VITROS Products Chemistry

INSTRUCTIONS FOR USE

HbA1c

VITROS Chemistry Products HbA1c Reagent Kit

Direct % Glycated Hemoglobin

REF **684 2905**

Rx ONLY

This device has significant negative interference with fetal hemoglobin (HbF). HbA1c results are invalid for patients with abnormal amounts of HbF including those with known Hereditary Persistence of Fetal Hemoglobin. Refer to the Limitations of the Procedure section of this Instruction For Use for details.

Intended Use

For *in vitro* diagnostic use only.

VITROS Chemistry Products HbA1c reagent is used on VITROS 5,1 FS Chemistry System, VITROS 4600 Chemistry System and the VITROS 5600 Integrated System for the quantitative determination of percent glycated hemoglobin A1c (DCCT/NGSP) and mmol/mol hemoglobin A1c (IFCC) in human whole blood.

The test is to be used as an aid in diagnosis of diabetes, as an aid in identifying patients who may be at risk for developing diabetes mellitus, and for the monitoring of long-term blood glucose control in individuals with diabetes mellitus.

Summary and Explanation of the Test

HbA1c is the major species of glycohemoglobin found in human blood, and is formed by the non-enzymatic glycation of the free amino group at the N-terminal of the hemoglobin A_0 β-chain. [1] The percentage of HbA1c (%A1c) is widely used as a retrospective index of glycemic control in diabetic patients. [2] Studies have shown that glycemic control results in reduction in diabetic complications. [3, 4] The glycation of hemoglobin depends on both the lifespan of the red blood cell and the blood glucose concentration. Since the rate of glycated hemoglobin formation is directly proportional to the concentration of glucose in the blood, the level of glycated hemoglobin is an index of the blood glucose concentrations over an extended period of time (~6 to 8 weeks). [5]

Principles of the Procedure

The determination of % glycated hemoglobin (HbA1c) is performed using the VITROS Chemistry Products HbA1c Reagent Kit in conjunction with the VITROS Chemistry Products Calibrator Kit 31 on the VITROS 5,1 FS/4600 Chemistry Systems and the VITROS 5600 Integrated System. The VITROS Chemistry Products HbA1c Reagents are two dual chambered packages containing ready-to-use liquid reagents. Whole blood samples are hemolyzed on the VITROS 5,1 FS/4600 Chemistry Systems and the VITROS 5600 Integrated System. The concentration of HbA1c and total Hb are measured in the hemolyzed samples, controls and calibrators.

Hemoglobin A1c and Hemoglobin

Whole blood samples are hemolyzed on the VITROS 5,1 FS/4600 Chemistry Systems and the VITROS 5600 Integrated System. Calibrators, controls and hemolyzed whole blood samples are mixed with Reagent 1 containing anti-HbA1c antibody to form a soluble antigen-antibody complex. Hemoglobin in the hemolyzed whole blood is converted with Reagent 1 to a hematin derivative that is measured bichromatically at 340 nm and 700 nm. Unbound anti-HbA1c antibody reacts with polyhapten (hexapeptide-glycan, A1c Reagent 2) to form an insoluble antibody-polyhapten immune complex, which is measured turbidimetrically at 340 nm. After a calibration has been performed for each reagent lot, the hemoglobin A1c and Hb concentrations in each unknown sample can be determined using the stored calibration curves and the measured absorbance obtained in the assay of the hemolyzed sample.

%A1c

%A1c is a derived test calculated from the quantitative measurements of hemoglobin and hemoglobin A1c.

HbA1c (SI Units) [6]

HbA1c (mmol of HbA1c/mol of Hb) in SI units is a derived test calculated from the quantitative measurements of hemoglobin and hemoglobin A1c.

Test Type and Conditions

HbA1c and Hb

Test Type	VITROS System	Approximate Incubation Time	Temperature	Wavelength	Reaction Sample Volume
End-Point	5600, 4600, 5,1 FS	7 minutes	37 °C (98.6 °F)	(HbA1c) 340 nm (Hb) 340/700 nm	5.0 µL

Reaction Scheme

Specimen Hemolysis

Whole Blood —(R1 (DIL5 Reagent 1) Hemolyzing Reagent)→ Hemolyzed whole blood

HbA1c Step 1: Immune Complex HbA1c & Hemoglobin Chromophore

Hemolyzed whole blood —(R1 (HbA1c Reagent 1) Excess anti-HbA1c)→ Hemoglobin Chromophore, HbA1c-anti-HbA1c immune complex and unbound anti-HbA1c (soluble)

HbA1c Step 2: Detect unbound (free) anti-HbA1c

Unbound anti-HbA1c —(R2 (HbA1c Reagent 2) Excess HbA1c polyhapten)→ HbA1c polyhapten/anti-HbA1c immune complex (turbid/insoluble)

Warnings and Precautions

For *in vitro* diagnostic use only.

WARNING: ***Take care when handling materials and samples of human origin. Since no test method can offer complete assurance that infectious agents are absent, consider all clinical specimens, controls, and calibrators potentially infectious. Handle specimens, solid and liquid waste, and test components in accordance with local regulations and CLSI Guideline M29[7] or other published biohazard safety guidelines.***

For specific warnings and precautions for calibrators, quality control materials and other components, refer to the Instructions for Use for the appropriate VITROS product or to other manufacturer's product literature.

Reagents

Reactive Ingredients

HbA1c Reagent 1 (R1): HbA1c antibody (ovine serum) ≥0.5 mg/mL
HbA1c Reagent 2 (R2): HbA1c Polyhapten ≥8 µg/mL
DIL5 (R1): Tetradecyltrimethylammonium bromide (TTAB) <1% (w/v).

Other Ingredients

HbA1c Reagent 1 (R1): Buffers, surfactant, stabilizers, and preservatives
HbA1c Reagent 2 (R2): Buffers, surfactant, stabilizers, and preservatives
DIL5 (R1): Surfactants, stabilizers, and preservatives

Reagent Handling

Caution: **Do not use reagent packs with damaged or incompletely sealed packaging.**

- Inspect the packaging for signs of damage.
- Be careful when opening the outer packaging with a sharp instrument so as to avoid damage to the individual product packaging.
- The reagents are supplied ready for use.
- Avoid agitation, which may cause foaming or the formation of bubbles.

Reagent Preparation

1. Remove from refrigerated storage.
2. Immediately load into Supply 3.

Reagent Storage and Stability

VITROS Chemistry Products HbA1c Reagents are stable until the expiration date on the carton when they are stored and handled as specified. Do not use beyond the expiration date. For additional information, refer to “Calibration”.

