

# **Evaluation Protocol**

- I. **Purpose:**  
To demonstrate that laboratory performance of accuracy and precision of the Metrika A1cNow™ test meets product claims found in the A1cNow™ product insert.
  
- II. **Definitions:**  
Accuracy: How close a result is to the “true” value.  
Bias: Describes how far the result is, on average, from the “true” value.  
Precision: Reproducibility, or how closely several results analyzed on the same sample agree.
  
- III. **References:**
  - A. EP15-P, User Demonstration of Performance for Precision and Accuracy; Proposed Guideline. (NCCLS, December, 1998)
  
  - B. National Glycohemoglobin Standardization Program (NGSP) Manufacturer Information Packet; University of Missouri, NGSP website ([http://web.missouri.edu/~diabetes/ngsp/cert/manu\\_info.html](http://web.missouri.edu/~diabetes/ngsp/cert/manu_info.html))
  
- III. **Materials Required:**
  - A. Four to six Metrika A1cNow Professional-use 10 test kits, P/N 03003 (40-60 individual tests) from the same lot.
  - B. Fresh patient samples at approximately 6% and 10% HbA1c for precision testing.
  - C. Twenty fresh, whole blood (EDTA) or fingerstick patient samples, in the ranges listed below.
    - Four samples in the 4.0 – 6.0% HbA1c range.
    - Six samples in the 6.0 – 8.0% HbA1c range.
    - Six samples in the 8.0 – 10.0% HbA1c range.
    - Four samples in the 10.0 – 12.0 % HbA1c range.
  
- IV. **Procedure**

This procedure is intended to be performed over a period of five days. Accuracy testing may take longer than five days, depending upon the availability of samples. Be sure the operator is familiar with all of the procedural steps outlined in the product insert and procedure card before beginning this evaluation. The operator may consult the website [www.FluVaccine.com](http://www.FluVaccine.com) for a video demonstration of the A1cNow test procedure. All samples must be well-mixed before testing. Please call Customer Support toll-free at 877-212-4968 with any questions.

  - A. **Precision** (*refer to the attached Precision Worksheet*)  
Using the patient samples (6% and 10% HbA1c), run four replicates per day of each level for a period of five days. Samples must be kept refrigerated (2-8°C) between days, so for best results, aliquot each patient sample into several tubes and allow each tube to come to room temperature each day of testing. Record the values on the attached precision worksheet. There should be a total of 20 results for each level of sample.

If the site desires to run one sample only, it is suggested that precision testing be performed on a patient sample at approximately 7% HbA1c. Four replicates per day for five days should be run, for a total of 20 results.

Calculate the mean, SD and %CV for each data set. Percent CV should not be statistically significantly greater than the values stated in the current product insert.

- B. Accuracy and Bias** (refer to the attached Accuracy Worksheet and Bias Graph) Metrika recommends performing comparison testing against a Tosoh 2.2 Plus, which is a National Glycohemoglobin Standardization Program (NGSP) certified method. If the site would like to run comparison to another instrument, it is recommended that the samples be split and an aliquot also be sent to a laboratory that performs HbA1c testing on the Tosoh 2.2 Plus.

Testing should include at least 20 patient samples in the ranges stated in the “Materials Required” section on page one of this protocol. Testing should be performed over a period of at least five days, on both methods (A1cNow and comparison method). Run four to seven samples per day. Record each result on the data sheet. The results may be plotted with the x-axis as the comparison method and the y-axis as the A1cNow. Linear regression may be performed, however, due to the low number of samples, this is not the best way to evaluate accuracy.

Calculate the difference ( $B = \text{bias}$ ) between each specimen’s results for the two methods ( $A1cNow \%HbA1c - \text{Comparison method } \%HbA1c = B$ ). Construct a plot of bias (vertical axis) vs. comparison method result (horizontal axis) for each sample. Examine the bias plot to determine if the difference between methods is relatively constant over the concentration of the range tested. If constant bias vs. concentration is observed, the mean bias (calculated in the next step) represents the average difference between the two methods.

Calculate the mean of each set of data points: comparison test result, A1cNow result, and bias between the results. Calculate the percent bias; it should be within the limits stated in the current product insert, allowing for the statistical uncertainty of 2-3% for a study with only 20 samples.

**C. Sources of Error**

Follow all instructions in the product insert for best results. The operator may refer to STAT Pharmaceuticals website ([www.FluVaccine.com](http://www.FluVaccine.com)) for a video demonstration of the procedure.

Accuracy testing is best performed using fingerstick samples for A1cNow. Otherwise, samples should be fresh, whole blood, preserved with EDTA (purple top tubes) less than 5 days of age. Samples should be stored in the refrigerator (2-8°C) and brought to room temperature and well-mixed before use.

For precision testing, Metrika recommends aliquoting the precision samples into several different tubes, and warming only enough sample to perform that day's testing.

Testing should be performed at room temperature (18-28°C). Allow all components and samples to equilibrate to room temperature for at least one hour before testing.

#### **D. Reference Range**

Reference ranges apply to expected values in a normal, healthy population. Since %HbA1c results are applicable to diabetic populations, conventional reference ranges are not relevant. However, for informational purposes, A1cNow was tested in 1999 with 118 non-diabetic individuals across three US sites. The mean %HbA1c result was 5.2% ( $\pm 0.71$ , 1 SD) and the 95% confidence limits were 3.9% to 6.5%. If your laboratory wishes to establish its own reference range, it is necessary to test approximately 120 non-diabetic individuals<sup>1,2</sup>.

