

DCA® Systems

Hemoglobin A_{1c} Reagent Kit

For Use With DCA® Analyzers

A Quantitative Assay for Hemoglobin A_{1c} in Blood

INTENDED USE:

This assay provides a convenient, quantitative method for measuring 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.

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.¹ 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.²

This assay is based on a latex immunoagglutination inhibition methodology.³ After loading the reagent test cartridge into the DCA® Analyzer, the test result is displayed in six minutes.

The DCA Hemoglobin A_{1c} assay is for use in laboratories such as physician office laboratories, clinics, and hospitals.

INFORMATION REGARDING CLIA WAIVER (US ONLY):

The DCA Vantage® system is CLIA-waived only when used with Siemens-branded DCA 2000+ or DCA Systems HbA_{1c} cartridges.

A certificate of CLIA waiver is required to perform the test in a waived setting.

To obtain a Certificate of Waiver, contact your state department of health or visit the CMS website for an application, form CMS-116.

Failure to adhere to the instructions for use, including instructions for limitations or intended use, and for performing QC testing, is considered as off-label use, resulting in the test being categorized as high complexity and subject to all CLIA regulation.⁵

SUMMARY AND EXPLANATION:

Hemoglobin A_{1c} is formed by the non-enzymatic glycation of the N-terminus of the β-chain of hemoglobin A_o.⁴ The level of hemoglobin A_{1c} is proportional to the level of glucose in the blood over a period of approximately two months.⁵ Thus, hemoglobin A_{1c} is accepted as an indicator of the mean daily blood glucose concentration over the preceding two months.^{6,7} Studies have shown that the clinical values obtained through regular measurement of hemoglobin A_{1c} lead to changes in diabetes treatment and improvement of metabolic control as indicated by a lowering of hemoglobin A_{1c} values.^{8,9}

CHEMICAL PRINCIPLES OF PROCEDURE:

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}.¹⁰

All of the reagents for performing both reactions are contained in the DCA Hemoglobin A_{1c} (HbA_{1c})

REAGENT CARTRIDGE Reagent Cartridge (Figure 1).

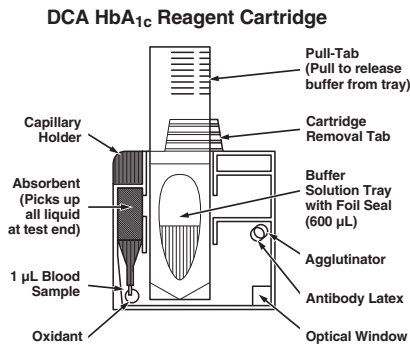


Figure 1

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 (Figure 2).

Assay Principle Inhibition Of Latex Agglutination

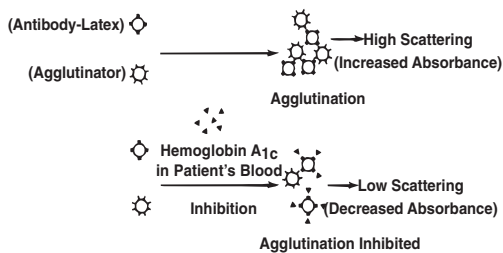


Figure 2

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:

$$\% \text{HbA}_{1c} = \frac{[\text{HbA}_{1c}]}{[\text{Total Hemoglobin}]} \times 100$$

The IFCC concentration in mmol/mol HbA_{1c} is calculated as follows:

$$\text{HbA}_{1c} \text{ mmol/mol} = (\text{HbA}_{1c} \text{ mmol}) / (\text{Total hemoglobin mol})$$

All measurements and calculations are performed automatically by the DCA Analyzer, and the screen displays the HbA_{1c} at the end of the assay. Values in this insert are in % HbA_{1c} NGSP and where shown in parentheses, as mmol/mol HbA_{1c} IFCC.

KIT CONTENTS:

10 Reagent Cartridges	
10 Capillary Holders	
Calibration Card	

REAGENTS:

Antibody Latex: 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 (10 µL dried in each reagent cartridge).

Agglutinator: 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 (10 µL dried in each cartridge).

Buffer Solution: 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 (10 µL dried in each cartridge).



H411 - Toxic to aquatic life with long lasting effects.

P273 - Avoid release to the environment.

P391 - Collect spillage.

P501 - Dispose of contents and container in accordance with all local, regional, and national regulations.

