PROFESSIONAL PROCEDURE GUIDE

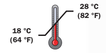
A1CNow+

PROFESSIONAL PROCEDURE GUIDE

Preparation

1 BEFORE YOU BEGIN

- Run the test with all parts of the test kit at the same temperature within the specified range.



- If the kit has recently been at high temperatures (above 82°F) or in the refrigerator, keep the kit at room temperature for at least one hour before use.
- Avoid running the test in direct sunlight, on hot or cold surfaces, or near sources of heat or cold.
- Quality control materials should be used to confirm the test kit is working properly. Refer to the product insert for information on when to run controls.

Monitor (back)

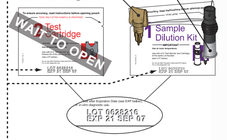
Complete test within 15 minutes.

Base (do not remove!)

Pouched Test Cartridge

Blood Collector

Sampler Body



Lot Number and Dating Label

Ensure Lot #'s match

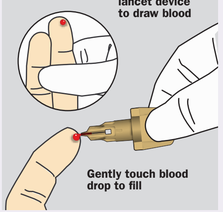
0628216

Blood Collection

2 COLLECT BLOOD

Fingerstick

Use your own lancet device to draw blood

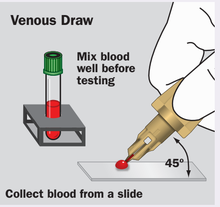


Gently touch blood drop to fill

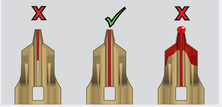
Or

Venous Draw

Mix blood well before testing



Collect blood from a slide



Too little add more blood

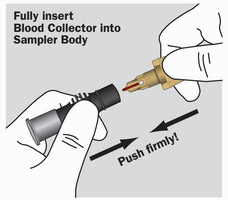
Just right

Too much wipe away excess

Blood Dilution

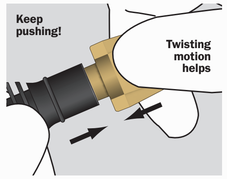
3 INSERT BLOOD COLLECTOR

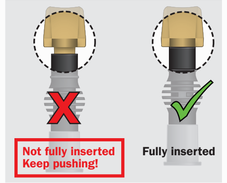
Fully insert Blood Collector into Sampler Body



Keep pushing!

Twisting motion helps



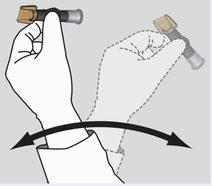


Not fully inserted Keep pushing!

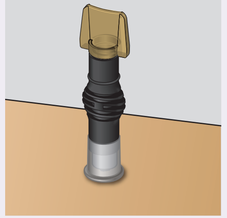
Fully inserted

4 SHAKE

Shake well 6-8 times This will mix the blood with the solution



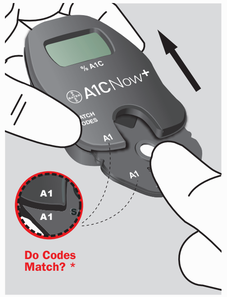
Stand Sampler on table while preparing Cartridge



5 INSERT CARTRIDGE

Use within 2 minutes.

"Click" Test Cartridge into place



Do Codes Match? *

Monitor and Test Cartridge codes must match

*** If not, Call Technical Support at 1-877-212-4968 x1**

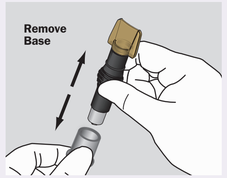
Blood Testing

6 PREPARE SAMPLER

WAIT for SMPL to display

Ready for Sampler

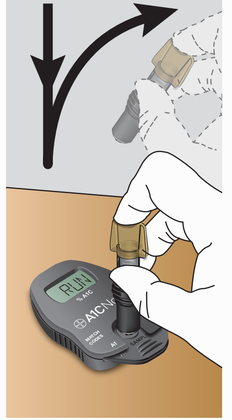
Remove Base



Ensure Monitor is on level surface

7 DISPENSE SAMPLE INTO CARTRIDGE

Push down completely to dispense diluted sample Remove quickly



Do not handle Monitor again until test is complete!

