouch, the reagent cartridge must be used within one hour.

Capillary Holders

Unused capillary holders may be saved and used with any lot of reagent cartridges. Each capillary holder is packaged separately in a blister package. To remove the capillary holder, remove the white plastic film from the clear plastic blister. DO NOT PUSH the capillary holder out of or through the plastic.

Before use, inspect the capillary holder for the presence of the following parts:

- absorbent pad
- glass capillary
- latching mechanism

If the capillary holder is missing any of the above parts, discard the capillary holder.

CALIBRATION

For detailed procedural information about performing a calibration, refer to the DCA Vantage Operator's Guide.

The DCA Vantage analyzer is calibrated by the manufacturer. Thereafter, the instrument automatically self-adjusts during the first-time power-up and during each assay. In the event the system is unable to make appropriate internal adjustments, an error message is displayed.

The values for the calibration parameters are encoded onto the calibration card provided with each lot of reagent cartridges. Before using a new lot of reagent cartridges, scan the calibration card into the analyzer.

Before you analyze samples, the reagent cartridge barcode (containing lot number and test name) is scanned. This accesses the appropriate calibration parameter values (calibration curve) for the particular lot number of reagent cartridges in use. If no calibration curve is in the instrument for the particular lot number of cartridges in use, the instrument prompts the user to scan the calibration card.

The instrument can store two calibrations for the DCA HbA_{1c} Assay. Each of two calibrations is for a different lot number.

When reagent cartridges are stored and used properly, acceptable performance up to the expiration date is ensured. To verify proper functioning of the DCA System, analyze DCA HbA_{1c} Controls (refer to Quality Control section).

Calibration Interval

Before using a new lot of reagent cartridges, scan the calibration card in to the analyzer.

Calibration Procedure

You must scan the calibration card for a lot of HbA_{1c} cartridges before you can use the lot on the DCA Vantage system. Scanning the calibration card enters the information on the DCA Vantage system.

1. Locate the dot on the system next to the barcode track.
2. Locate the barcode on the calibration card.
3. Hold the card so that the barcode faces to the right.
4. Insert the Calibration card into the top of the barcode track.
5. Hold the Calibration card gently against the right side of the track and smoothly slide the card down.

   A beep sounds to signal a successful scan.

   NOTE: If no beep sounds, repeat the scanning procedure. If you repeatedly fail to hear a beep, refer to the Troubleshooting section in the DCA Vantage Operator's Guide.
6. To return to the Home screen, select **OK**.

Accessing Calibration Data

Use the Calibration Data screen to access calibration data including scan date and time, and lot number for DCA HbA_{1c}.

1. At the Recall menu, select **Calibration Data**. The Calibration Data screen displays.
2. Select **HbA_{1c}**.

   The HbA_{1c} calibration data displays.

3. Highlight the calibration data that you want to display, and select **View**. The Calibration Data screen displays.
4. To print the calibration data, select **Print**.
5. To return to the Recall menu, select **Recall**.

QUALITY CONTROL (QC)

Quality Control (QC)

For detailed QC procedural information, refer to the DCA Vantage Operator's Guide and the HbA_{1c} instructions for use.

To assure quality of both testing procedures and patient results for hemoglobin A_{1c}, the DCA System performs 48 optical, electronic, mechanical, and reagent systems checks during the course of each specimen assay. These checks include calibration verification during every test. If an assay or system error occurs during any individual measurement, the system automatically reports an error message, preventing the reporting of erroneous patient results.

QC Materials

DCA HbA_{1c} Normal & Abnormal Control Kit, 5068A.

QC Frequency

- **DAILY** - On the days when patient testing is performed
- With each new shipment of reagents
- With each new lot of reagent
- Each time a calibration card is scanned
- To train and confirm performance acceptability of new users
- When results do not match the patients' clinical condition or symptoms
- Following maintenance or repair procedures

Troubleshooting Out-of-Range QC Values

If the control results fall outside the values stated in the package insert, the following sources of error may have occurred:

- Deterioration of the reagent cartridge test areas due to exposure to light, ambient moisture, or heat.
- Deterioration of the control solution.
- Use a new reagent cartridge to repeat the quality control procedure.
- Use a fresh box of reagent cartridges, or a new lot. If the new reagent cartridge fails to give results within the expected values, proceed to the next possible cause.
- Use a fresh control solution to repeat the quality control procedure.
- Review these instructions to ensure that the test was performed according to the procedures recommended by Siemens Medical Solutions.
- Verify that the materials are not expired.
- Verify that required maintenance was performed.

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DAILY START-UP

You must perform required control tests when they are scheduled. Patient test is disabled until the required control is performed and passed.

1. Locate the control card.

   NOTE: One side of the control card is for a normal control and the other side is for an abnormal control.

