

# A1CNow<sup>®</sup>+

## Procedure: A1CNow<sup>®</sup>+ Test System

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### Approved

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## Table of Contents

I.	Purpose .....	3
II.	Principle .....	3
III.	Specimen Collection .....	3
	A. Fingertstick (Capillary) Whole Blood Samples .....	3
	B. Venous Whole Blood Samples .....	4
IV.	Warnings and Precautions .....	4
	A. Limitations .....	4
V.	Equipment/Materials/Reagents .....	5
	A. Equipment.....	5
	B. Materials .....	5
	C. Storage and Handling Requirements .....	5
VI.	Quality Control (QC).....	6
VII.	Result Interpretation .....	6
VIII.	Step-by-Step Procedure to Run A1CNow Tests .....	7
IX.	Procedural Notes .....	8
X.	Maintenance.....	8
	A. A1CNow Kit Storage and Handling.....	8
XI.	Performance .....	9
	A. Expected Values (non-diabetic population).....	9
	B. Linearity .....	9
	C. Interference Testing/Specificity .....	9
	D. Precision.....	10
	E. Accuracy.....	10
XII.	References .....	10

Refer to the A1CNow Professional Procedure Guide package insert for complete instructions for instrument and test performance.

## I. Purpose

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.

High levels of blood glucose result in over-glycation of proteins throughout the body, including hemoglobin. Glycation of hemoglobin can occur at the amino termini of the alpha and beta chains, as well as other sites with free amino groups. Hemoglobin A undergoes 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.

## II. Principle

PTS Diagnostics has developed an enabling technology that incorporates microelectronics, optics, and dry-reagent chemistry strips within a reusable, self-contained, integrated handheld 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 ( $A1C \div \text{total Hb} \times 100$ ).

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.

## III. Specimen Collection

**Note:** No fasting or special diet is necessary.

### A. Fingerstick (Capillary) Whole Blood Samples

The A1CNow+ test requires 5 microliters ( $\mu\text{L}$ ) 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.

## **B. Venous Whole Blood Samples**

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.

## **IV. 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 is 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.

Note: Do not mix Monitors with Cartridges and Sample Dilution Kits from different lots.

## **A. Limitations**

1. This test is NOT for the screening or diagnosis of diabetes.
2. If the patient has high levels of Hemoglobin F, Hemoglobin S, Hemoglobin C, or other hemoglobin variants, the A1CNow system may report incorrect results.
3. 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.
4. Percent A1C results are not reliable in patients with chronic blood loss and consequent variable erythrocyte life span.
5. 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.
6. This test is not a substitute for regular healthcare provider visits and blood glucose monitoring.
7. As with any laboratory procedure, a large discrepancy between clinical impression and test results usually warrants investigation.

## V. Equipment/Materials/Reagents

### A. Equipment:

- 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)

### B. Materials:

- Fingerstick sample
  - Lancet or other blood fingerstick collection device
- Venous Sample
  - Heparin (sodium or lithium ["green top"]) preferred
  - Venous collection supplies
- Gauze pad or cotton ball
- Bandage
- Liquid control solution
  - Contact Customer Service (1-877-870-5610) for a list of liquid controls that may be used

### C. Storage and Handling Requirements:

- 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.

## VI. Quality Control (QC)

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:

- With each new shipment
- With each new lot
- With each new operator
- Whenever problems (storage, operator, instrument, or other) are identified
- 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 PTS Customer Service (1-877-870-5610).

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.

## VII. 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.<sup>1</sup> 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.<sup>1</sup> Levels can be as high as 20% in people with poorly controlled diabetes.<sup>8</sup>

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.<sup>9</sup>

## VIII. Step-by-Step Procedure to Run A1CNow Tests

1. Preparation
  - Run the test with all parts of the test kit at the same temperature: 18-28°C/64-82°F.
  - 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.
  - Complete the test within 15 minutes.
2. Blood Collection
  - Fingertick
    - a. Use your own lancet device to draw blood
    - b. Gently touch the tip of the blood collector to the blood drop to fill
  - Venous Draw
    - a. Mix heparinized blood well before testing
    - b. Hold the blood collector at a 45° angle to collect blood from a slide
3. Blood Dilution: Insert Blood Collector
  - Fully insert Blood Collector into Sampler Body
  - Push firmly
  - Keep pushing, a twisting motion helps
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
  - Open the Test Cartridge pouch
  - Use within 2 minutes of opening the pouch
  - Insert the Test Cartridge into the Monitor until the Cartridge is *CLICKED* into place
  - Monitor and Test Cartridge codes must match  
**Note:** If codes do not match, call Customer Service at 1-877-870-5610.
6. Blood Testing: Prepare Sampler
  - Wait for "SMPL" to display on the Monitor
  - When Monitor displays "SMPL," it is ready for the sample.
  - Remove the base of the Sampler
  - Ensure the Monitor is on a level surface
7. Dispense the sample into the cartridge
  - Place the tip of the plunger into the corresponding hole in the Test Cartridge
  - Push down completely to dispense diluted sample  
**Note:** Remove quickly after the sample is dispensed
  - Do not handle Monitor again until test is complete
8. Results will display in five minutes
  - The Monitor counts down from five minutes
  - After the countdown, the display will cycle: A1C result, "QCOK," and number 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 under troubleshooting section in the Professional Procedure Guide  
**Note:** If you cannot resolve an error, please call Customer Service at 1-877-870-5610.
9. Reuse of Monitor
- Save Monitor
  - Discard Test Cartridge
  - To run another test, use a new Sampler and Test Cartridge from the same kit and return to Step 1, PREPARATION.

## IX. Procedural Notes

1. The Lot number on the Monitor, Test Cartridge pouch, and Sample Dilution Kit should always match.
2. Use Monitor only with the materials included in the original kit.
3. The Monitor will expire after the programmed number of tests have been run.  
**Note:** If another Test Cartridge is inserted, the Monitor will display “00 TL.”

## X. Maintenance

There is no user calibration possible with the A1CNow+ Monitor. All calibration is performed prior to leaving the manufacturer’s facility.

### A. A1CNow Kit Storage and Handling

1. 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.
2. 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.
3. 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.
4. Leave all components in their sealed pouches until use. If refrigerated, ensure pouches are at room temperature before use.
5. Do not mix pouches and Monitors from different lots.

## **XI. Performance**

### **A. 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.

### **B. 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).

### **C. 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.

<b>INTERFERENT</b>	<b>TEST CONCENTRATION</b>
<b>Bilirubin</b>	<b>20 mg/dL</b>
<b>Triglyceride</b>	<b>3000 mg/dL</b>
<b>Hemoglobin</b>	<b>500 mg/dL</b>
<b>Acetaminophen</b>	<b>80 µg/mL</b>
<b>Ascorbic acid</b>	<b>5 mg/dL</b>
<b>Ibuprofen</b>	<b>120 µg/mL</b>
<b>Acetylsalicylic acid</b>	<b>1 mg/dL</b>
<b>Glyburide (Glibenclamide)</b>	<b>240 ng/mL</b>
<b>Metformin (1,1 –dimethylbiguanide HCl)</b>	<b>25 µg/mL</b>

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.

#### **D. 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.

#### **E. 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 ( $x = \text{reference results}$ ), bias calculation, and Bland Altman limits. The detailed data is included in the product Insert.

## **XII. References**

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