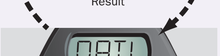
Results

8 5 MINUTES TO RESULTS

Display counts down

QC test result*

Result



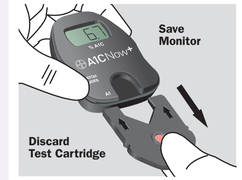
# of tests left

This result cycle remains displayed for 60 minutes or until the next Test Cartridge is inserted.

* If "QCOK" is not displayed, please see list of error codes on reverse side.

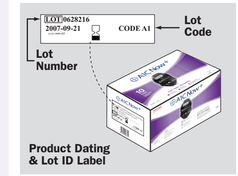
If you cannot resolve an error, please call Technical Support at 1-877-212-4968 x1.

9 REUSE MONITOR



THE MONITOR IS REUSABLE

To run another test, use a new Sampler and Test Cartridge from the same kit and return to Step 1, "**PREPARATION**."



ALWAYS MATCH LOT NUMBERS

Use Monitor only with the materials included in the original kit. The Monitor will expire after the programmed number of tests have been run. If another Test Cartridge is inserted, the Monitor will display "00 TL."

90821B 04/2008

A1CNow+ PROFESSIONAL-USE PRODUCT INSERT

This test is WAIVED under the Clinical Laboratory Improvement Amendments of 1988 (CLIA).

If a laboratory modifies the test instructions, the test will no longer be considered waived.

Intended Use

The A1CNow+® test provides quantitative measurement of the percent of glycated hemoglobin (%A1C) levels in capillary (fingerstick) or venous whole blood samples. The test is for professional use to monitor glycemic control in people with diabetes.

Summary and Explanation

High levels of blood glucose result in over-glycation of proteins throughout the body including hemoglobin.[1] Glycation of hemoglobin can occur at the amino termini of the alpha and beta chains, as well as other sites with free amino groups.[1] Hemoglobin A under goes a slow glycation with glucose that is dependent on the time-average concentration of glucose over the 120-day life span of red blood cells.

The most prevalent and well-characterized species of glycated hemoglobin A is A1C, making up approximately 3% to 6% of total hemoglobin in healthy individuals.[1] The correlation of A1C and blood glucose levels make it a useful method of monitoring long-term blood glucose levels in people with diabetes.[2] Previous studies, such as the Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS), used glycated hemoglobin as a way to measure overall glycemic control during the studies. These studies, and others, have shown that tight glycemic control is associated with fewer diabetes-related complications (e.g., vision problems, cardiovascular problems, and kidney problems).[3] The National Glycohemoglobin Standardization Program (NGSP) was established to assure traceability of hemoglobin A1C (A1C) results to the DCCT. Studies show a direct relationship from %A1C to average blood glucose (MBG) levels. For every 1% change in A1C there is a change of about 30 mg/dl in MBG.[4] The formula used to calculate the mean (average) blood glucose levels from the A1C levels is MBG = (31.7 x HbA1c) - 66.1. To convert to mean plasma glucose (MPG) use[5] MPG = MBG x 1.11.

A1C can be measured by a variety of techniques, and over the past decade they have expanded to include point-of-care assays. Point-of-care assays are well suited to environments such as healthcare providers' offices and clinics, because they are generally easy to perform, require no laboratory equipment, and provide rapid turn-around-time from sampling to result.[6] This immediate feedback of results enhances provider/patient interaction and, therefore better enables disease management.[7]

Principle of the Assay

Bayer has developed an enabling technology that incorporates microelectronics, optics, and dry-reagent chemistry strips within a reusable, self-contained, integrated hand held monitor and a single-use test cartridge. An unmeasured whole blood mixture (diluted) is directly applied to the sample port, and results are displayed in numeric form on the Monitor's liquid crystal display after 5 minutes. Having no switches or buttons, the Monitor self-activates upon insertion of the Test Cartridge. The A1CNow+ Monitor utilizes both immunoassay and chemistry technology to measure A1C and total hemoglobin, respectively. Upon the addition of a diluted blood sample, blue microparticles conjugated to anti-A1C antibodies migrate along the reagent strips. The amount of blue microparticles captured on the strips reflects the amount of A1C in the sample.