2. Locate the dot on the system next to the barcode track.
3. Locate the barcode on the Control card.
4. Hold the card so that the desired control level barcode faces to the right.
5. Insert the Control card into the top of the barcode track.
6. Hold the Control card gently against the right side of the track and quickly slide the card down.
7. A beep sounds to signal a successful scan. **NOTE:** If no beep sounds, repeat the scanning procedure. If you repeatedly fail to hear a beep, refer to the Troubleshooting section in the DCA Vantage Operator's Guide.
8. Scan the reagent cartridge containing the control to be run. (See Performing the HbA_{1c} Test)
9. Enter user ID when required

PROCEDURE

1. Open the plastic wrap of the capillary holder by tearing the wrap at the serrated edge with the arrow.
2. Inspect the capillary holder for the presence of the following parts:
   - absorbent pad
   - glass capillary
   - latching mechanism

If the capillary holder is missing any of the above parts, discard the capillary holder.

Filling the Capillary with Whole Blood from a Fingerstick

When the glass capillary is filled with the sample, **analysis must begin within 5 minutes**.

NOTE: 1 µL of blood is required to fill the capillary.

1. Hold the capillary holder at an angle.
2. Touch only the tip of the capillary to a small drop of blood on the finger until the capillary fills.

Filling the Capillary with Blood obtained by Venipuncture:

1. Mix the sample well (by inversion or use of a tube mixer) to prevent separation of red blood cells and plasma.
2. Remove the stopper from the blood collection tube in such a way that a small sample of blood remains on the stopper.
3. Hold the capillary holder at an angle.
4. Touch only the tip of the capillary to the blood sample on the stopper.

NOTE: Do not attempt to fill the capillary by touching the glass capillary to blood in a blood collection tube. Attempting to fill the capillary in this manner most often results in blood touching the capillary holder. If blood touches the capillary holder, discard the capillary holder.

5. Using a lint-free tissue, carefully wipe the outside of the glass capillary.

   NOTE: Do not allow the tissue to touch the open end of the glass capillary. Contact with the open end of the capillary could result in loss of sample (by wicking into the tissue). If sample loss is obvious, discard the capillary holder. Repeat the procedure using a new capillary holder.

6. Inspect the glass capillary for the presence of bubbles.
7. If bubbles are obvious, discard the capillary holder and repeat the procedure using a new capillary holder.

Inserting Capillary Holder into Reagent Cartridge

Avoid harsh insertion of the capillary holder. Do not dislodge the sample from the glass capillary or erroneous results may occur.

1. Carefully insert the capillary holder into the reagent cartridge until the holder gently snaps into place.

Scanning the Reagent Cartridge

1. Locate the dot (on the system) next to the barcode track.
2. Locate the barcode on the reagent cartridge.
3. Hold the reagent cartridge so that the barcode faces to the right.
4. Insert the reagent cartridge (above dot) into the barcode track.
5. Quickly and smoothly, slide the reagent cartridge down. A beep sounds to signal a successful scan.

   NOTE: If no beep sounds, repeat procedure. If a beep repeatedly fails to sound, refer to the Troubleshooting section in the DCA Vantage Operator's Guide.

Inserting the Reagent Cartridge into the System

1. Open the cartridge compartment door.
2. Hold the reagent cartridge so that the barcode faces to the right.
3. Insert the reagent cartridge into the cartridge compartment until a gentle snap is heard or felt.

   NOTE: The cartridge is designed to fit only one way into the system. Do not force the cartridge into system.

4. Using a smooth, slow, continuous motion, pull the flexible pull-tab completely out of the reagent cartridge.
5. Close the door and dispose of the flexible pull-tab. Five 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 plastic tab, you have 5 seconds to re-open the door and pull the tab.

Entering Sample Data

The Sample Data menu screen displays when the system detects the system door closes, and indicates a test is in progress after the 5-second delay.

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You are **required** to enter the following sample demographic information:

- Patient ID
- Last Name
- User ID

1. Select **Patient ID**. The Patient ID screen displays.
2. Enter the **Patient ID.**
3. Select **Enter**. The Patient ID displays next to the Patient ID button.
4. Select **Last Name**. The Last Name screen displays.
5. Select **User ID**. The User ID screen displays.
6. Select **Comments 1(**OPTIONAL**)** if a comment is desired

Removing the Reagent Cartridge

1. Open the cartridge compartment door.
2. Locate the button on the right side of the cartridge compartment.
3. Push and hold it down with your right hand.
4. With your left hand, gently push the tab on the cartridge to the right.

   This action releases (unlocks) the cartridge.
5. Pull the reagent cartridge out of the compartment.
6. Close the system door.
7. Discard the cartridge in a proper container, according to your standard laboratory procedures.

Cancelling a test

You can cancel a test anytime.

To cancel a test, select **Cancel**.

NOTE: If a test in progress is cancelled, you must discard the sample.