IMPORTANT: *Do Not Freeze.*

Reagent	Storage Condition		Stability
Unopened	Refrigerated	2–8 °C (36–46 °F)	Until expiration date
Opened	On-analyzer	System turned on	≤ 28 days
	On-analyzer	System turned off	≤ 30 minutes

Verify performance with quality control materials after reloading reagents that have been removed from Supply 3 and stored for later use.

Specimen Collection, Preparation and Storage

Specimens Recommended

EDTA Whole Blood

- K_2EDTA
- K_3EDTA

IMPORTANT: *Certain collection devices have been reported to affect other analytes and assays [8]. Owing to the variety of specimen collection devices available, Ortho-Clinical Diagnostics is unable to provide a definitive statement on the performance of its products with these devices. Confirm that your collection devices are compatible with this assay.*

Specimens Not Recommended

- Heparinized Whole Blood
- Sodium Fluoride/Potassium Oxalate Whole Blood

Whole Blood

IMPORTANT: *Samples must be transferred to sample cups for analysis. Use of the original blood tube could cause separation of the blood during instrument handling and may lead to invalid results.*

Specimen Collection and Preparation

Collect specimens using standard laboratory procedures. [9, 10]

Patient Preparation

No special patient preparation is necessary.

Special Precautions

None.

Specimen Handling and Storage

- Handle and store specimens in stoppered containers to avoid contamination and evaporation.
- Samples should be fully resuspended prior to use.
- Samples should be free of clots.

Specimen Storage and Stability

Storage	Temperature	Stability
Original Specimen		
Room Temperature	18–28 °C (64–82 °F)	≤ 3 days
Refrigerated	2–8 °C (36–46 °F)	≤ 3 days

Testing Procedure

Materials Provided

VITROS Chemistry Products HbA1c Reagent Kit

Materials Required but Not Provided

- VITROS Chemistry Products FS Reconstitution Diluent
- VITROS Chemistry Products Calibrator Kit 31
- Quality control materials, such as VITROS Chemistry Products %A1c Performance Verifiers I and II
- Isotonic saline

Operating Instructions

- Check reagent inventories at least daily to ensure that quantities are sufficient for the planned workload.
- For additional information, refer to the operating instructions for your system.

IMPORTANT: *Bring all fluids and samples to room temperature, 18–28 °C (64–82 °F), prior to analysis.*

Specimen Testing

IMPORTANT: *Whole blood specimens must be placed in sample cups for analysis. Do not place an evaporation cap on the sample cup.*

Note: If using VITROS MicroSample Cups for analysis, you must use the VITROS FS Adaptor for MicroSample Cups.

1. Fully resuspend whole blood samples **immediately** prior to transfer to sample cups.
2. Samples should be free of clots.
3. For the system to properly mix the whole blood sample prior to aspiration, pipette 350 µL into a sample cup. Sample volumes less than 300 µL or greater than 400 µL may result in improper mixing of the whole blood sample.

Sample Programming

To report results in %A1c units, select the **%A1c** button in sample programming or via a LIS download.
To report results in HbA1c (mmol/mol) SI units select the **HbA1c** button in sample programing or via a LIS download.

Sample Dilution

On-Analyzer Sample Dilution

On-analyzer dilution is not recommended.

Manual Sample Dilution

If either component concentration (Hb or HbA1c) exceeds the system's measuring (reportable) range:

1. Manually dilute the original, whole blood sample with an equal volume of saline. Ensure original sample is resuspended prior to dilution.
2. Resuspend the diluted whole blood sample by gentle inversion and re-analyze.
3. Do not multiply the results by the dilution factor to obtain an estimate of the original sample's %A1c or HbA1c (mmol/mol) value. Hemoglobin and glycated hemoglobin are both diluted; therefore it is not required to multiply by the dilution factor in order to obtain an estimate of the original sample's value.

Calibration

Required Calibrators

- VITROS Chemistry Products Calibrator Kit 31

Calibrator Preparation, Handling, and Storage

Refer to the Instructions for Use for VITROS Chemistry Products Calibrator Kit 31.

Calibration Procedure

Refer to the operating instructions for your system.

When to Calibrate

Calibrate:

- When the reagent lot changes.

- When critical system parts are replaced due to service or maintenance.
- When government regulations require.
  For example, in the USA, CLIA regulations require calibration or calibration verification at least once every six months.

The VITROS HbA1c assay may also need to be calibrated:

- If quality control results are consistently outside acceptable range.
- After certain service procedures have been performed.

For additional information, refer to the operating instructions for your system.

Calculations

Components

HbA1c

Absorbance is measured at 340 nm after a fixed incubation time. After a calibration has been performed for each reagent lot, HbA1c concentration in the unknown samples can be determined using the stored calibration curve and the measured absorbance obtained in the assay of each sample.

Hb

Absorbance is measured bichromatically at 340 nm and 700 nm after a fixed incubation time. After a calibration has been performed for each reagent lot, Hb concentration in the unknown samples can be determined using the stored calibration curve and the measured absorbance obtained in the assay of each sample.

Derived Tests

Two reporting units are supported by your system.

- HbA1c in SI units
- %A1c in NGSP units

HbA1c

SI Units

$$\text{HbA1c (mmol/mol)} = \frac{\text{HbA1c [g/dL]}}{\text{Hb [g/dL]}} \times 1000$$

%A1c

NGSP units

$$\%\text{A1c} = (\text{IFCC}^{*} \times 0.09148) + 2.152$$

*IFCC=HbA1c (mmol/mol) SI units

Validity of a Calibration

Calibration parameters are automatically assessed by the system against a set of quality parameters detailed in the Review Assay Data screen (found via Options → Review/Edit Calibrations → Review Assay Data). Failure to meet any of the pre-defined quality parameters results in a failed calibration. The calibration report should be used in conjunction with quality control results to determine the validity of a calibration.

Linearity

Evaluation of the linearity of the VITROS Chemistry Products HbA1c assay was performed based on CLSI EP06-A. [11] For each assay component (Hb, HbA1c, %A1c), a low pool and a high pool were prepared with the assay component outside the measuring range. The low and high concentration pools were mixed to give 16 further pools of intermediate concentrations. The VITROS HbA1c assay was tested using each VITROS System.