For the total hemoglobin (Hb) portion of the test, the sample diluent converts Hb to met-Hb. The intensity of met-Hb color measured on the reagent strips is proportional to the concentration of hemoglobin in the sample. Test results are expressed as %A1C (A1C ÷ total Hb x 100).

Calibration of the A1CNow+ is performed with a set of blood samples that have been value-assigned by a National Glycohemoglobin Standardization Program (NGSP) certified laboratory using an NGSP reference method. Total Hb calibration values for those samples are obtained with a Total Hb analyzer (HemoCue Hemoglobin Test System, HemoCue, Inc., Lake Forest, CA). The calibration of the A1CNow+ test is thus traceable to the NGSP and to an NGSP Certified Network reference method.

Specimen Collection and Storage

Note: No fasting or special diet is necessary

Fingerstick

The A1CNow+ test requires 5 microliters (µL) of whole blood (1 large drop). Fingerstick blood is obtained by standard techniques with any lancing system. If alcohol is used for cleansing, be sure the finger is completely dry before lancing.

Venipuncture/Sample Collection for Venous Draw

Venous blood should be collected into heparin tubes (sodium or lithium, "green tops"). Blood samples should be well-mixed and tested at room temperature. Venous blood samples are stable for up to 8 hours at room temperature and up to 14 days in the refrigerator.

Warnings and Precautions

1. For in vitro diagnostic use only.
2. Carefully read and follow the Professional Procedure Guide to ensure proper test performance.
3. If refrigerated, bring sealed pouches and Monitor to room temperature for one hour.
4. The A1CNow+ Monitor and Test Cartridges should not be used if either are cracked or broken.
5. The Test Cartridges should not be used if the foil pouch is damaged.
6. Add sample to A1CNow+ Test Cartridge within 2 minutes after pouch is opened.
7. All components of the A1CNow+ system are potentially biohazardous. Dispose of as biohazardous waste.
8. The Dilution Buffer in the Sampler contains ferricyanide in a buffered detergent solution. Do Not Ingest. In case of contact with skin or eyes, flush the area with large amounts of water.
9. Do not reuse Test Cartridges or Sample Dilution Kits.

Do not mix Monitors with Cartridges & Sample Dilution Kits from different lots.

Kit Storage and Stability

- Pouched Test Cartridges, A1CNow+ Monitors, and Sample Dilution Kits may be stored at room temperature (18-28°C) for up to **four months** prior to use. Monitors, Test Cartridges, and Dilution Kits stored at room temperature must be thrown away if not used within the **four months**.
- If the temperature label, placed on the outside of every kit, is exposed to a temperature in excess of 122°F/50°C, the dot on the label will turn red and the product should not be used.
- The Monitors, Test Cartridges, and Sample Dilution Kits may be used until the expiration date printed on the box and pouches when stored refrigerated (2-8°C). Monitors, Test Cartridges, and Sample Dilution Kits stored in the refrigerator must be thrown away if not used by the expiration date.
- Leave all components in their sealed pouches until use. If refrigerated, ensure pouches are at room temperature before use.
- Do not mix pouches and Monitors from different lots.

Package Components

- A1CNow+ Monitor (1)
- A1CNow+ Test Cartridges (10, or 20) Each Test Cartridge includes the following chemistries:
  - antibody to HbA1c, antigen conjugate that binds to the antibody, and membranes.
- Sample Dilution Kit (10, or 20), each containing:
  - Sampler (1) containing 0.37 ml of buffered detergent solution with ferricyanide
  - Blood Collector (1)
- Product insert (1)
- Patient result labels (10, or 20)

Materials Required but Not Supplied

- Fingerstick sample: lancet, or other blood fingerstick collection device or,
- Venous Sample: Heparin (sodium or lithium ["green top"]) preferred, venous collection supplies.
- Gauze pad or cotton ball
- Bandage
- Liquid control solution. Contact Bayer Technical Support (877-212-4968) for a list of liquid controls that may be used.

