

**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[®] InView™ 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 hemoglobin¹. Glycation of hemoglobin can occur at the amino termini of the alpha and beta chains, as well as other sites with free amino groups¹. 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.

The most prevalent and well-characterized species of glycated hemoglobin A is A1C, making up approximately 3% to 6% of total hemoglobin in healthy individuals¹. The correlation of A1C and blood glucose levels make it a useful method of monitoring long-term blood glucose levels in people with diabetes². Previous studies, such as the Diabetes Complications and Control 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 MBG⁴. The formula used to calculate the mean (average) blood glucose levels from the A1C levels is $MBG = (31.7 \times HbA1c) - 66.1$. To convert to mean plasma glucose (MPG) use⁵ $MPG = MBG \times 1.11$.

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 physicians' offices and clinics, because they are generally easy to perform, require no laboratory equipment, and provide rapid turn-around-time from sampling to result⁶. This immediate feedback of results enhances physician/patient interaction and, therefore better enables disease management⁷.

Principle of the Assay

Metrika has developed an enabling technology called MODM™ (Micro-Optical Detection Method) 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 InView 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 InView 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 B-Hemoglobin System, HemoCue Ab, Ångelholm, Sweden). The calibration of the A1cNow InView 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 InView test requires 10 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

Venous blood is to be collected in an EDTA tube ("Purple Top"). Blood 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 if refrigerated (2-8°C).

Warnings and Precautions

1. For in vitro diagnostic use only.
2. Carefully read and follow the "Procedure" section to ensure proper test performance.
3. If refrigerated, bring sealed pouches to room temperature for one hour.
4. The A1cNow InView 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 InView Test Cartridge within 2 minutes after pouch is opened.
7. Handle and dispose of all samples and pipets following appropriate biohazard procedures.
8. The Dilution Buffer 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 InView monitors, and Sample Dilution Kits may be stored at room temperature (18-28°C) for up to **three months** prior to use. Monitors, Test Cartridges, and Dilution Kits must be thrown away if not used within the **three months**.

• 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 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 InView Monitor (1)
- A1cNow InView 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:
 - Tube (1), containing 0.69 mL of buffered detergent solution with ferricyanide
 - Capillary (1)
 - Dropper (1)
 - Tube Holder (1)
- Product insert (1)
- Procedure card (1)
- Patient result labels (10, or 20)

Materials Required but Not Supplied

- Fingerstick sample: lancet, or other blood fingerstick collection device or,
- Venous sample: EDTA tube ("purple top"), venous collection supplies, as well as:
- Gauze pad or cotton ball
- Bandage

PROCEDURE

Make sure all parts are the same lot number. Always run the test with all parts of the test kit at room temperature (18°–28°C, 64°– 82° F). If the kit has been recently at high temperatures (above 82°F) or in the refrigerator allow ALL parts to come to room temperature in their sealed foil pouches for at least one hour before running the test.

Avoid running the test in direct sunlight, on hot or cold surfaces, or near sources of heat or cold.

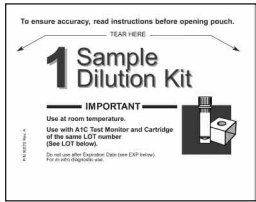
Once the A1cNow InView Monitor, Test Cartridge and Sample Dilution Kit have been at room temperature for at least one hour, open the Sample Dilution Kit and place the parts on a clean, flat surface. Test immediately.

PREPARE

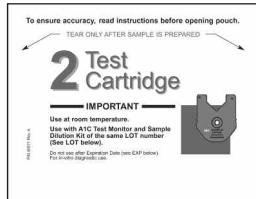
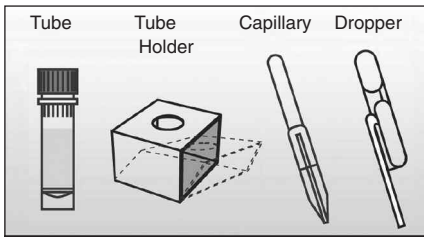
the following items. Have them all at room temperature:



1. The Monitor



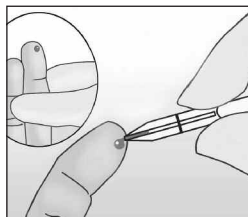
2. Sample Dilution Kit (Open Pouch)



3. Test Cartridge (Wait to open)

GET BLOOD

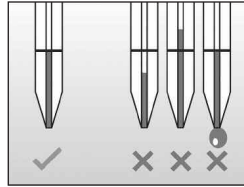
OPEN POUCH #1, then squeeze the folded Tube Holder to open it. Remove cap. Place Tube in Holder.