The VITROS HbA1c assay is linear through the following ranges:

- Linear range for Hb: 4.972–30.318 g/dL (component)
- Linear range for HbA1c: 0.080–2.530 g/dL (component)
- Linear range for %A1c: 3.034–15.444% (NGSP derived test)
- Linear range for HbA1c: 9.6–145.3 mmol/mol (SI derived test)

%A1c, NGSP

Analyzer	Intercept	Slope	r^2	Concentration Range Tested
VITROS 5,1 FS	0.005	0.9995	0.999	2.39–17.35 %A1c
VITROS 4600	0.030	0.9957	0.997	3.03–15.44 %A1c
VITROS 5600	0.012	0.9988	0.999	2.89–15.79 %A1c

mmol/mol, SI

Analyzer	Intercept	Slope	r^2	Concentration Range Tested
VITROS 5,1 FS	0.044	0.9995	0.999	2.6–166.1 mmol/mol
VITROS 4600	0.223	0.9957	0.997	9.6–145.3 mmol/mol
VITROS 5600	0.099	0.9988	0.999	8.1–149.1 mmol/mol

Measuring (Reportable) Range

- Measuring range for Hb: 6.0–22.0 g/dL (component)
- Measuring range for HbA1c: 0.2–2.5 g/dL (component)
- Measuring range for derived tests:

%A1c NGSP* Units	HbA1c (mmol/mol) SI** Units
4–14	20–130

* NGSP – National Glycohemoglobin Standardization Program

** SI – Systéme International

Traceability of Calibration

The values assigned to the VITROS Chemistry Products Calibrator Kit 31 for %A1c are traceable to the IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) Reference Method. [1] The derived result (%A1c) is calculated from the individual quantitative results for hemoglobin (Hb) and glycated hemoglobin (HbA1c). NGSP results are derived from the International Federation of Clinical Chemistry (IFCC) HbA1c (mmol/mol) SI units using the Master Equation; [6, 12, 13]

%NGSP = (0.09148 × IFCC) + 2.152.

Quality Control

Quality Control Material Selection

IMPORTANT: *VITROS Chemistry Products %A1c Performance Verifiers are recommended for use with the VITROS 5,1 FS/4600 Chemistry and VITROS Integrated Systems. Evaluate the performance of other commercial control fluids for compatibility with this assay before using for quality control.*

- Control materials other than VITROS Chemistry Products %A1c Performance Verifiers may show a difference when compared with other %A1c Performance Verifiers methods if they:
  - Depart from a true human matrix.
  - Contain high concentrations of preservatives, stabilizers, or other nonphysiological additives.
- Use of either pre-hemolyzed or whole blood quality control materials is acceptable. Ensure during sample programming that the appropriate configuration is selected.

Quality Control Procedure Recommendations

IMPORTANT: *Either pre-hemolyzed or whole blood quality control materials are acceptable for this assay. Ensure that the system is configured correctly for the type of control used. To configure quality control fluids, go to QC→Define Controls→Wh Blood. If using a pre-hemolyzed control, check the box next to "**Pretreated**." If using a whole blood control, leave the box next to **"Pretreated"** unchecked.*

- Choose control levels that check the clinically relevant range.
- Analyze quality control materials in the same manner as pretreated whole blood, before or during patient sample processing.
- To verify system performance, analyze control materials:
  - After calibration.
  - According to local regulations or at least once each day that the assay is being performed.
  - After specified service procedures are performed. Refer to the operating instructions for your system.
- If control results fall outside your acceptable range, investigate the cause before deciding whether to report patient results.

- For general quality control recommendations, refer to *Statistical Quality Control for Quantitative Measurements: Principles and Definitions; Approved Guideline-Third Edition* [14] or other published guidelines.
- For additional information, refer to the operating instructions for your system.

Quality Control Material Preparation, Handling, and Storage

Refer to the Instructions for Use for VITROS Chemistry Products %A1c Performance Verifiers I and II or to other manufacturer's product literature.

Results

Reporting Units

The VITROS 5600 Integrated, VITROS 4600 Chemistry and the VITROS 5,1 FS Chemistry Systems can be programmed to report VITROS Chemistry Products HbA1c assay results in NGSP equivalent %A1c units (by selecting the **%A1c** button) and/or HbA1c (mmol/mol) SI units (by selecting the **HbA1c** button).

Unit Conversion from HbA1c (mmol/mol) SI Units to NGSP %A1c

NGSP %A1c results are derived from the International Federation of Clinical Chemistry (IFCC) HbA1c (mmol/mol) SI units using the Master Equation: [6, 12, 13] %A1c (NGSP) = (IFCC* × 0.09148) + 2.152.

where IFCC* = HbA1c (mmol/mol) SI Units

HbA1c in (mmol/mol) SI units can be calculated from the %A1c units using the Master Equation to yield the following equation: HbA1c (mmol/mol) = (%A1c (NGSP) x 10.93) – 23.50

Limitations of the Procedure

Known Interferences

Glycated HbF is not detected as it does not contain the glycated β-chain that characterizes %A1c. However, HbF is measured in the total Hb assay and as a consequence, specimens containing high amounts of HbF (>7%) may result in lower than expected mmol/mol HbA1c values (IFCC) and % A1c values (NGSP).

For substances that were tested and did not interfere, refer to “Specificity.”

Other Limitations

- The test is intended for the calculation of %A1c or mmol A1c/mol Hb. For patient samples, do not report the individual results for total hemoglobin (Hb) or hemoglobin A1c (HbA1c).
- The result from this or any other diagnostic test should be used and interpreted only in the context of the overall clinical picture.
- The design of VITROS HbA1c assay allows its use in patients with hemoglobin variants S, C, D and E traits. However, in case of disease condition such as HbSS, HbCC, or HbSC, even if the assay does not interefere with their variant, these patients may suffer anemia, increased red blood cell turnover, and transfusion requirements which can adversely affect HbA1c as a marker of long-term glycemic control. Any cause of shortened erythrocyte survival or decrease in mean erythrocyte age (e.g. recovery from acute blood loss, hemolytic anemia) will reduce exposure of erythrocytes to glucose with a consequent decrease in mmol/mol HbA1c values (IFCC) and %A1c values (DCCT/NGSP). Therefore, health care providers should not use the HbA1c test for these patients. Alternative forms of testing such as glycated serum protein or glycated albumin should be considered for these patients. [15, 16]
- Hemoglobin F does not contain β chains and is not detected by the VITROS HbA1c assay, but it is measured in the total Hb assay. The calculation of mmol A1c/mol Hb values (IFCC) and %A1c values (DCCT/NGSP) relative to total amount of Hb in the sample, produces falsely decreased HbA1c values in specimens with high amounts of HbF. [17]
- Hemoglobin A1c should not be used to diagnose gestational diabetes. It reflects the average blood glucose levels over the preceding 3 months (the average life of a red blood cell), and therefore may be falsely low during pregnancy or any other condition associated with recent onset of hyperglycemia. [18]
- In very rare cases of rapidly evolving type 1 diabetes, the increase of HbA1c values might be delayed compared to the acute increase in glucose concentrations. In these conditions diabetes mellitus must be diagnosed based on plasma glucose concentrations and/or the typical clinical symptoms. [19]
- HbA1c does not provide a measure of glycemic variability or hypoglycaemia. Glycemic control in these type 1 or type 2 diabetic patients prone to glycemic variability is best evaluated by a combination of results from self-monitoring and HbA1c. [20]
- Hemoglobin A1c should not be used to diagnose diabetes mellitus in patients with malignancies or severe chronic hepatic and renal disease. [21]
- Hemoglobin A1c testing should not replace glucose testing for type 1 diabetes, in pediatric patients and in pregnant women.