Result Interpretation

Percent A1C monitors glucose control over the last three months. About 50% of the A1C result is from the past 30 days; about 25% is from the past 30-60 days and about 25% is from the past 60-120 days.[1] Depending on the test methodology used, laboratory methods show that the reference range of the A1C test is approximately 4.0-6.5% A1C, and 6% to 9% in people with well to moderately controlled diabetes.[1] Levels can be as high as 20% in people with poorly controlled diabetes.[8] The American Diabetes Association's (ADA's) most recent Clinical Practice Recommendation for diabetes specifies a treatment goal for patients in general of less than 7% with a treatment goal for the individual patient of as close to normal (less than 6%) as possible without significant hypoglycemia.[9]

Troubleshooting

See the table below for a description of A1CNow+ operating and error codes (OR =Out of Range; QC = Quality Control, E= Monitor Error)

MESSAGE	DESCRIPTION AND RESOLUTION
OR 1	The blood sample may have too little hemoglobin (less than 20% hematocrit), not enough blood was collected, or the blood was not well mixed inside the Sampler.* You may wish to check hematocrit by another method.
OR 2	The blood sample may have too much hemoglobin (greater than 60% hemocrit), or excess blood was collected.* You may wish to check hemocrit by another method.
OR 3	The blood sample may have too little A1C, or insufficient blood was collected.*
OR 4	The blood sample may have too much A1C, or excess blood was collected.*
OR 5	The Monitor temperature is below 18°C (64°F). Repeat the test at room temperature (18-28°C).
OR 6	The Monitor temperature is above 28°C (82°F). Repeat the test at room temperature (18-28°C).
<4.0	The %A1C is less than 4%.
>13.0	The %A1C is greater than 13%.
QC 2	Occurs when you insert a Test Cartridge that already has sample added to it. Do not remove and reinsert a Test Cartridge after adding sample.*
QC 6	Sample was added to Test Cartridge before "SMPL" display. This counts down one test on the Monitor. Remove and discard Test Cartridge. To avoid this error, do not add sample until the "WAIT" prompt clears and "SMPL" appears.
QC 7	The Test Cartridge remained in the Monitor without sample addition for 2 minutes after "SMPL" prompt. This counts down one test on the Monitor. Discard the Test Cartridge and insert a fresh one when you are ready to dispense the Sampler.
QC30-33	The Monitor was unable to obtain a valiid initial reading. Be sure to remove the Sampler within one second after dispensing it into the sample port, and do not disturb the Monitor while the test is running.*
QC 50 to 51 QC 55 to 56	Insufficient sample was delivered to the Test Cartridge. To avoid this error be sure to fully insert the Blood Collector into the Sampler and shake immediately.*
All other QC codes	The quality control checks did not pass. Call Bayer Technical Support toll free at 877-212-4968 x1. The test will have to be repeated with another Test Cartridge and Sample Dilution Kit.
E1 to E99	The Monitor has a Fatal Error. Call Bayer Technical Support toll-free at 877-212-4968 x1.

*Carefully repeat the test using a new Test Cartridge and a new Sample Dilution Kit.

Limitations

- This test is NOT for the screening or diagnosis of diabetes.
- If the patient has high levels of Hemoglobin F, Hemoglobin S, Hemoglobin C, or other hemoglobin variants, the A1CNow system may report incorrect results.
- Any cause of shortened red cell survival (e.g., hemolytic anemia or other hemolytic diseases, pregnancy, recent significant blood loss, etc.) will reduce exposure of red cells to glucose. This results in a decrease in %A1C values. Percent A1C results are not reliable in patients with chronic blood loss and consequent variable erythrocyte life span.
- Rheumatoid Factor in high amounts will cause low results, or an error code. It is recommended that A1C be re-checked by alternate methodology such as boronate affinity.
- This test is not a substitute for regular healthcare provider visits and blood glucose monitoring.
- As with any laboratory procedure, a large discrepancy between clinical impression and test results usually warrants investigation.

Controls

Each A1CNow+ Monitor performs over 50 internal chemical and electronic quality control checks, including potential hardware and software errors (e.g. cartridge alignment, programming), and potential reagent strip errors (e.g. insufficient sample volume, invalid calculations). The Monitor has been programmed to report an error code if these quality checks are not passed.