Safety data sheets (MSDS/SDS) available on www.siemens.com/poc



CAUTION:

DCA HbA_{1c} Reagent Cartridges are for **IVD** *in vitro* diagnostic use.

Safety glasses, gloves and lab coat are recommended when using the DCA System.



WARNING:

To prevent injury, do not force removal of a cartridge from the instrument. Consult the operator's guide to verify the proper removal technique. Contact your technical service provider if the problem cannot be solved.

TEMPERATURE INDICATOR:


Upon receipt of this 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.

STORAGE:

2°C/36°C Store reagent cartridges refrigerated at 2–8°C (36–46°F).

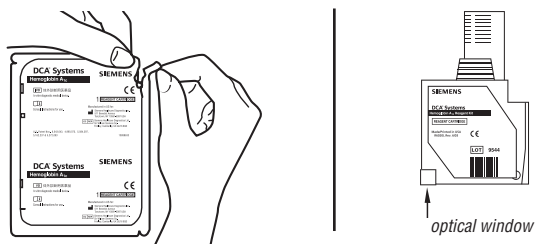
15°C/30°C Capillary holders may be stored refrigerated or at room temperature (15–30°C/59–86°F).

USE LIFE:

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

RECOMMENDED PROCEDURES FOR 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 the cartridge is damaged, the pull-tab is loose or missing, the desiccant is missing, or if loose desiccant particles are found inside the foil pouch.

Upon removal from refrigerated storage, allow the reagent cartridge to warm up at room temperature for 10 minutes (in the unopened foil pouch) or 5 minutes (if removed from the foil pouch). **After opening the foil pouch, the reagent cartridge must be used within (1) hour.**

RECOMMENDED PROCEDURES FOR HANDLING 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.



Discard the plastic capillary holder if any of the following are missing from the holder: (a) glass capillary, (b) absorbent pad, (c) latching mechanism.

STABILITY OF REAGENT CARTRIDGES:

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

SPECIMEN COLLECTION AND PREPARATION:

The provided glass capillary (within plastic capillary holder) holds 1 µL of whole blood. The blood sample may be obtained by finger stick or venipuncture. Acceptable anticoagulants are EDTA, heparin, fluoride/oxalate, and citrate.

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

EDTA, heparin, fluoride/oxalate, and citrate preserved whole blood may be stored at -70–5°C (-94–41°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.

TESTING PROCEDURE:

 See the Quick Reference Guide and Operator's Guide for detailed illustrated directions.

CALIBRATION:

Instrument: The DCA Analyzer is calibrated by the manufacturer. Thereafter, the instrument automatically self-adjusts during 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.

Reagent: Before reagent cartridges are released by the manufacturer, each lot of reagent cartridges undergoes a thorough analysis and characterization. Values of calibration parameters based on a DCCT reference method are determined that provide for optimal reagent performance. DCA HbA_{1c} test method is National Glycohemoglobin Standardization Program (NGSP) Certified and is traceable to International Federation of Clinical Chemistry (IFCC) reference materials and test methods. The values for the calibration parameters are encoded onto the calibration card provided with each lot of reagent cartridges. Prior to use of each new lot of reagent cartridges, scan the calibration card into the analyzer.

Before the sample can be analyzed, 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).

QUALITY CONTROL:

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.

CLIA WAIVED LABORATORIES:

It is recommended that quality control specimens be tested with each new lot of reagents, new shipment of reagents and monthly for reagents that have been stored for more than 30 days. QC testing is recommended to ensure reagent storage integrity, train and confirm performance acceptability for new users, and when patients' clinical conditions or symptoms do not match. Additional QC intervals may be required as per your laboratory procedures. Liquid ready-to-use controls are available; contact technical support for recommendations.

Compare QC results to those listed as acceptable by the QC manufacturer. If control results are not acceptable, do not test patient samples until the problem is resolved. Repeat control testing until results are acceptable.

For technical support assistance call (877) 229-3711.

ALL OTHER LABORATORIES:

The staff at each laboratory site can benefit by establishing a quality assurance plan, based on their institution's policies. Run quality control specimens under the following conditions:

- At regular intervals determined by the laboratory procedures
 - With each new shipment of reagents
 - With each new lot of reagents
 - Each time a calibration card is scanned
 - To train and confirm performance acceptability for new analysts
 - When results do not match the patient's clinical condition or symptoms.
- Good laboratory practices include a well-designed and implemented quality control process.