- Hemoglobin A1c should not be used to diagnose diabetes mellitus in patients with a hemoglobinopathy but normal red cell turnover (e.g. sickle cell trait).
- Hemoglobin A1c should not be used in patients with homozygous sickle cell trait, hemolytic anemia, or other hemolytic diseases and recent significant or chronic blood loss.

Expected Values

Reference Interval

The expected normal HbA1c range in adults is 4.0–6.0% (NGSP) or 20–42 mmol/mol (IFCC units). [22] Each laboratory should confirm the validity of these intervals for the population it serves. The Standards of Medical Care in Diabetes - 2015 recommend to diagnose diabetes using a HbA1c method that is NGSP-certified and standardized to the DCCT assay and a cut point of HbA1c ≥6.5%. [19, 20]

	Current*	IFCC traceable methods
Reference interval (non-diabetics)	4–6%	20–42 mmol/mol

* Refer to methods aligned to the US National Glycohemoglobin Standardization Program.

To provide clinical decision guidance for laboratories reporting results in %A1c (NGSP units), the NGSP interval was published by the National Glycohemoglobin Standardization Program (NGSP). [20] The reference interval is applicable to methods traceable to the Diabetes Control and Complications Trial (DCCT). [3]

%A1c (NGSP)	Interpretation
≥6.5	Action Suggested

To provide clinical decision guidance for laboratories reporting results in HbA1c mmol/mol (SI units), the following values were calculated from the NGSP %A1c cut point using the Master Equation. [6, 12] The HbA1c cut point is ≥48 mmol/mol (SI Units).

Calculated HbA1c (mmol/mol) SI Units	Interpretation
≥48	Action Suggested

Performance Characteristics

Limit of Quantitation/Detection

The Limit of Detection (LoD) for VITROS Chemistry Products HbA1c Reagent is 2.580% (NGSP Units), determined consistent with CLSI document EP17 [23] and with proportions of false positives (α) less than 5% and false negatives (β) less than 5%; based on 500 determinations, 5 low-level samples. The Limit of Blank (LoB) is 2.396% (NGSP Units) based on 100 determinates with 5 blank samples. The Limit of Quantitation (LoQ) determined consistent with the guidelines in CLSI document EP17 [23], based on 100 determinations with the 5 LoD pools using the classical Westgard model is 2.370% (NGSP Units). The associated bias and precision components were 0.064% NGSP and 0.121% NGSP respectively, which yielded a Westgard model TE of 0.3060 and a %TE of 12.57. Because the determined LoQ is less than the LoD, the final LoQ was set equal to the LoD of 2.580% (NGSP).

The Limit of Detection (LoD) for the Hb component of the VITROS Chemistry Products HbA1c Reagent is 0.312 g/dL, determined consistent with CLSI document EP17 [23] and with proportions of false positives (α) less than 5% and false negatives (β) less than 5%; based on 500 determinations, 5 low-level samples. The Limit of Blank (LoB) is 0.186 g/dL based on 100 determinates with 5 blank samples. The Limit of Quantitation (LoQ) determined consistent with the guidelines in CLSI document EP17 [23], based on 126 determinations with six LoQ pools; and a precision goal of 8.3% using the functional sensitivity method is 2.117 g/dL.

The Limit of Detection (LoD) for the HbA1c component of the VITROS Chemistry Products HbA1c Reagent is 0.072 g/dL, determined consistent with CLSI document EP17 [23] and with proportions of false positives (α) less than 5% and false negatives (β) less than 5%; based on 500 determinations, 5 low-level samples. The Limit of Blank (LoB) is 0.042 g/dL based on 100 determinates with 5 blank samples. The Limit of Quantitation (LoQ) determined consistent with the guidelines in CLSI document EP17 [23], based on 500 determinations with 5 LoD pools; and a precision goal of 14.6% using the functional sensitivity method is 0.133 g/dL.

Limit of Blank, Limit of Detection and Limit of Quantitation – Hb

LoB		LoD*		LoQ	
g/dL	g/L	g/dL	g/L	g/dL	g/L
0.186	1.86	0.312	3.12	2.117	21.17

* Proportions of false positives (α) and false negatives (β) were less than 5%; based on 500 determinations, with 5 low-level samples.

Limit of Blank, Limit of Detection and Limit of Quantitation – HbA1c

LoB		LoD*		LoQ	
g/dL	g/L	g/dL	g/L	g/dL	g/L
0.042	0.42	0.072	0.72	0.133	1.33

* Proportions of false positives (α) and false negatives (β) were less than 5%; based on 500 determinations, with 5 low-level samples.

Limit of Blank, Limit of Detection and Limit of Quantitation – %A1c

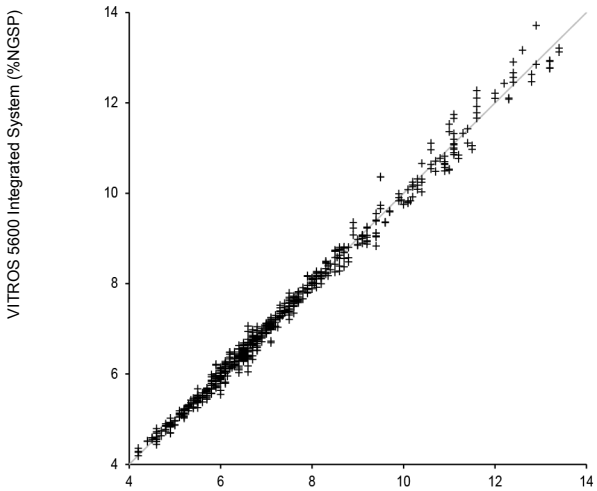
LoB		LoD*		LoQ	
NGSP	SI (mmol/mol)	NGSP	SI (mmol/mol)	NGSP	SI (mmol/mol)
2.396	2.67	2.580	4.68	2.580	4.68

* Proportions of false positives (α) and false negatives (β) were less than 5%; based on 500 determinations, with 5 low-level samples.

