HbA1c

Cat. No.	Pack Name	Packaging (Content)
XSYS0054	HBA1C	R1: 1 x 24 ml, R2a: 1 x 8 ml, R2b: 1 x 4 ml, R3: 2 x 50 ml

EN

CE IVD

INTENDED USE
Diagnostic reagent for quantitative in vitro determination of hemoglobin A1c in whole blood on photometric systems.

CLINICAL SIGNIFICANCE
Hemoglobin A1c (HbA1c) is a glycated hemoglobin which is formed by the non-encymatic reaction of glucose with native hemoglobin. This process runs continuously throughout the circulatory life of the red cell (average life time 100 - 120 days). The rate of glycation is directly proportional to the concentration of glucose in the blood. The blood level of HbA1c represents the average blood glucose level over the preceding 6 to 8 weeks (due to the kinetics of erythrocyte turnover this period is more affected by the blood glucose level than the preceding weeks). Therefore, HbA1c is suitable for retrospective long-term monitoring of blood glucose concentration in individuals with diabetes mellitus. Clinical studies have shown that lowering of HbA1c level can help to prevent or delay the incidence of late diabetic complications.
As the amount of HbA1c also depends on the total quantity of hemoglobin the reported HbA1c value is indicated as a percentage of the total hemoglobin concentration.
Falsely low values (low HbA1c despite high blood glucose) may occur in people with conditions with shortened red blood cell survival (hemolytic diseases) or significant recent blood loss (higher fraction of young erythrocytes). Falsely high values (high HbA1c despite normal blood glucose) have been reported in iron deficiency anemia (high proportion of old erythrocytes). These circumstances have to be considered in clinical interpretation of HbA1c values.

METHODOLOGY
Particle enhanced immunoturbidimetric test.
HbA1c is determinated directly without measurement of total hemoglobin.

PRINCIPLE
Total Hb and HbA1c in hemolyzed blood bind with the same affinity to particles in R1.The amount of binding is proportional to the relative concentration of both substances in the blood. Mouse anti-human HbA1c monoclonal antibody (R2a) binds to particle bound HbA1c. Goat anti--mouse IgG polyclonal antibody (R2b) interacts with the monoclonal mouse anti-human HbA1c antibody and agglutination takes place. The measured absorbance is proportional to the HbA1c bound to particles, which in turn is proportional to the percentage of HbA1c in the sample.

STANDARDIZATION
The assay is standardized according to the approved IFCC reference method [3]. Calibration according to DCCT/NGSP is also possible. Corresponding calibrator values are listed in the package insert of the calibrator set HbA1c liquid.
DCCT/NGSP and IFCC values show a linear relationship and can therefore be calculated from each other. Also new IFCC recommended units mmol/mol (mmol HbA1c / mol Hb) can be easily calculated from IFCC values. Recalculation equations are following:
IFCC = (NGSP – 2.15) / 0.915
NGSP = 0.915 x IFCC + 2.15
mmol/mol = 10 x IFCC
IFCC: International Federation of Clinical Chemistry [3,4]
DCCT: Diabetes Control and Complications Trial [5]
NGSP: National Glycohemoglobin Standardization Program [6]

REAGENT COMPOSITION

R1:	Buffer	20 mmol/l
	Latex	1.5 %
R2a:	Buffer	10 mmol/l
	Mouse anti-human HbA1c monoclonal antibody	5.5 mg/dl
R2b:	Buffer	1 mmol/l
	Goat anti-mouse IgG polyclonal antibody	67 mg/dl
	Stabilizers	
R3:	Hemolysing solution	

REAGENT PREPARATION
Transfer 4 ml of R2b into bottle R2a and mix well immediately.
Ratio between R2a and R2b must be 2/1. Stability of premixed R2a/R2b: One month stored at 2–8°C.

STABILITY AND STORAGE
The reagents are stable until expiry date when kept at 2–8°C. Stability in the instrument is at least 4 weeks if contamination is avoided. Do not freeze.

SPECIMEN COLLECTION
Whole blood collected with EDTA.
Sample preparation:
Hemolyzing Solution (R3) 500 µl
Sample/calibrator/control 10 µl
Mix and allow to stand for 5 minutes or until complete lysis is apparent.
In the case of instruments which can process 3 reagents, sample preparation can be performed on board.