These practices, for example, may involve:

- Proper storage and handling of reagent kits
- Careful sample collection and handling procedures
- Training of testing personnel
- Routine review of sample and control results
- Periodic quality system reviews
- Retention of quality control testing records.

If the problem cannot be corrected, or the reason for an out-of-limits result cannot be determined, contact the Authorized Representative nearest you.

RESULTS:

The displayed test result requires no further calculation. HbA_{1c} concentrations in the following range are reported: 2.5–14.0% HbA_{1c} NGSP (HbA_{1c} range 4–130 mmol/mol IFCC).

The test is linear throughout this range.

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} NGSP (4 mmol/mol HbA_{1c} IFCC). This method does not provide for re-assay using a larger sample aliquot. Results less than 2.5% HbA_{1c} NGSP (4 mmol/mol HbA_{1c} IFCC) 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 preceded 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} NGSP (130 mmol/mol HbA_{1c} IFCC). 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.

LIMITATIONS OF PROCEDURE:

The DCA HbA_{1c} assay gives accurate and precise results over a range of total hemoglobin of 7–24 g/dL. Most patients will 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 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, Dylemor, glipizide.

EXPECTED VALUES:

The expected normal range for % HbA_{1c} using the DCA HbA_{1c} test was determined by assaying blood samples from 103 apparently healthy individuals (fasting blood glucose < 120 mg/dL). No significant differences in normal range were observed among males and females, geographical location, or age groups evaluated. The mean HbA_{1c} value was 5.0% ± 0.35% (1 S.D.). The range was 4.2–6.5%. The 95% confidence limits (mean ± 2 S.D.) were 4.3–5.7%. These values are similar to those reported in the literature.⁷

Depending on the assay methodology used, HbA_{1c} is approximately 3–6% in non-diabetics, 6–8% in controlled diabetics and can be as much as 20% or higher in poorly controlled diabetics.¹¹ However, each laboratory should determine normal ranges to conform with the population being tested.

SPECIFIC PERFORMANCE CHARACTERISTICS:

The precision and correlation data are results of studies conducted by the staff at separate physician offices. The statistical calculations were performed following Clinical Laboratory Standards Institute (CLSI) procedures.

Precision: Multiple DCA 2000 HbA_{1c} assays of two different commercially prepared whole blood controls were performed by three independent investigators. The assigned values listed were determined from studies conducted by the manufacturer. Within-run precision was evaluated by including Normal and Abnormal controls, in duplicate, in each run of clinical specimens.

Control	Site No.	Assigned Value (HbA _{1c})	Mean Value (HbA _{1c})	No. Runs	No. Assays	Within-Run S.D.	Within-Run %C.V.	Between-Run S.D.	Between-Run %C.V.
Normal	1	5.2	4.95	21	42	0.16	3.3	Neg.*	Neg.*
Normal	2	5.2	5.10	22	44	0.11	2.2	0.06	1.2
Normal	3	5.2	5.11	22	44	0.12	2.3	0.06	1.1
Abnormal	1	11.9	11.32	21	42	0.34	3.0	Neg.*	Neg.*
Abnormal	2	11.9	11.86	22	44	0.33	2.8	0.51	4.3
Abnormal	3	11.9	11.81	22	44	0.44	3.7	0.11	0.9

*Negligible

Correlation: The percentage of HbA_{1c} in clinical specimens ranging from 3.8–14.0% HbA_{1c} (both venous and capillary) was determined using the DCA 2000 HbA_{1c} System (y) and ion exchange high performance liquid chromatography (HPLC) (x). Results are as follows:

Site No.	Sample Type	No. of Assays	Regression Line	Standard Error of Estimate	Correlation Coefficient
1	venous	50	$y = 0.91x + 0.26$	0.42	0.98
1	capillary	50	$y = 0.94x + 0.00$	0.51	0.98
2	venous	47	$y = 0.89x + 0.42$	0.39	0.98
2	capillary	47	$y = 0.91x + 0.34$	0.50	0.97
3	venous	49	$y = 0.94x + 0.34$	0.42	0.98
3	capillary	50	$y = 0.91x + 0.58$	0.52	0.97

In addition, a correlation study was performed at a university diabetes center using the DCA 2000 HbA_{1c} System (y) and a reference HPLC¹² (x) used during the DCCT:

