ISE S.r.l. CUSTOMISED SOLUTIONS FOR YOUR LABORATORY	EN	HbA1C				Instruction For Use	CE IVD	Rev.E - 15-11-22
			R3330000001	R1: 1x 18.5 mL	R2: 1x 4.0 mL	Lys: 2x 16.0 mL		
		REF	A-R0000000029	R1: 1x 21.0 mL	R2: 1x 4.5 mL	Lys: 2x 32.0 mL		
			A-R0000000030	R1: 1x 21.0 mL	R2: 1x 5.0 mL	Lys: 4x 50.0 mL		

Intended Use

Quantitative determination of Hemoglobin A1c (HbA1c) in human blood by turbidimetric immunoassay. The determination of HbA1c is most commonly performed for the evaluation of glycemic control in diabetes mellitus. HbA1c values provide an indication of glucose levels over the preceding 4-8 weeks. A higher HbA1c value indicates poorer glycemic control.

For professional in vitro diagnostic use only.

Diagnostics Implications

Throughout the circulatory life of the red cell, HbA1c is formed continuously by the adduction of glucose to the N-terminal of the haemoglobin beta chain. The process, which is non-enzymatic, reflects the average exposure of haemoglobin to glucose over an extended period. In a classical study, Trivelli *et al.*[1] showed HbA1c in diabetic subjects to be elevated 2-3 folds over the levels found in normal individuals. Several investigators have recommended that HbA1c serves as an indicator of metabolic control of the diabetic, since HbA1c levels approach normal values for diabetics in metabolic control[2,3,4]. HbA1c has been defined operationally as the "fast fraction" haemoglobins (HbA1a, A1b, A1c) that elute first during column chromatography with cation-exchange resins. The non-glycosylated haemoglobin, which consists of the bulk of the haemoglobin has been designated HbA0.

Method

This method utilizes the interaction of antigen and antibody to determine the HbA1c in whole EDTA blood. HbA1c in test samples is absorbed onto the surface of latex particles, which react with Anti-HbA1c (antigen-antibody reaction) and gives agglutination. The amount of agglutination is measured as absorbance. The HbA1c value is obtained from a calibration curve.

Reagents Provided

The same reagents are supplied in different volume formats depending on the I.S.E. S.r.l. analysers utilised and installed reagent support.

Supplied Volumes

	Product Code		
	A-R0000000029	*A-R0000000030*	*R3330000001*
Vial size	*35 / 15 mL*	*50 / 20 mL*	*18 / 18 mL*
Reagent 1	1 x 21.0 mL	1x 21.0 mL	1x 18.5 mL
Reagent 2	1x 4.5 mL	1x 5.0 mL	1x 4.0 mL
Lysing Sol.	2x 32.0 mL	4x 50.0 mL	2x 16.0 mL

Reagent format

Reagent	*Format*	*Code*
Reagent 1 (liquid)	Ready to Use	179C03
Reagent 2 (liquid)	Ready to Use	179C02
Lysing Solution (liquid)	Ready to Use	181D05

Reagent Contents

Reagent 1:	*Conc.*	*U.M.*
Latex	-	-
Sodium Azide	0.95	g/L
Reagent 2:		
Anti-human Hemoglobin A1c Mouse Monoclonal	-	-
Stabilizers.	-	-
Reagent 3:		
Lise Solution. Stabilizers.	-	-

Stability and Storage

The reagents are stable until expiry date when kept at 2-8°C. After opening the reagents are stable for 1 month at 2-8°C. Do not freeze.

Reagents required but not supplied

1. Calibrators and Controls*

Key Reference	*Description*
R0300000003	Hemoglobin A1c Standard Set
R0400000007	Hemoglobin A1c Control Low
R0400000004	Hemoglobin A1c Control High

** Hemolysates from packed human erythrocytes lyophilized and stabilized. Values are stated in the supplied insert.*

2. Hemolysing Solution for sample preparation (additional)

Key Reference	*Description*
R3140000042	Hemolysing Solution for HbA1c (500 mL)

Sample collection and preparation

Use fresh EDTA blood. To determine HbA1c, a hemolysate must be prepared for each sample:

Manual Lysing Procedure
for A-R0000000030:
1. Dispense 2 mL Lysing Solution into a test tube.
2. Place 20 µL of well mixed whole EDTA blood (samples, standards and controls) into the test tube.
3. Mix. Allow to rest for 5 minutes or until complete lysis is evident.
4. Stability of the hemolysate: 72 hours at 2-8 °C.

for A-R0000000029:
1. Dispense 0.5 mL Lysing Solution into a test tube.
2. Place 5 µL of well mixed whole EDTA blood (samples, standards and controls) into the test tube.
3. Mix. Allow to rest for 5 minutes or until complete lysis is evident.
4. Stability of the hemolysate: 72 hours at 2-8 °C.

The following list highlights which I.S.E. S.r.l. systems require manual lysing and automatic lysing.

Systems	*Lysing Procedure*	*Procedure*
Miura 500 SA/DA	MANUAL	Follow Manual Lysing method
Miura 200 SA/DA	MANUAL	Follow Manual Lysing method
Miura One	MANUAL	Follow Manual Lysing method
Hemo One	AUTOMATIC (on board)	Included with protocols

General Assay Procedure

The I.S.E. S.r.l. HbA1c kit is designed and validated using the automated systems from I.S.E. S.r.l. Application sheets are available upon request for use with I.S.E. S.r.l. systems. All applications not explicitly approved by I.S.E. S.r.l. cannot be guaranteed in terms of performance and must therefore be established by the operator.
Wavelength λ=578nm.