Quality control testing should be performed at the following times:

1. With each new shipment.
2. With each new lot.
3. With each new operator.
4. Whenever problems (storage, operator, instrument, or other) are identified.
5. To ensure that storage conditions have not affected the product, run a control sample before running a patient sample if the test kit has been stored for more than a month and it has been at least a month since the last control testing.

The measured value should be within the acceptable limits stated for the control material. If the results obtained are outside the acceptable limit, please review the procedure and re-test the control material. If the measured value continues to fall outside the acceptable limit, please refrain from analyzing additional patient samples and contact Bayer Technical Support (877-212-4968).

Good laboratory practices include a complete quality control program. This entails proper sample collection and handling practices, ongoing training of testing personnel, ongoing evaluation of control results, proper storage of test kits, etc. A permanent record of control results should be retained.

Performance

Expected Values (non-diabetic population)

The expected normal range for %A1C using the A1CNow system was determined by testing blood samples from 118 presumptively non-diabetic individuals (fasting glucose levels <127 mg/dL) across three US sites. The population included 33 males and 85 females, and an age range from 19 to 76 with a mean age of 43. The mean %A1C result was 5.2% ±0.71% (1 SD). The 95% confidence limits were 3.9% to 6.5%. These values are similar to those reported in the literature. Each laboratory should determine its own reference range to conform to the population being tested.

Linearity

Studies were performed to evaluate the linearity of the A1CNow system across its dynamic range. Clinical samples representing low and high %A1C levels were identified, and were mixed in various proportions into nine preparations. These samples were tested in replicates of at least five (n = 5). The observed results were compared to the expected results and analyzed in terms of percent recovery. The test is linear for %A1C levels between 4% and 13%, and produces reliable results with hematocrits between 20% and 60% packed cell volume (PCV).

Interference Testing/Specificity

Studies were performed to assess the effect of common test interferents, various common over-the-counter therapeutic agents, and oral antihyperglycemic agents commonly used to treat Type II diabetes. Two levels of %A1C (low and high, approximately 4% and 10%, respectively) were tested. See table below.

INTERFERENT	TEST CONCENTRATION
Bilirubin (unconjugated)	20 mg/dL
Triglyceride	3000 mg/dL
Hemoglobin	500 mg/dL
Acetaminophen	80 µg/mL
Ascorbic acid	5mg/dL
Ibuprofen	120 µg/mL
Acetylsalicylic acid	1mg/dL
Glyburide (glibenclamide)	240 ng/mL
Metformin (1.1-dimenthylbiguanide HCl)	25 µg/mL

The studies showed no effect from any of these potential interferents at concentrations up to approximately 5-times their normal levels or therapeutic doses.

Studies showed no interference from modified hemoglobins, including labile glycated hemoglobin when tested at two levels of %A1C (low and high, approximately 5% and 11% respectively). The modified hemoglobins, and the levels evaluated, were: labile hemoglobin with 1400 mg/dL glucose, carbamylated hemoglobin at a final concentration of 5 mM potassium cyanate, and acetylated hemoglobin at a final concentration of 14 mM acetylsalicylic acid.

There were mixed results from the testing of high levels of Hemoglobin F, Hemoglobin S, and Hemoglobin C. Unreliable results may be obtained from patients with elevated levels of variant hemoglobins.

Precision

Precision testing was done under a specialized protocol. Following this protocol, two whole blood samples, one of approximately 6 %A1C (low), and one of approximately 9 %A1C (high), were tested over 20 days and four runs per day, for a total of 80 assays per level. The overall imprecision (including within-day and between-day) was 3.00% CV at the low level and 4.02% CV at the high level. This performance meets the requirements of NGSP certification.

Accuracy

Accuracy studies were conducted with 189 diabetic and non-diabetic subjects across three US sites. Fingerstick sampling was performed on each subject for testing with A1CNow+, and venous blood was collected from each subject for comparative testing using an NGSP-certified method. A1CNow+ results were compared to the NGSP reference results. The A1C results ranged from 5.0 %A1C to 12.8 %A1C, with a mean of 7.3 %A1C (reference results). Data analysis consisted of least squares linear regression (x = reference results), bias calculation, and Bland Altman limits. The data are provided below.