REPORTING OF RESULTS

Result preceded by a less than sign (<):

A less than sign in the display indicates a concentration below the lower limit of the test (under range). Report the result as being less than 2.5% HbA_{1c}. This method does not provide for re-assay using a larger sample aliquot. Results less than 2.5% HbA_{1c} are rare and may indicate that the sample contains substantial amounts of fetal hemoglobin (does not react in the immunoassay); or that the patient may be suffering from hemolytic anemia or polycythemia (conditions which often result in a significant decrease in the life span of red blood cells).

Result proceeded by a greater than sign (>):

A greater than sign in the display indicates a concentration above the upper limit of the test (over range). Report the result as being more than 14.0% HbA_{1c}. This method does not provide for re-assay using a diluted sample. To obtain a more quantitative test value, use another test method.

All laboratory tests are subject to random error. If the test result is questionable, or if clinical signs and symptoms appear inconsistent with test results, re-assay the sample or confirm the result using another method.

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Reference Interval

HbA_{1c} concentrations in the following range are reported: 2.5% to 14.0% HbA_{1c}.

The test is linear throughout this range.

PROCEDURE NOTES

Calculations

All measurements and calculations are performed automatically by the DCA Analyzer, and the screen displays percent HbA_{1c} at the end of the assay.

Disposal

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner, and in compliance with all federal, state, and local requirements.

MAINTENANCE

Weekly - see OPERATOR'S GUIDE, MAINTENANCE section for details on performance

- Cleaning the Barcode Window
- Cleaning the Exterior

Monthly – see OPERATOR'S GUIDE, MAINTENANCE section for details on performance

- Removing and cleaning the cartridge spring
- Changing the Air Filter
- Running the Optical Test

As needed – see OPERATOR'S GUIDE, MAINTENANCE section for details on performance

- Cleaning the Exterior
- Changing the Air Filter
- Removing and cleaning the cartridge spring
- Cleaning the Barcode Window
- Running the Optical Test
- Calibrating the Touch screen
- Replacing the Fuse

METHOD LIMITATIONS

The DCA HbA_{1c} assay gives accurate and precise results over a range of **total hemoglobin** of 7 to 24 g/dL. Most patients will have hemoglobin concentrations within these values. However, patients with severe anemia may have hemoglobin concentrations lower than 7 g/dL, and patients with polycythemia may have hemoglobin concentrations above 24 g/dL. Patients known to have these conditions should be assayed by a test employing a different assay principle if their hemoglobin concentrations are outside of the acceptable range.

Glycated **hemoglobin F** is not measured by the DCA HbA_{1c} assay.

At levels of hemoglobin F less than 10%, the DCA system accurately indicates the patient's glycemic control. However, at very high levels of hemoglobin F (>10%), the amount of HbA_{1c} is lower than expected because a greater proportion of the glycated hemoglobin is in the form of glycated hemoglobin F. HbA_{1c} results for such patients do not accurately indicate the patient's glycemic control and should not be compared to published normal or abnormal values.

Conditions such as hemolytic anemia, polycythemia, homozygous HbS, and HbC, can result in decreased life span of the red blood cells, which causes HbA_{1c} results to be lower than expected, regardless of the method used, and not be related to glycemic control, when using published reference ranges.

Bilirubin, up to a level of 20 mg/dL, does not interfere with this assay.

Triglycerides, up to 1347 mg/dL in fresh whole blood, do not interfere with this assay. Highly lipemic blood samples stored for long periods of time or frozen should not be assayed using this method.

Rheumatoid factor, up to 1:5120 titer, does not interfere with this assay.

Expected serum levels of the following **drugs** commonly prescribed to persons with diabetes do not interfere with this assay: Diabinese, Orinase, Tolinase, Micronase, Dymelor, glipizide.

EQUIPMENT / REPLACEMENT PARTS

The accessory items available for the DCA Vantage system are listed below.

Hemoglobin A_{1c}

Part Number	Description
5035C	DCA Reagent Kit
5068A	DCA Normal & Abnormal Control Kit

Replacement Parts

The replacement parts available for the DCA Vantage system are listed below.

Part Number	Description
00142617F	NA Power Cord for System
00171415A	Euro Power Cord for System
06498298	UK Power Cord for System
06498417	Air Filter Holder
06489248	Cartridge Return Spring
04469001	Fuse: T-1.25 A, Slow Blow; 250 volt
06488209	Cleaning Sticks (10)
06489221	Optical Test Cartridge
122521	Air Filter (2 pack) Replacement Kit
5773	Printer Paper (5 pack)
1759	Printer Paper (self adhesive, 5 pack)

TECHNICAL SUPPORT

Siemens Medical Solutions Technical Care Center: 1-877-229-3711

Customer Service: 1-800-255-3232

REFERENCES

1. Siemens Medical Solutions DCA Hb1Ac, 5051GD Rev. 02/07.
2. Siemens Medical Solutions DCA Vantage Operator's Guide, Ref 06489264 Rev. A, 2007-05.
3. Clinical and Laboratory Standards Institute. *Laboratory Documents: Development and Control; Approved Guideline—Fifth Edition*. CLSI document GP2-A5 [ISBN 1-56238-600-X]. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2006.