A more useful clinical practice tool would be to set ranges for treatment goals. Because A1cNow is directly calibrated to an NGSP-certified method and because this calibration cannot be changed by the user in the field, Metrika recommends using the American Diabetes Association (ADA) treatment goal of less than 7%, and suggests additional action when the %HbA1c level is above 8%<sup>3</sup>.

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<sup>1</sup> NCCLS C28-A2, How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline – Second Edition. (2000)

<sup>2</sup> Tietz Textbook of Clinical Chemistry, Third Edition. W.B. Saunders Company, 1999

<sup>3</sup> American Diabetes Association Practice Recommendations, 2001

**Precision Data A1cNow™**

Date	Day	Replicate	Level 1	Level 2	Operator
	1	1			
		2			
		3			
		4			
	2	1			
		2			
		3			
		4			
	3	1			
		2			
		3			
		4			
	4	1			
		2			
		3			
		4			
	5	1			
		2			
		3			
		4			
		Mean			
		SD			
		%CV			

Use appropriate statistical software, or the following formulas to calculate the mean, SD and %CV of the data.

Percent CV should not be statistically significantly greater than the values stated in the product insert.

**Mean:** average of all points.  
 = sum of all points ÷ n (the number of measurements)

**SD** = Standard deviation

$$= \sqrt{\frac{\sum (x - \text{mean})^2}{n - 1}}$$

Where:  $\sum$  = sum  
 x = the value of each individual measurement  
 Mean = the mean value of all measurements (calculated, above)  
 n = the number of measurements

**%CV** = percent coefficient of variation  
 = (SD ÷ Mean) x 100

**Accuracy Data A1cNow™**

Date	Sample ID	Tosoh %HbA1c	A1cNow %HbA1c	Bias (A1cNow - Tosoh)	Operator
	1				
	2				
	3				
	4				
	5				
	6				
	7				
	8				
	9				
	10				
	11				
	12				
	13				
	14				
	15				
	16				
	17				
	18				
	19				
	20				
	Mean				
				%bias	

Use appropriate statistical software, or the following formulas to calculate the mean and %bias of the data.

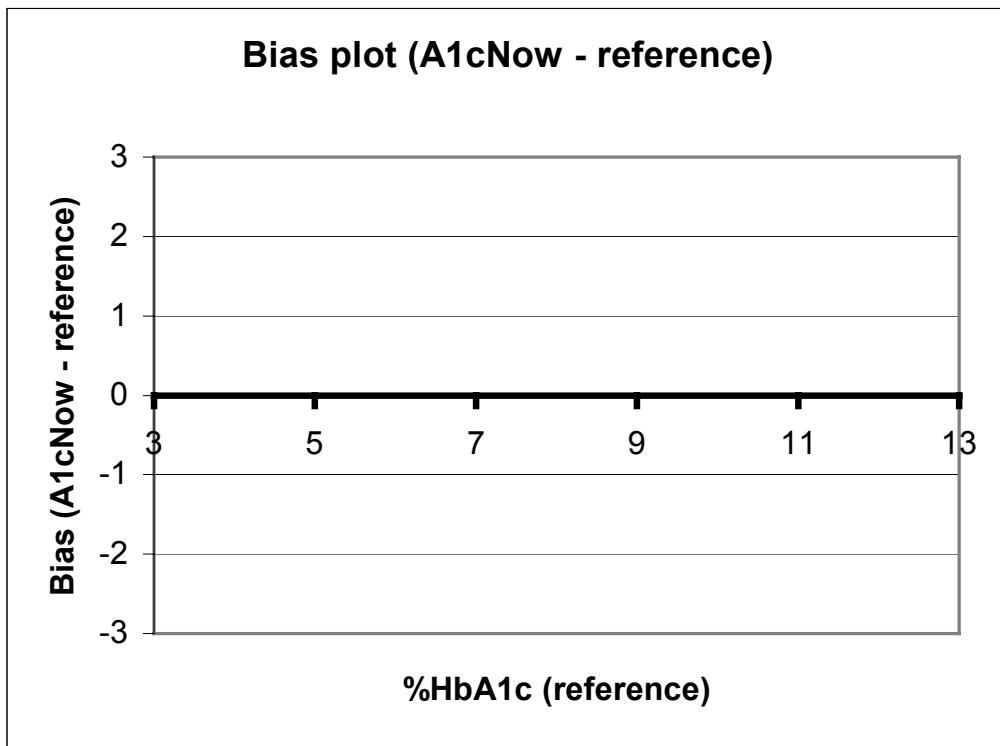
Percent bias should be within limits stated in the A1cNow™ product insert.

**Mean:** average of all points.

Mean = sum of all points ÷ n (the number of measurements)

**%bias** =  $\frac{(\text{Mean A1cNow result} - \text{mean Tosoh result})}{\text{Mean Tosoh result}} \times 100$

Plot reference %HbA1c vs. bias (A1cNow – reference) on the graph below.



Alternatively, data for precision and accuracy may be sent to Metrika, Inc. for analysis. Contact Customer Support at (800) 748-5665. Results will be returned promptly.

Toll free customer service: (800) 748-5665  
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