CLEAN AND DRY the patient's finger. Lance the finger to obtain a large (10 µL) drop of blood. Do not milk the finger.

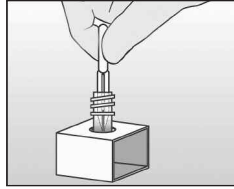
DO NOT SQUEEZE THE BULB of the Capillary during this process. Place the Capillary at a slight angle against the blood drop or blood volume and let capillary action fill the Capillary just to the black line. Pull the Capillary away and **DO NOT SQUEEZE THE BULB**.

NOTE: For Venous Draw Procedure: obtain a blood sample by standard venipuncture technique in an EDTA (purple top) tube. Mix the sample well before testing. A standard laboratory precision pipet may be used to transfer 10 µL from an EDTA tube into the Dilution Buffer tube, instead of the capillary pipet.



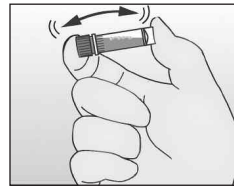
Do not underfill, overfill, or leave a hanging drop!

ADD BLOOD TO TUBE



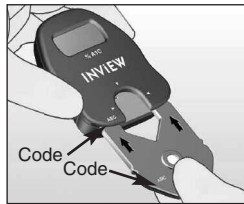
PUT CAPILLARY TIP into the liquid in the Tube. Leaving it submerged, squeeze the bulb firmly two or three times to rinse all of the blood from the Capillary into the Tube.

RECAP TUBE, SHAKE!



RECAP THE TUBE tightly and shake it vigorously 6-8 times. It's OK to have some bubbles. The diluted sample will be red-orange in color. Replace the Tube in the Tube Holder.

INSERT CARTRIDGE



OPEN POUCH #2 and insert Cartridge into Monitor immediately. The display will turn on and show "SMPL".

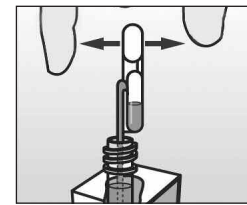
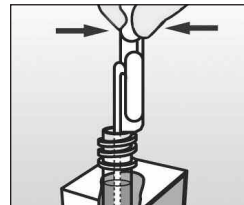
Place on a flat surface. The Monitor is now ready for use. (If you see any other message on the display, go to "Troubleshooting").

IMPORTANT: ADD DILUTED SAMPLE WITHIN 2 MINUTES!



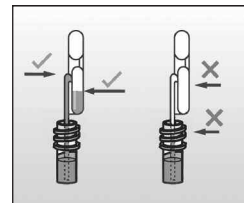
NOTE: The code on the Monitor must match the code on the Cartridge. If the code numbers do not match, DO NOT continue with the test.

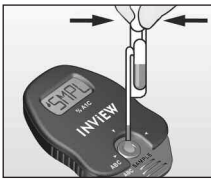
FILL DROPPER



REMOVE THE CAP from the Tube. Squeeze the top bulb of the Dropper and submerge it in the liquid. Release the bulb to fill the Dropper as shown. The Dropper must be completely filled including some

overflow. If not, empty and refill as described above. Two or three bubbles are OK; if there are lots of bubbles, empty and refill as described above.





ADD DILUTED SAMPLE

DO NOT TOUCH the Cartridge with the Dropper (hold the Dropper slightly above the sample well of the Cartridge). Squeeze the bulb firmly to add all of the sample **ALL AT ONCE**. Liquid will stay in the overflow bubble.



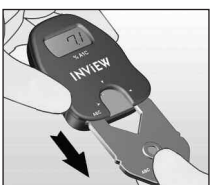
The display will start counting down from 5 minutes, then show a test result alternating with the letters "QC OK". Do not handle the Monitor until the result is displayed. The results remain displayed for 30 minutes or until the next Test Cartridge is inserted.

RECORD RESULT

RECORD THE RESULT immediately on the patient label provided. Results are expressed as % (percent) A1C.

REMOVE CARTRIDGE

KEEP MONITOR



THE MONITOR IS REUSABLE until all the Cartridges are used up. To run a new test, start with a new Sample Dilution Kit and Test Cartridge and go to "Prepare".



The monitor will display 3 TL, 2 TL, 1 TL and 0 TL, alternating with "QC OK" and the result when there are 3, 2, 1 and 0 tests left, respectively. After all Cartridges in the kit are used, the Monitor will expire. (If you insert a new Cartridge, the display will show "0 TL" (Zero Tests Left) for five minutes and then shut down permanently.)

