



KEEPING YOU INFORMED

Choose an A1c Method that Gives You the Complete Picture in the presence of HbF

Fetal hemoglobin (HbF) is a type of hemoglobin that is present in fetuses during gestation and in newborns up to six months after birth. Factors that affect HbF levels in adults: Sickle cell disease^{1, 2, 3}, β -Thalassemia^{4, 5}, Hereditary Persistence of Fetal Hemoglobin (HPFH)⁶, some cases of malignancies⁷, presence of elevated HbF in some ethnicities⁸.

It is important to choose an A1c method that gives you an accurate result in the presence of elevated HbF and provides the complete hemoglobin picture.

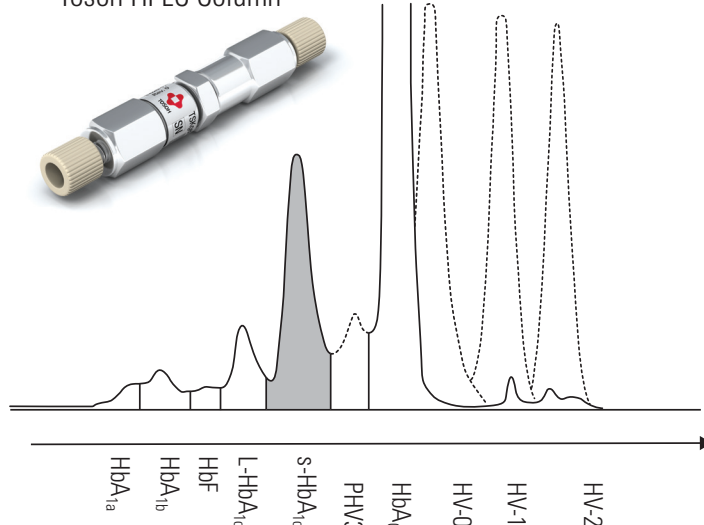
Immunoassays have a low tolerance to HbF.

Literature suggests that HbF levels of approximately 10-15% can interfere with A1c results. Some immunoassay methods notify users that their method interferes with A1c results at levels as low as 7% HbF, leading to inaccurately lower reported A1c values⁹.

Tosoh A1c by HPLC is accurate in the presence of high levels of fetal HbF.

With the Tosoh G8 A1c analyzer 5.24F assay there is no interference with %A1c values in the presence of HbF of up to 25%. In addition, there is a publication showing that there is no interference with %A1c in the presence of HbF levels up to 30%¹⁰.

Tosoh HPLC Column



Tosoh's cation exchange HPLC column is packed with proprietary non-porous resin to deliver chromatograms with clear separation.

G8 HPLC Analyzer

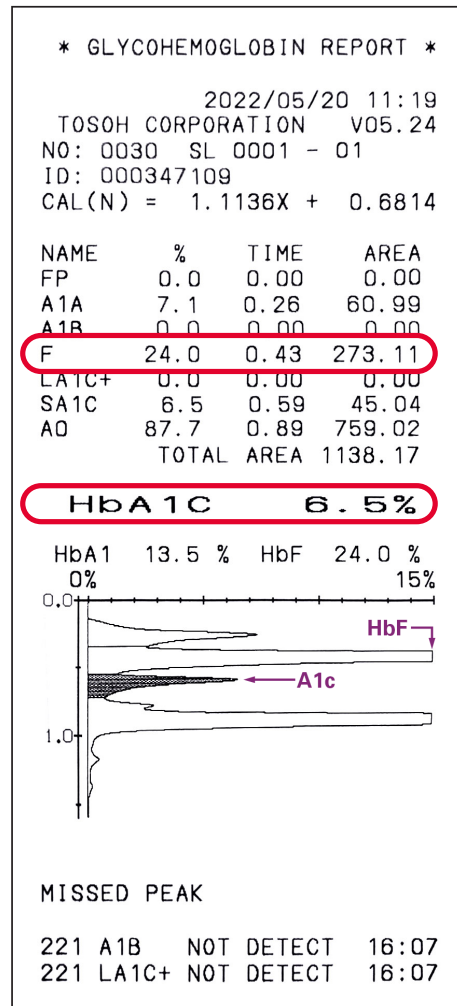
With a consistent accuracy of less than 2% CVs and an analysis time of only 1.6 minutes per sample the G8 is a low maintenance and a small footprint analyzer making it the ideal solution for laboratories of all sizes.