Specimen stability:
Whole blood 1 week at 2–8°C
Hemolysate 10 hours at 15–25°C
Hemolysate 10 days at 2–8°C

ASSAY PROCEDURE
Application sheets for automated systems are available on request.
Wavelength 660 nm
Optical path 1 cm
Temperature 37 °C
Measurement Against air
3-component system – ready-to-use

Sample or calibrator	20 µl
Reagent 1	750 µl
Mix, incubate for 2 min., then add	
Reagent 2a	250 µl
Mix, incubate for 3 min., then add:	
Reagent 2b	125 µl
Mix, read absorbance after **exactly** 2 min.	

2-component system - premixed R2a/R2b

Sample or calibrator	30 µl
Reagent 1	1000 µl
Mix, incubate for 5 min., then add:	
Reagent 2a/2b	500 µl
Mix, read absorbance after **exactly** 5 min.	

MATERIALS REQUIRED BUT NOT PROVIDED
General laboratory equipment.

CALIBRATION
The concentration of HbA1c in unknown samples is derived from a calibration curve using an appropriate mathematical model such as spline. The calibration curve is obtained with 4 calibrators at different levels and NaCl solution (9 g/l) for determination of the zero value.
Stability of calibration: 3-component system 8 weeks
2-component system 6 days
For calibration use the HBA1C CAL SET with 4 different concentrations (XSYS0057).

CONTROLS
For internal quality control use the HBA1C CON L (XSYS0055), HBA1C CON H (XSYS0056).

CALCULATION
Results are calculated automatically by the instrument.

EXPECTED VALUES[7]
Reference intervals should be established or verified by the laboratory based on an appropriate non-diabetic patient population.

	% NGSP	% IFCC	mmol/mol
Non-diabetics	4 - 6	3 - 4	30 - 40
Target of therapy	< 7	< 5	< 50
Change of therapy	> 8	> 6	> 60

PERFORMANCE DATA
Data contained within this section is representative of performance on ERBA XL systems. Data obtained in your laboratory may differ from these values.

Accuracy
A comparison between XL-Systems HbA1c (y) and commercially available test (x) using 40 samples were carried. Serum samples were assayed in parallel and the results compared by linear regression analysis according to Passing-Bablok. The following statistics were obtained:
r = 0.927
y = 1.000 x – 3.0 mmol/mol

QUALITY SYSTEM CERTIFIED
ISO 9001 ISO 13485

Measuring Range
The test has been developed to determine concentrations of HbA1c within a measuring range from 3.98 – 15.42 % DCCT/NGSP, 2 – 14.5 % IFCC, 20 –145 mmol/mol.
The assay is applicable for hemoglobin concentrations in blood from 6 to 26 g/dl.

Specificity / Interferences
Due to its antibodies, HbA1c is a specific immunoassay for human HbA1c. No interference was observed by ascorbic acid up to 60 mg/dl, conjugated and unconjugated bilirubin up to 40 mg/dl, lipemia up to 2000 mg/dl triglycerides, RF up to 250 IU/ml, carbamylated Hb up to 7.5 mmol/l, and acetylated Hb up to 5.0 mmol/l.
No interference is observed by uremia, labile intermediates (Schiff base), and Hemoglobin variants HbS and HbA2. Elevated levels of HbF may lead to falsely low HbA1c values. Alcoholism and ingestion of large doses of aspirin may lead to inconsistent results [1].

Sensitivity /Limit of Detection
The limit of detection is 6.6 mmol/mol HbA1c.

PRECISION
Values according to IFCC

Within-run precision n = 20	Mean (mmol/mol)	SD (mmol/ml)	CV (%)
Sample 1	24.6	1.02	4.15
Sample 2	98.8	1.61	1.63

Between day precision n = 20	Mean (mmol/mol)	SD (mmol/ml)	CV (%)
Sample 1	30.3	1.23	4.07
Sample 2	111	3.34	3.01

WARNING AND PRECAUTIONS
For *in vitro* diagnostic use. To be handled by entitled and professionally educated person.
Components of the kit are not classified as dangerous but reagents R1, R2a and R2b contain less than 0.1% sodium azide - classified as very toxic and dangerous substance for the environment.
In very rare cases, samples of patients with gammopathy might give falsified results.
Immediately after HbA1c measurement cleaning of cuvettes is necessary. Use the alkaline cuvette washing solution which is recommended by the analyzer manufacturer.
Take necessary precautions for use of laboratory reagent.

WASTE MANAGEMENT
Please refer to local legal requirements.

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