NOTE: If any contamination is visible inside the Monitor, call Metrika at 1-877-212-4968.

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 days¹. 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 diabetes¹. Levels can be as high as 20% in people with poorly controlled diabetes⁸. Reference range studies conducted by Metrika using the A1cNow system showed the normal range to be 3.9-6.5% A1C in the non-diabetic population tested. Reference ranges should be determined by each laboratory to conform to the population being tested.

Troubleshooting

See the table below for a description of A1cNow InView 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), or there was under-sampling of whole blood.* You may wish to check hemocrit by another method.
OR 2	The blood sample may have too much hemoglobin (greater than 60% hemocrit), or there was over-sampling of whole blood.* You may wish to check hemocrit by another method.
OR 3	The blood sample may have too little A1C, or there was under-sampling of whole blood.*
OR 4	The blood sample may have too much A1C, or there was over-sampling of whole blood.*
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 1 to QC99	The quality control checks did not pass. Call Metrika Technical Support toll free at 877-212-4968. 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 Metrika Technical Support toll-free at 877-212-4968

*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.
- This test is designed to be run at 18-28°C (64-82°F) and 15-80% humidity. Using the monitor outside this temperature range will give an error code.
- This test is not a substitute for regular doctor 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 InView monitor performs over 25 internal chemical and electronic quality control checks, including potential hardware and software errors (e.g., autostart leads, 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.

If external quality control testing is desired, commercial controls may be purchased from other vendors. Please contact Metrika Customer Service for recommendations. Metrika recommends that external controls be tested at the following times:

- Whenever laboratory or room conditions have been above 28°C if stored at room temperature.
- To perform training or retraining of testing personnel.
- Whenever A1cNow InView results do not match other clinical findings or symptoms.

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

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.

The expected %A1C value for patients with diabetes will depend on physician discretion. The American Diabetes Association's (ADA's) most recent Clinical Practice Recommendation for diabetes specifies a treatment goal of less than 7%, and suggests additional action when the A1C level is above 8%⁹.

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 mg/dL
Ascorbic acid	5 mg/dL
Ibuprofen	120 mg/dL
Acetylsalicylic acid	1 mg/dL
Glyburide (glibenclamide)	240 mg/dL
Metformin (1.1-dimethylbiguanide HCl)	25 mg/dL

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 2.97% CV at the low level and 4.14% 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 InView, and venous blood was collected from each subject for comparative testing using an NGSP-certified method. A1cNow InView 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 InView Fingerstick Comparative Testing
(NGSP-certified method is the Tosoh A1c 2.2 Plus)**

n	189	Bias at 6% A1C (% difference)	5.89 (- 1.83%)
slope	1.02	Bias at 7% A1C (% difference)	6.91 (-1.29%)
y-intercept	- 0.23	Bias at 9% A1C (% difference)	8.95 (- 0.56%)
"r"	0.95	Avg. % diff. - 1.23%	

The results showed that the accuracy of A1cNow InView, 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 InView 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 System Venous Comparative Testing
(NGSP-certified method is the Tosoh A1c 2.2 Plus)**

In a separate study, venous blood was collected from 50 diabetic subjects, and each sample was tested twice by three different lots (total of six results, two replicate tests from one dilution). Aliquots of the venous samples were also tested by the NGSP-certified method, providing approximately 300 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	299	Bias at 6% A1C (% difference)	5.77 (- 3.83%)
slope	1.023	Bias at 7% A1C (% difference)	6.801 (-2.86%)
y-intercept	-0.361	Bias at 9% A1C (% difference)	8.84 (- 1.78%)
"r"	0.90	Avg. % diff. - 2.82%	

The results showed that the accuracy with venous sampling was, on average, 97%. This means that, on average, a true 7 %A1C could read approximately 6.8 %A1C. An individual result may differ by -1.3 %A1C and +0.9% A1C from the true result. The A1cNow system may be used with either fingerstick (capillary) or venous (EDTA 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 InView 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 InView and an NGSP-certified method
(Tosoh A1c 2.2 Plus)**

n	188	Bias at 6% A1C (% difference)	6.02 (+ 0.33%)
slope	0.99	Bias at 7% A1C (% difference)	7.01 (+0.14%)
y-intercept	0.08	Bias at 9% A1C (% difference)	8.99 (- 0.11%)
"r"	0.93	Avg. % diff. + 0.12%	

The results showed that untrained users could perform A1cNow InView testing on themselves with the same accuracy as trained individuals.

References

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