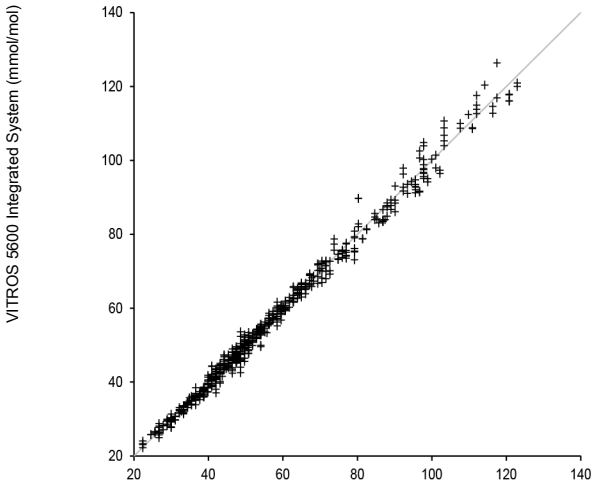
Method Comparison

The plots and table show the results of a method comparison study with whole blood samples analyzed on the VITROS 5600 Integrated System with those analyzed using a secondary reference laboratory of the NGSP. The table also shows the results of comparisons with whole blood samples on the VITROS 4600 Chemistry System and the VITROS 5,1 FS Chemistry System with a secondary reference laboratory of the NGSP, and the results of comparisons with whole blood samples on the VITROS 4600 Chemistry System and the VITROS 5,1 FS Chemistry System with the VITROS 5600 Integrated System. The testing followed CLSI Protocol EP9. [24] The VITROS HbA1c assay is certified by the National Glycohemoglobin Standardization Program and is traceable to the Diabetes Control and Complications Trial Reference Method.

NGSP Units



HbA1c SI Units



					NGSP Units				HbA1c SI Units (mmol/mol)			
	n	Slope	+/- 95% range of slope	Correlation Coefficient	Range of Samples	Inter-cept	+/- 95% range of intercept	Sy.x	Range of Samples	Inter-cept	+/- 95% range of intercept	Sy.x
5600 vs. comparative method*	357	1.00	0.99–1.02	0.996	4.2–13.4	-0.06	-0.13–0.02	0.18	22–123	-0.4	-0.9–0.2	1.9
4600 vs. comparative method*	357	1.02	1.01–1.03	0.995	4.2–13.4	-0.14	-0.22– -0.06	0.19	22–123	-0.9	-1.5– -0.3	2.1
5,1 FS vs. comparative method*	357	1.01	0.99–1.02	0.996	4.2–13.4	-0.08	-0.16–0.01	0.18	22–123	-0.5	-1.1–0.1	1.9
4600 vs. 5600	125	1.00	0.99–1.01	0.998	4.3–13.1	-0.01	-0.09–0.06	0.10	23–120	-0.1	-0.7–0.4	1.1
5,1 FS vs. 5600	126	0.99	0.97–1.00	0.998	4.3–13.8	0.01	-0.09–0.12	0.12	23–127	-0.2	-1.0–0.6	1.3

* Secondary Reference Laboratory of the NGSP

Precision

Precision was evaluated with quality control materials (hemolysate and whole blood-based) for 20-days and whole blood patient samples for 4-days on the VITROS 5,1 FS Chemistry System, VITROS 4600 Chemistry System and VITROS 5600 Integrated System following CLSI Protocol EP5. [25]

These results are guidelines. Variables such as instrument maintenance, environment, reagent storage/handling, control material reconstitution, and sample handling can affect the reproducibility of test results.

VITROS 5,1 FS Chemistry System – Combined (%A1c, NGSP)

Mean	Repeatability		Between Run		Between Day		Between Lot		Between Analyzer		Total			
HbA1c	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**N**	**Days**
VITROS PV1* 5.65%	0.033	0.58	0.037	0.65	0.039	0.69	0.045	0.80	0.085	1.50	0.115	2.03	756	21
Control* 4.96%	0.026	0.52	0.032	0.64	0.029	0.58	0.043	0.87	0.063	1.27	0.091	1.83	792	22
Control* 6.38%	0.048	0.75	0.052	0.82	0.050	0.78	0.057	0.89	0.084	1.32	0.133	2.09	756	21
Control* 8.17%	0.065	0.80	0.041	0.50	0.065	0.80	0.081	0.99	0.178	2.18	0.220	2.69	792	22
Control* 11.85%	0.147	1.24	0.092	0.78	0.119	1.00	0.102	0.86	0.058	0.49	0.241	2.03	720	20
Patient** 5.02%	0.023	0.46	0.031	0.62	0.032	0.64	0.033	0.66	0.094	1.87	0.111	2.21	144	4
Patient** 6.51%	0.038	0.58	0.038	0.58	0.042	0.64	0.053	0.81	0.099	1.52	0.131	2.01	144	4
Patient** 8.16%	0.054	0.66	0.071	0.87	0.031	0.38	0.064	0.78	0.107	1.31	0.157	1.92	144	4
Patient** 11.93%	0.171	1.43	0.173	1.45	0.090	0.75	0.114	0.96	0.100	0.84	0.301	2.52	144	4

Within Lab precision (Total) was determined using three lots of reagents on three analyzers on each platform, using a single calibration per lot.

* Control Fluid

** Whole Blood

VITROS 4600 Chemistry System – Combined (%A1c, NGSP)

Mean	Repeatability		Between Run		Between Day		Between Lot		Between Analyzer		Total			
HbA1c	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	N	Days
VITROS PV1* 5.63%	0.035	0.62	0.021	0.37	0.029	0.52	0.033	0.59	0.043	0.76	0.074	1.32	756	21
Control* 4.94%	0.028	0.57	0.017	0.34	0.024	0.49	0.033	0.67	0.028	0.57	0.059	1.19	792	22
Control* 6.35%	0.050	0.79	0.045	0.71	0.034	0.54	0.055	0.87	0.044	0.69	0.103	1.62	756	21
Control* 8.07%	0.071	0.88	0.037	0.46	0.046	0.57	0.043	0.53	0.054	0.67	0.115	1.43	792	22
Control* 11.89%	0.177	1.49	0.069	0.58	0.109	0.92	0.095	0.80	0.000	0.00	0.239	2.01	720	20
Patient** 4.98%	0.027	0.54	0.025	0.50	0.016	0.32	0.012	0.24	0.036	0.72	0.055	1.10	144	4
Patient** 6.47%	0.049	0.76	0.019	0.29	0.020	0.31	0.034	0.53	0.040	0.62	0.077	1.19	144	4
Patient** 8.06%	0.090	1.12	0.064	0.79	0.032	0.40	0.052	0.65	0.044	0.55	0.134	1.66	144	4
Patient** 11.96%	0.157	1.31	0.142	1.19	0.117	0.98	0.064	0.54	0.000	0.00	0.250	2.09	144	4

Within Lab precision (Total) was determined using three lots of reagents on three analyzers on each platform, using a single calibration per lot.