<https://ngsp.org/>



The difference in the A1c results between methods can be misleading to the clinician. Choose an A1c method that provides the complete picture

Chromatogram: Tosoh G8 HPLC Analyzer



- Whole blood patient sample was run on the G8
- An HbA1c result of 6.5% was obtained.
- The chromatogram resulted in a presumptive identification of HbF at 24%

An HbA1c of 6.5% indicates a diabetes diagnosis and patient treatment per ADA guidelines (<https://diabetes.org/about-diabetes/diagnosis>).

The same sample was run on an Immunoassay method for A1c testing.

Family Medical Clinic

FINAL COPY

Patient: Birth: Acc #:
Patient #: Age: Collection Date: 5/20/2022 1:59 PM ED
Doctor: Gender:
Home Phone

LabCorp Results

Test Name	Result	Units	Flag	Reference Range	Site ID	Status
Hemoglobin A1c	5.1	%		4.8-5.6	MB	F

Reported On: 5/21/2022 7:08 AM

Prediabetes: 5.7 - 6.4
Diabetes: >6.4
Glycemic control for adults with diabetes: <7.0

MB: Labcorp

- The immunoassay method gave a lower A1c result of 5.1%
- The method did not detect variants or Hemoglobin F.
- The A1c result is only a number.
- Immunoassay method used: Roche Tina Quant

<https://www.labcorp.com/tests/001453/hemoglobin-hb-a-sub-1c-sub>

Electrophoresis method confirmed that the peak identified as HbF by Tosoh G8 HPLC was a true HbF.

Family Medical Clinic				FINAL COPY		
Patient:		Acc #:				
Patient #:	Birth:	Collection Date: 5/24/2022 8:12 AM		DH		
Doctor:	Age:					
	Gender:					
Test Name	Result	Units	Flag	Reference Range		
HemoCue (whole blood)	74.0	mg/dL		Run by: DH on 5/24/2022 10:04 AM		
HemoCue				60.0 - 110.0		
LabCorp Results				MB	F	
Test Name	Result	Units	Flag	Reference Range	Site ID	Status
Hemoglobinopathy Profile				Reported On: 5/26/2022 8:17 AM		
Hgb Fractionation Cascade				Reported On: 5/25/2022 3:08 PM		
Hgb F	25.4	%	H	0.0-2.0	MB	F
Hgb A	72.5	%	L	96.4-98.8	MB	F
Hgb A2	2.1	%		1.8-3.2	MB	F
Hgb S	0.0	%		0.0	MB	F
Interpretation:	Comment				MB	F
Hemoglobin pattern and concentrations reveal an elevation of Hgb F which may indicate the presence of a hereditary persistence gene. However, elevations of Hgb F have also been reported to occur with certain anemias, pregnancy, porphyrias, and some malignancies.						
MB:	Labcorp					

Considering the significant difference in A1c results between both methods, the sample was sent for confirmatory testing using electrophoresis to determine if the sample had elevated HbF. The method confirmed that the peak identified as HbF in the HPLC method was indeed a true HbF peak, at a level of 25.4%.

Tosoh HPLC delivers A1c results with a detailed chromatogram, allowing you to see all that is behind the A1c number.

With immunoassays, the result is only a number and does not provide information on presence of hemoglobin variants and HbF. Immunoassays have a low tolerance to elevated hemoglobin F as stated previously.

The difference in the A1c results in this example can be misleading to the clinician: the immunoassay result falls under the normal patient category and the HPLC result falls under the diabetic category. An HbA1c of 6.5% indicates a diabetes diagnosis and patient treatment per ADA guidelines (<https://diabetes.org/about-diabetes/diagnosis>)

Cation-exchange HPLC is an effective and sophisticated method for hemoglobin A1c testing, offering additional information on hemoglobin fractions and presumptive identification of hemoglobin variants and elevated HbF, making it a valuable tool in clinical laboratories.