A1CNow+ Fingerstick Comparative Testing

(NGSP-certified method is the Tosoh A1c 2.2 P

n	189	Bias at 6% A1C (% difference)	5.8
Slope	1.02	Bias at 7% A1C (% difference)	6.9
y-intercept	- 0.23	Bias at 9% A1C (% difference)	8.9
"r"	0.95	Avg. % diff.	- 1.2

The results showed that the accuracy of A1CNow+, with fingerstick samples was, on average, 99%. This means that, on average, a true 7 %A1C could read approximately 6.9 %A1C. An individual A1CNow+ result may differ by as much as -1.0 %A1C to +0.8 %A1C from the true result. This represents the 95% confidence limits of a Bland-Altman plot.

A1CNow+ Venous Comparative Testing

(NGSP-Certified method is the Tosoh A1c 2.2 Plus)

Venous blood was collected from 110 diabetic subjects, and each sample was tested on one of three different lots. Aliquots of the venous samples were also tested by the NGSP-certified method, providing comparative results. Data analysis again consisted of least squares linear regression (x = reference results), bias calculation and Bland-Altman limits. The data are provided below.

n	110	Bias at 6% A1C (% difference)	5.95 (-0.8%)
Slope	1.03	Bias at 7% A1C (% difference)	6.98 (-0.3%)
y-intercept	-0.237	Bias at 8% A1C (% difference)	8.01 (+0.1%)
"r"	0.97	Avg. % diff.	-0.3%

The results showed that the accuracy with venous sampling was, on average, 99.7%. An individual result may differ by -0.8 %A1C to +0.7 %A1C from the true result. This represents the 95% confidence limits of the Bland-Altman plot. A1CNow+ may be used with either fingerstick (capillary) or venous (heparin-anticoagulated) whole blood samples.

Expected Performance in Waived Laboratories

Clinical studies were performed at three US sites with over 180 untrained people (most with diabetes). These study subjects read the instructions and then performed one A1CNow+ test on themselves. A venous blood sample was collected from each subject, and this sample was tested by an NGSP-certified laboratory method for %A1C. The two results were then compared.

Untrained User A1CNow+ and an NGSP-certified method

(Tosoh A1c 2.2 Plus)

accuracy as trained individuals.

References

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2. Nathan, D.M., et al. *The clinical information value of the glycosylated hemoglobin assay.* N Engl J Med 1984; 310; 341-346.
3. The Diabetes Control and Complications Trial Research Group. *The effect of intensive treatment of diabetes on the development and progression of longterm complication in insulin-dependent diabetes mellitus.* N Engl J Med 1993; 329; 977-986.
4. American Diabetes Association. *Standards of medical care for patients with diabetes mellitus.* Diabetes Care.1999; 22 (suppl 1): S32–S41.
5. Fogh-Anderson, N., D'Orazio, P. *Proposal for standardizing direct-reading biosensors for blood glucose.* Clin Chem 1998; 44(3); 655-659.
6. MLO Supplement. *Point-of-Care Testing*, 1992.
7. Cagliero, E., Levina, E.V., Nathan, D.M. *Immediate feedback of A1C levels improves glycemic control in type 1 and insulin-treated type 2 diabetic patients.* Diabetes Care 1999; 22(11): 1785-1789.
8. Goldstein, D.E., Little, R.R., Wiedmeyer, H.M., et al. *Glycated hemoglobin: Methodologies and clinical applications.* Clin Chem 1986; 32: B64-B70.
9. American Diabetes Association: *Clinical Practice Recommendations 2006.* Diabetes Care, 2006; 29 (Suppl. 1).

INTERNATIONAL SYMBOLS	
	MANUFACTURER
Σ_N	CONTAINS SUFFICIENT FOR <n> TESTS
IVD	IN VITRO DIAGNOSTIC MEDICAL DEVICE
EC REP	AUTHORIZED REPRESENTATIVE IN THE EUROPEAN COMMUNITY
2°C 8°C	STORE REFRIGERATED (2-8°C, 36-46°F)

Wilburn Medical USA
146 Furlong Industrial Drive
Kernersville, NC 27284
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Toll Free Fax: 1-866-FAXWILBURN (1-866-329-9452)
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