Quality control

Human control serum with known levels of HbA1c is commercially available for quality control purposes. Data sheets are included, listing the values and the confidence limits. Normal and abnormal control sera are available from I.S.E. S.r.l. "Low control" code R0400000007 and "High control" code R0400000004. Obtained values must be within the range of acceptability.
If erratic results occur, please contact an authorised ISE representative.

Calibration Stability

For the instrumentation series, the calibration is recommended to be done every 15 days.

Conversion Formulas

Method NGSP unit: %HbA1c = 0,0915*(Ris. in mmol/mol) + 2,15%
Method IFCC unit: mmol/mol = 10,93*(Ris. in %) - 23.5mmol/mol

Normal Ranges

HbA1c	*%*	*mmol/mol*
Non Diabetics	< 6%	< 42.1 mmol/mol
Therapeutic Diabetics	> 7%	> 53.1 mmol/mol

The above values are an indication. Each laboratory should establish its own normal values based on its local population.

Performances

Linearity: 0 - 15 %.
Specificity: Under the conditions of the assay system this method is specific for HbA1c.
Accuracy-Recovery: The recovery of pure HbA1c added to normal sample at known titres was 101.7%.

Precision of the method

Condition	*U.M.*	*Low*	*Medium*	*High*
Inter-Run	CV%	1.42	0.90	1.49
Intra-Run	CV%	0.79	0.73	0.88

Accuracy of the method

Control	*U.M.*	*Measured*	*Assigned*
Low	%	5.58	5.1 – 6.9
High	%	13.47	11.8 – 16.0

Interferences: High lipid (3000 formazin turbidity units) or bilirubin (conjugated type 30 mg/dL, free type 30 mg/dL) counts in blood samples do not affect the test results.

I.S.E S.r.l.
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Manufactured for I.S.E. S.r.l. in Industriepark-West 58, 9100 Sint-Niklaas, Belgium

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Sensitivity: At λ=578nm sensitivity was calculated as 0.2%.
Comparative method: The I.S.E. S.r.l. method was compared to a method in use.
Comparison with ISE, 138C012: y = 1.0823x – 0.1887 / r = 0.9828
Stability at 2 - 8°C: at least 2 years after production.

Precautions and Warnings

1. In vitro diagnostic use only.
2. Refer to the safety data sheets (SDS) and take the necessary precautions for the use of laboratory reagents.
3. Do not use after expiry date and do not interchange reagents from different lots.
4. Replace caps on reagents immediately after use. Do not switch caps.
5. Do not pipet by mouth. Do not smoke, eat, drink or use cosmetics during the use of the reagent. Do not swallow.
6. Sodium azide has been reported to form lead or copper azide in laboratory plumbing which may explode on percussion. Flush drains whit water thoroughly after disposing of fluids containing sodium azide.
7. Each donor unit used in the preparation of the reagents, standards and controls was found to be negative for the presence of HIV1 and HIV2 antibodies, as well as for the hepatitis B surface antigen and anti-hepatitis C antibodies, using a method approved by the FDA. However, the material must be considered potentially hazardous and handled with the same care as samples taken from patients.
8. Cuts, abrasions, and other skin lesions should be properly protected with an appropriate waterproof dressing.
9. Take care to avoid self-inoculation, splashing of mucous membranes or generation of aerosols. Laboratory gloves should be worn while handling patients' samples or disposing of solid or liquid wastes.
10. In addition to the eventual risk indications regarding the active components, the reagents contain inactive components such as preservatives (e.g. sodium azide or others) and detergents. The total concentrations of these components are lower than the limits reported by the current directive sand following modification and amendments. However, it is recommended to handle reagents carefully, to avoid ingestion and contact with eyes, skin and mucus membranes and to use laboratory reagents according to good laboratory practice.
11. All human samples must be handled and disposed of as potentially infectious materials.

Disposal of reagent

Disposal of reagents must be performed in accordance with the EC regulations regarding waste, or the local national or regional legislation.
The product is in conformity with D.L: 8 September 2000, no. 332 "Actuation of the directive 98/79/EC regarding in vitro medical diagnostic devices".

Reporting of serious incidents

The user must report (through the distributor) any serious accident occurring in relation to the device to both the manufacturer and the competent authority of the European Union Member State in which the user and / or patient is established. For other jurisdictions, reports of serious incidents must be produced in accordance with regulatory requirements.

Symbols on labels and packaging

IVD	In vitro diagnostic medical device
REF	Catalog Number
LOT	Lot number
	Manufacturer
	Expiry date
	Temperature limitation
	Consult Instructions for use
Rn	Reagent "n"

References

1. Trivelli, L.A., Ranney, H.M., and Lai, H.T., New Eng. J. Med. 284, 353 (1971).
2. Gonen, B., and Rubenstein, A.H., Diabetologia 15, 1 (1978).
3. Gabbay, K.H., Hasty, K., Breslow, J.L., Ellison, R.C., Bunn, H.F., and Gallop, P.M., J. Clin. Endocrinol. Metab. 44, 859 (1977).
4. Bates, H. M., Lab. Mang., Vol 16 (Jan. 1978)

Revision history		
Rev.E	15-11-2022	Revision of the document

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