Advantages of Tosoh Cation Exchange-HPLC for A1c testing

- 1. Charge-based Separation:** It separates hemoglobin molecules based on their charge, which is particularly useful for detecting hemoglobin variants that differ in charge from normal hemoglobin.
- 2. Presumptive Identification of Hemoglobin Variants:** Besides providing an A1c value, cation-exchange HPLC can presumptively identify commonly occurring hemoglobin variants that might interfere with A1c results. This can help in providing the full patient picture.
- 3. Chromatogram Detail:** The method provides a detailed chromatogram with clear separation of peaks, offering a comprehensive view of the hemoglobin profile.
- 4. Automation and Compatibility:** Advanced HPLC analyzers allow for automated chromatogram review and are compatible with track-line systems, making them suitable for high-volume laboratories.
- 5. Quality Control:** Attention to the quality, type, and size of resin used in column packing, as well as consistency measures in the packing process, ensures reliable and reproducible results.
- 6. Resolution and Accuracy:** Quality of peak resolution in the chromatogram is crucial for accurate results. Well-resolved peaks lead to more accurate A1c measurements.

Not all HbA1c testing methods are the same. Using the right method gives you confidence in the results you deliver. HPLC is the gold standard method for hemoglobin A1c testing.

Choose wisely. Choose Tosoh.



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Limitations of immunoassay method for A1c testing

- 1. Low tolerance for elevated Hemoglobin F:** Literature suggests that HbF levels of approximately 10-15% can interfere with A1c results. Some immunoassay methods notify users that their method interferes with A1c results even at levels as low as 7% HbF, leading to lower reported A1c values.⁹
- 2. Lack of Presumptive Identification of Variants:** Immunoassays cannot detect the presence of common variant hemoglobins or other variants that may interfere with A1c results. This can be a significant limitation in certain clinical scenarios.
- 3. Result is only a number:** The A1c result obtained from an immunoassay is a numerical value and does not provide additional details or information about the potential presence of specific hemoglobin variants and/or HbF present.
- 4. Two-step assay:** In immunoassay methods for measuring glycated hemoglobin (HbA1c), it is common to run two separate tests: one for total hemoglobin and another for glycated hemoglobin. The total hemoglobin is usually performed using a colorimetric method and the glycated hemoglobin test usually relies on antibodies that are specific to the glycated form of hemoglobin.

References

1. <https://www.nhlbi.nih.gov/health/sickle-cell-disease> (accessed on 03/15/2024)
2. Akinsheye, I. et al. Fetal hemoglobin in sickle cell anemia, *Blood*. 2011 Jul 7; 118(1): 19–27.
3. Steinberg, H.M. Fetal hemoglobin in sickle cell anemia. *Blood*. 2020 Nov 19; 136(21): 2392–2400.
4. Rees, DC et al. Why are hemoglobin F levels increased in HbE/ beta thalassemia? *Blood*, 1999 Nov 1;94(9):3199-204.
5. Rochette, J. et al. Fetal hemoglobin levels in adults. *Blood Rev*, 1994 Dec;8(4):213-24.
6. Sharma, DC, et al. Hereditary persistence of fetal hemoglobin. *Asian J Transfus Sci*. 2020 Jul-Dec; 14(2): 185–186.
7. M Wolk, J E Martin, and C Reinus. Development of fetal haemoglobin - blood cells (F cells) within colorectal tumour tissues. *J Clin Pathol*. 2006 Jun; 59(6): 598–602v.
8. Sivaraman, P. Choosing a test method to measure HbA1c, *Medical Laboratory Observer*, June 26, 2020
9. https://www.accessdata.fda.gov/cdrh_docs/reviews/K121610.pdf Accessed on 03/25/2024
10. Little R. et al. The Effect of Increased Fetal Hemoglobin on 7 Common Hb A1c Assay Methods. *Clin Chem*. 2012, 58: 945-6.

Note: The data is available in Tosoh Bioscience Inc. internal records.