* Control Fluid

** Whole Blood

VITROS 5600 Integrated System – Combined (%A1c, NGSP)

Mean	Repeatability		Between Run		Between Day		Between Lot		Between Analyzer		Total			
HbA1c	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	N	Days
VITROS PV1* 5.66%	0.036	0.64	0.013	0.23	0.020	0.35	0.031	0.55	0.041	0.72	0.067	1.18	756	21
Control* 4.98%	0.034	0.68	0.011	0.22	0.017	0.34	0.058	1.17	0.000	0.00	0.070	1.41	792	22
Control* 6.37%	0.057	0.90	0.029	0.46	0.034	0.53	0.033	0.52	0.041	0.64	0.089	1.40	756	21
Control* 8.10%	0.072	0.89	0.017	0.21	0.030	0.37	0.054	0.67	0.037	0.46	0.103	1.27	792	22
Control* 11.89%	0.178	1.50	0.055	0.46	0.067	0.56	0.018	0.15	0.066	0.56	0.209	1.76	720	20
Patient** 5.04%	0.027	0.54	0.013	0.26	0.021	0.42	0.032	0.64	0.020	0.40	0.053	1.05	144	4
Patient** 6.53%	0.042	0.64	0.026	0.40	0.019	0.29	0.038	0.58	0.032	0.49	0.073	1.12	144	4
Patient** 8.14%	0.062	0.76	0.062	0.76	0.000	0.00	0.044	0.54	0.032	0.39	0.104	1.28	144	4
Patient** 12.04%	0.173	1.44	0.089	0.74	0.088	0.73	0.064	0.53	0.070	0.58	0.234	1.94	144	4

Within Lab precision (Total) was determined using three lots of reagents on three analyzers on each platform, using a single calibration per lot.

* Control Fluid

** Whole Blood

VITROS 5,1 Chemistry System – Combined (mmol/mol, SI Units)

Mean	Repeatability		Between Run		Between Day		Between Lot		Between Analyzer		Total			
mmol/mol	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	N	Days
VITROS PV1* 38.3	0.36	0.9	0.40	1.0	0.43	1.1	0.49	1.3	0.93	2.4	1.26	3.3	756	21
Control* 30.7	0.28	0.9	0.35	1.1	0.32	1.0	0.47	1.5	0.69	2.2	0.99	3.2	792	22
Control* 46.2	0.52	1.1	0.57	1.2	0.55	1.2	0.62	1.3	0.92	2.0	1.45	3.1	756	21
Control* 65.7	0.71	1.1	0.45	0.7	0.71	1.1	0.89	1.4	1.95	3.0	2.40	3.7	792	22
Control* 106.0	1.61	1.5	1.01	1.0	1.30	1.2	1.11	1.0	0.63	0.6	2.63	2.5	720	20
Patient** 31.3	0.25	0.8	0.34	1.1	0.35	1.1	0.36	1.2	1.03	3.3	1.21	3.9	144	4
Patient** 47.7	0.42	0.9	0.42	0.9	0.46	1.0	0.58	1.2	1.08	2.3	1.43	3.0	144	4
Patient** 65.6	0.59	0.9	0.78	1.2	0.34	0.5	0.70	1.1	1.17	1.8	1.72	2.6	144	4
Patient** 106.9	1.87	1.7	1.89	1.8	0.98	0.9	1.25	1.2	1.09	1.0	3.29	3.1	144	4

Within Lab precision (Total) was determined using three lots of reagents on three analyzers on each platform, using a single calibration per lot.

* Control Fluid

** Whole Blood

VITROS 4600 Chemistry System – Combined (mmol/mol, SI Units)

Mean	Repeatability		Between Run		Between Day		Between Lot		Between Analyzer		Total			
mmol/mol	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	N	Days
VITROS PV1* 38.0	0.38	1.0	0.23	0.6	0.32	0.8	0.36	0.9	0.47	1.2	0.81	2.1	756	21
Control* 30.5	0.31	1.0	0.19	0.6	0.26	0.9	0.36	1.2	0.31	1.0	0.64	2.1	792	22
Control* 45.9	0.55	1.2	0.49	1.1	0.37	0.8	0.60	1.3	0.48	1.0	1.13	2.5	756	21
Control* 64.7	0.78	1.2	0.40	0.6	0.50	0.8	0.47	0.7	0.59	0.9	1.26	1.9	792	22
Control* 106.5	1.93	1.8	0.75	0.7	1.19	1.1	1.04	1.0	0.00	0.0	2.61	2.5	720	20
Patient** 30.9	0.30	1.0	0.27	0.9	0.17	0.6	0.13	0.4	0.39	1.3	0.60	1.9	144	4
Patient** 47.1	0.54	1.1	0.21	0.4	0.22	0.5	0.37	0.8	0.44	0.9	0.84	1.8	144	4
Patient** 64.6	0.98	1.5	0.70	1.1	0.35	0.5	0.57	0.9	0.48	0.7	1.46	2.3	144	4
Patient** 107.2	1.72	1.6	1.55	1.4	1.28	1.2	0.70	0.7	0.00	0.0	2.73	2.5	144	4

Within Lab precision (Total) was determined using three lots of reagents on three analyzers on each platform, using a single calibration per lot.

* Control Fluid

** Whole Blood

VITROS 5600 Integrated System – Combined (mmol/mol, SI Units)

Mean	Repeatability		Between Run		Between Day		Between Lot		Between Analyzer		Total			
mmol/mol	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	N	Days
VITROS PV1* 38.4	0.39	1.0	0.14	0.4	0.22	0.6	0.34	0.9	0.45	1.2	0.73	1.9	756	21
Control* 30.9	0.37	1.2	0.12	0.4	0.19	0.6	0.63	2.0	0.00	0.0	0.77	2.5	792	22
Control* 46.1	0.62	1.3	0.32	0.7	0.37	0.8	0.36	0.8	0.45	1.0	0.97	2.1	756	21
Control* 65.0	0.79	1.2	0.19	0.3	0.33	0.5	0.59	0.9	0.40	0.6	1.13	1.7	792	22
Control* 106.4	1.95	1.8	0.60	0.6	0.73	0.7	0.20	0.2	0.72	0.7	2.28	2.1	720	20
Patient** 31.5	0.30	1.0	0.14	0.4	0.23	0.7	0.35	1.1	0.22	0.7	0.58	1.8	144	4
Patient** 47.8	0.46	1.0	0.28	0.6	0.21	0.4	0.42	0.9	0.35	0.7	0.80	1.7	144	4
Patient** 65.4	0.68	1.0	0.68	1.0	0.00	0.0	0.48	0.7	0.35	0.5	1.14	1.7	144	4
Patient** 108.1	1.89	1.7	0.97	0.9	0.96	0.9	0.70	0.6	0.77	0.7	2.56	2.4	144	4

Within Lab precision (Total) was determined using three lots of reagents on three analyzers on each platform, using a single calibration per lot.