Site No.	Sample Type	No. of Assays	Regression Line	Standard Error of Estimate	Correlation Coefficient
4	venous	100	$y = 1.02x - 0.00$	0.45	0.98

CLIA WAIVER ACCURACY:

To evaluate the expected performance of the Siemens Healthcare Diagnostics DCA Hemoglobin A_{1c} product used on the DCA 2000 analyzer in a CLIA-waived setting, a lay user field study was performed at three non-laboratory study sites. The 68 participants represented diverse demographics, had no previous laboratory experience, and received no training for the study. Participants were provided with six (6) masked whole blood hemolysates with established target concentrations for HbA_{1c} to be used as patient specimens: 4.36, 6.25, 8.18, 8.88, 9.94, and 11.63% HbA_{1c}. The lay user results were compared to target values traceable to the high pressure liquid chromatography (HPLC) reference method used at the Glycohemoglobin Reference Laboratory at the University of Missouri Medical Center.

A summary of the performance is shown below.

Lay User Results: 408 Lay Users: 68

The overall accuracy and imprecision rates for HbA_{1c} were:

Target Level (% HbA _{1c})	Mean (% HbA _{1c})	Accuracy	95% CI*	SD	Imprecision	% CV
4.36	4.35		4.30–4.40	0.24		5.4
6.25	6.14		6.10–6.18	0.18		2.9
8.18	8.10		8.04–8.16	0.23		2.9
8.88	8.97		8.90–9.04	0.28		3.2
9.94	9.96		9.88–10.02	0.30		3.0
11.63	11.71		11.61–11.81	0.39		3.4

*95% Confidence Interval

Statistical analysis (t-statistics) demonstrated that the observed differences among the three study sites were not significant.

Specificity:

Effect of Hemoglobin Variants: The antibody in the DCA HbA_{1c} assay is specific for the first few amino acid residues of the glycosylated amino-terminus of the β-chain of hemoglobin A. Any glycosylated hemoglobin molecule having this same structure will be measured in the assay. Most glycosylated hemoglobin variants are immunoreactive in the DCA HbA_{1c} assay (such as, HbS_{1c}, HbC_{1c}, HbE_{1c}). 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 HbA_{1c} assay. The DCA reports %HbA_{1c} values that reflect the glycemic control of patients with these hemoglobinopathies.^{13,14}

Effect of Pre-HbA_{1c} (Labile Fraction): The labile fraction (Schiff base attachment of glucose to HbA, or pre-HbA_{1c}) does not affect the assay result because the antibody is specific for the stable ketoamine.¹³

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 HbA_{1c}.^{15,16}

AVAILABILITY:

DCA HbA_{1c} Reagent Kit is available as [REF] 5035C (10's). DCA [CONTROL] [NORMAL] Normal and [CONTROL] [ABNORMAL] Abnormal Control kit is available as [REF] 5068A.

To receive a hardcopy of this document, please contact your local technical support provider or distributor.

GLOSSARY OF ACRONYMS

ADA: American Diabetes Association • **CLIA:** Clinical Laboratory Improvement Amendments • **CLSI:** Clinical and Laboratory Standards Institute

DCCT: Diabetes Control and Complications Trial • **IFCC:** International Federation of Clinical Chemistry • **NGSP:** National Glycohemoglobin Standardization Program

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
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Made in US

 Siemens Healthcare Diagnostics Inc
511 Benedict Avenue
Tarrytown, NY 10591-5097 USA



Siemens Healthcare Diagnostics Ltd.
Sir William Siemens Sq.
Frimley, Camberley, UK GU16 8QD

シーメンスヘルスケア・
ダイアグノスティクス株式会社
東京都品川区大崎1-11-1
Siemens Healthcare Diagnostics


www.siemens.com/poc

**Global Siemens
Headquarters**
Siemens AG
Wittelsbacherplatz 2
80333 Muenchen
Germany

**Global Siemens
Healthcare Headquarters**
Siemens AG
Healthcare
Henkestrasse 127
91052 Erlangen
Germany
Phone: +49 9131 84-0
www.siemens.com/healthcare

Global Division
Siemens Healthcare
Diagnostics Inc.
511 Benedict Avenue
Tarrytown, NY 10591-5097
USA
www.siemens.com/diagnostics