* Control Fluid

** Whole Blood

Specificity

Substances that Do Not Interfere

- Samples containing hemoglobin variants HbS up to 41%, HbC up to 38%, HbD up to 38% and HbE up to 26% of the total hemoglobin concentration do not interefere.
- The anti-HbA1c antibodies used in this kit do not cross-react with HbA0 up to 90%, HbA1a up to 1.5%, HbA1b up to 4% of the total hemoglobin concentration.
- This method is unaffected by the presence of acetylated hemoglobin, carbamylated hemoglobin, and labile glycated hemoglobin when samples were treated with 50 mg/dL Acetaldehyde, 150 mg/dL Urea, and 1500 mg/dL Glucose for four hours at 37 °C, respectively.
- The substances listed in this table were tested with the VITROS Chemistry Products HbA1c assay at %A1c values of approximately 6.5% and 8.5% (48 to 69 HbA1c (mmol/mol)), using protocols based on CLSI Protocol EP7 [26], and found not to interfere, bias ≤0.5 %A1c and ≤0.6 %A1c (≤5 and ≤7 HbA1c (mmol/mol)), at the concentration shown.

Substance*	Concentration	
Acetaminophen	20 mg/dL	1.32 mmol/L
Acetylsalicylic acid	100 mg/dL	5.55 mmol/L
Ampicillin	100 mg/dL	2.86 mmol/L
Ascorbic Acid	80 mg/dL	4.54 mmol/L
Bilirubin	50 mg/dL	0.86 mmol/L
Ca-dobesilate	20 mg/dL	0.48 mmol/L
Cefoxitin sodium	250 mg/dL	5.56 mmol/L
Cholesterol	350 mg/dL	9.1 mmol/L
Cyclosporin	0.5 mg/dL	4.16 µmol/L
Doxycyclin hyclate	5 mg/dL	0.10 mmol/L
Glucose	1000 mg/dL	55.5 mmol/L
Glycated Albumin	500 mg/dL	0.07 mmol/L
Ibuprofen	50 mg/dL	2.42 mmol/L
Insulin	592.8 µIU/mL	3926 pmol/L
Intralipid	500 mg/dL	Not Applicable
Levodopa	2 mg/dL	0.10 mmol/L

Substance*	Concentration	
Metformin	4 mg/dL	0.31 mmol/L
Methyldopa	2 mg/dL	94.7 µmol/L
Metronidazole	20 mg/dL	1.17 mmol/L
N-Acetylcysteine	166.3 mg/dL	10.2 mmol/L
Phenylbutazone	40 mg/dL	1.30 mmol/L
Rheumatoid Factor	750 IU/mL	750 kIU/L
Rifampicin	6 mg/dL	72.9 µmol/L
Rosiglitazone maleate	0.8 mg/dL	16.9 µmol/L
Theophylline	10 mg/dL	0.56 mmol/L
Total Protein	5 g/dL	50 g/L
Total Protein	9 g/dL	90 g/L
Triglyceride	1000 mg/dL	11.3 mmol/L

* These results are intended to be representative; however, your results may differ somewhat due to reagent lot and specimen-related differences. The degree of interference at concentrations other than those listed for the substance and analyte may not be predictable.

VITROS 5,1 FS Analyzer

Hemoglobin Variant	# samples tested	Variant Concentration Range (%)	Range of %A1c Concentration	Relative % Difference from Reference Concentration at Low and High HbA1c Concentrations			
				~6.0 %HbA1c		~9.0 %HbA1c	
				Relative %Bias	Range %Bias	Relative %Bias	Range %Bias
HbA0	70	49.3–90.4	5.8–13.4	-1.11	-6.15–2.91	-0.90	-1.93–2.04
HbA1a		0.1–1.5					
HbA1b		1.3–4.1					
HbA2	22*	4.9–6.1	5.7–9.0	-1.80	-7.25–0.68	-2.67	-5.08– -2.13
HbC	31**	24.5–38.4	5.1–9.8	-2.56	-7.13–3.24	-2.85	-4.00–0.30
HbD	21	29.0–38.0	5.2–11.3	-1.52	-7.65–2.86	-2.95	-4.34–0.05
HbE	30***	14.3–26.3	5.4–9.1	-0.37	-6.45–3.95	-1.00	-1.26–6.01
HbS	40	28.2–41.6	4.6–12.7	0.76	-4.72–5.95	0.70	-1.60–7.86
HbF	43	0.2–34.8	5.5–12.8	-5.31	-29.17–2.65	-2.58	-24.47–1.85

* All twenty-two HbA2 patient samples were spiked.

** Nineteen native HbC patient samples and 12 spiked patient samples.

*** Eighteen native HbE patient samples and 12 spiked patient samples.

VITROS 4600 Analyzer

Hemoglobin Variant	# samples tested	Variant Concentration Range (%)	Range of %A1c Concentration	Relative % Difference from Reference Concentration at Low and High HbA1c Concentrations			
				~6.0 %HbA1c		~9.0 %HbA1c	
				Relative %Bias	Range %Bias	Relative %Bias	Range %Bias
HbA0	70	49.3–90.4	5.8–13.4	-0.91	-6.91–4.53	-0.58	-0.52–3.07
HbA1a		0.1–1.5					
HbA1b		1.3–4.1					
HbA2	22*	4.9–6.1	5.7–9.0	-1.78	-7.07–1.60	-1.81	-4.52– -1.40
HbC	31**	24.5–38.4	5.1–9.8	-1.92	-6.44–2.68	-2.67	-2.58– -0.26
HbD	21	29.0–38.0	5.2–11.3	-1.33	-6.09–1.25	-3.74	-4.61– -2.58
HbE	30***	14.3–26.3	5.4–9.1	2.38	-3.41–7.92	-0.69	-5.13–1.46
HbS	40	28.2–41.6	4.6–12.7	-0.84	-4.07–4.29	0.30	-1.05–7.85
HbF	43	0.2–34.8	5.5–12.8	-4.88	-28.65–4.29	-2.63	-23.35–2.14

* All twenty-two HbA2 patient samples were spiked.

** Nineteen native HbC patient samples and 12 spiked patient samples.

*** Eighteen native HbE patient samples and 12 spiked patient samples.

VITROS 5600 Analyzer

Hemoglobin Variant	# samples tested	Variant Concentration Range (%)	Range of %A1c Concentration	Relative % Difference from Reference Concentration at Low and High HbA1c Concentrations			
				~6.0 %HbA1c		~9.0 %HbA1c	
				Relative %Bias	Range %Bias	Relative %Bias	Range %Bias
HbA0	70	49.3–90.4	5.8–13.4	0.06	-5.83–4.53	0.03	0.03–2.79
HbA1a		0.1–1.5					
HbA1b		1.3–4.1					
HbA2	22*	4.9–6.1	5.7–9.0	-3.25	-8.12–1.33	-4.12	-8.03– -1.66
HbC	31**	24.5–38.4	5.1–9.8	-2.86	-6.46–1.18	-3.52	-4.05– -1.26
HbD	21	29.0–38.0	5.2–11.3	-2.71	-7.18–0.45	-5.37	-6.65– -4.98
HbE	30***	14.3–26.3	5.4–9.1	-0.49	-6.15–3.94	-2.39	-7.11– -0.62
HbS	40	28.2–41.6	4.6–12.7	-0.74	-7.40–4.24	-1.87	-1.83–4.21
HbF	43	0.2–34.8	5.5–12.8	-4.30	-30.03–4.24	-2.03	-25.67–2.06

* All twenty-two HbA2 patient samples were spiked.

** Nineteen native HbC patient samples and 12 spiked patient samples.

*** Eighteen native HbE patient samples and 12 spiked patient samples.

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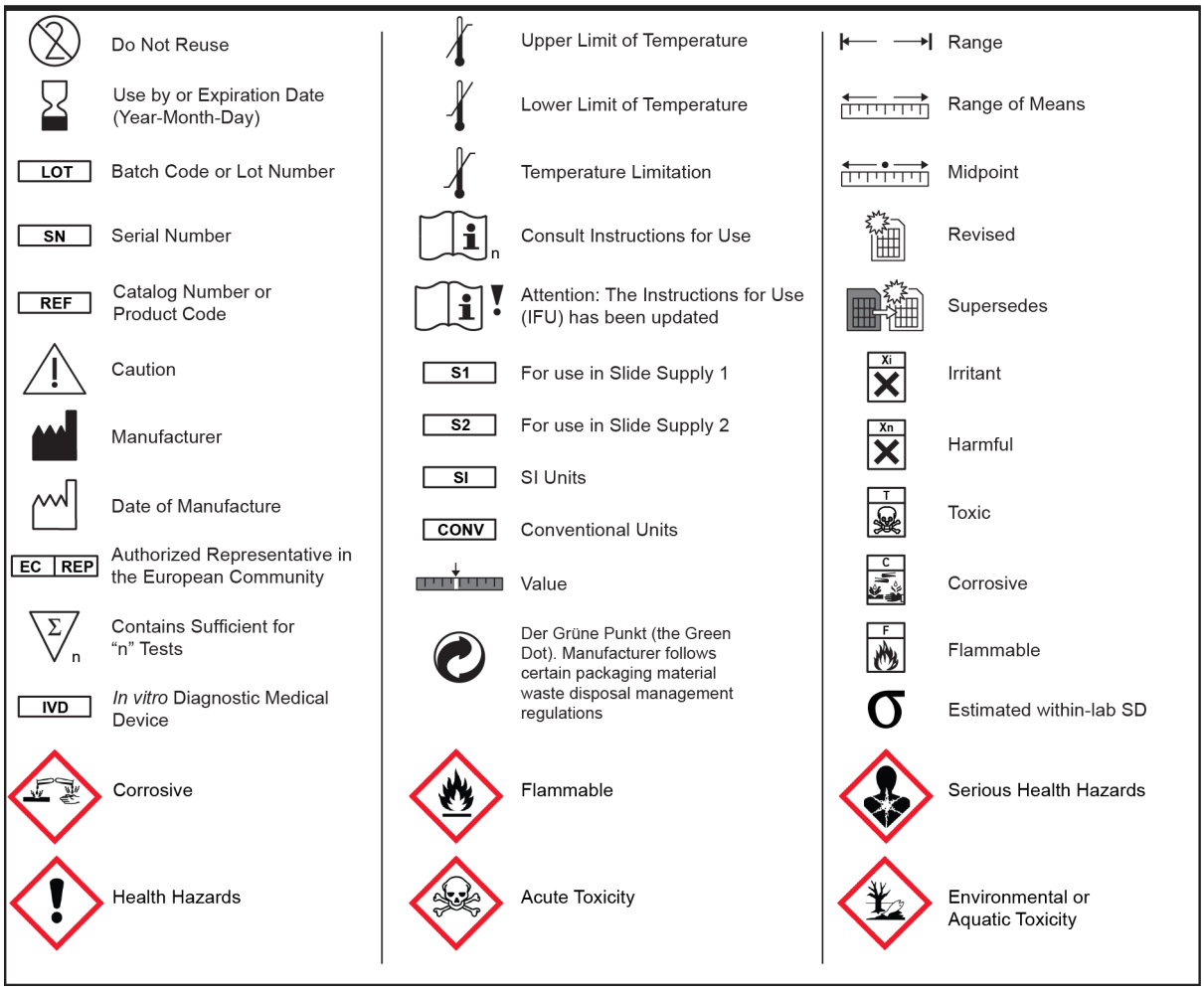
Glossary of Symbols

The following symbols may have been used in the labeling of this product.



Revision History

Date of Revision	Version	Description of Technical Changes*
2015-09-11	3.0	Substances that Do Not Interfere: Triglyceride changed from 500 mg/dL (5.7 mmol/L) to 1000 mg/dL (11.3 mmol/L)
2015-06-04	2.0	• First release of document in US • Prescription Use Statement added • Black Box Warning added • Intended Use: updated wording • Warnings and Precautions: updated to align with the new Safety Data Sheets • Specimens Recommended: added K_2EDTA and K_3EDTA • Calibration: added Linearity section • Results: – Reporting Units updated – Unit Conversion updated • Other Limitations: added statements • Expected Values: Reference Interval section updated to include new table and wording • Method Comparison: – updated wording – updated plot diagrams – updated table • Precision: – updated wording – added new tables • Substances that Do Not Interfere: – changed HbD variant concentration level from 37% to 38% – new tables added • References: – updated references 7, 19, 20 – added reference 11, 22 • Glossary of Symbols: added Globally Harmonized Symbols to comply with the Classification, Labelling and Packaging (CLP) Regulations
2014-05-19	1.0	First release of document

* The change bars indicate the position of a technical amendment to the text with respect to the previous version of the document.

When this Instructions For Use is replaced, sign and date below and retain as specified by local regulations or laboratory policies, as appropriate.

_____ _____

Signature Obsolete Date

CE

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