HbA1c – Immunoturbidimetric Assay

Instructions for use (IFU)

IVD CE

ORDERING INFORMATION

	Code	Composition
OPEN KONELAB INDIKO	REF B75182586	n° 2 vials x 20 mL R.A n° 2 vials x 7 mL R.B n° 2 vials x 100 mL (Lysing Reagent)
CHEMILAB	REF B82181041	n° 1 vial x 15 mL R.A n° 1 vial x 5 mL R. B

INTENDED USE

Immunoturbidimetric diagnostic test for the quantitative determination of the percentage of Hemoglobin A1c (HbA1c) on human whole blood. The measurement of HbA1c percentage is used for long-term monitoring of blood glucose in diabetic patients. All results should be interpreted in relation to the clinical context. FOR PROFESSIONAL USE ONLY.

CLINICAL SIGNIFICANCE

HbA1c is formed by the reaction of glucose with the N-terminal amino group of the hemoglobin beta chain. The Diabetes Control and Complications Trial (DCCT) research group has previously shown a relationship between HbA1c percentage and average blood glucose levels during the previous 2 to 3 months. The DCCT study also showed that long-term control of diabetes can prevent complications such as cardiovascular disease, retinopathy and neuropathy. Measuring the percentage of HbA1c is the method of choice for monitoring the therapy of diabetic patients.

PRINCIPLE OF THE METHOD

The HbA1c test uses the interaction between antigen and antibody to directly determine the concentration of HbA1c (%) in whole blood. Whole blood samples are treated with Lysing Reagent to lyse red blood cells. The lysed sample is then incubated with latex microparticles (reagent A). Hemoglobin and HbA1c are captured on microparticles. When the anti-HbA1c monoclonal (reagent) antibody (reagent B) is added, the latex complex - HbA1c - antibody is formed. The amount of agglutination is measured turbidimetrically and is proportional to the amount of HbA1c absorbed on the surface of the microparticles.

Storage and stability

= storage temperature 2-8°C

Stored at 2-8°C avoiding direct light, reagents are stable until the expiration date printed on the label. A slight variation in composition, from batch to batch, does not affect the test results. Do not mix reagents of different batches. Reagents should not be left on the analyser after use but should be stored refrigerated at 2-8°C. Latex can settle, stir gently before use.

Concentrations

Reagent A:		
	Conc.	**U.M.**
Latex microparticles	0,13	%
Stabilizers		
Reagent B:		
Mouse anti human HbA1c (monoclonal)	0,05	mmol/L
Anti mouse IgG (polyclonal)	0.08	mmol/L
Buffer		
Stabilizers		
Reagent C (Lysing Reagent)		
Buffer		
Stabilizers (Sodium Azide)	< 0,01	%

Reagents included in the kit

The reagent is described above.

Materials required but not supplied in the kit

Controls, calibrators and pipettes with adequate volume.

PRECAUTIONS and WARNINGS

1. Reagents and waste materials shall be disposed of in accordance with Community waste provisions or national or regional provisions.
2. Reagents may contain non-active components such as preservatives and detergents. The total concentration of these components is below the limits set out in Regulation 1272/2008 EC and subsequent amendments and additions.
3. It is recommended that the reagent be handled in accordance with the rules of good laboratory practice and that appropriate personal protective equipment be used.
4. Do not use the reagent if it is visibly degraded (e.g. presence of corpuscles).
5. All human samples shall be handled and disposed of as potentially infectious material.
6. The kit should only be used by qualified and properly trained technical personnel.
7. Diagnoses shall be carried out exclusively by authorised and qualified personnel.
8. Comply with national directives on occupational safety and quality assurance.
9. Use equipment that complies with current regulations.

Reporting of serious incidents

In the event of a serious incident in relation to the use of the device, please inform the manufacturer (via your distributor) and the competent authority of the European Union member state where the incident occurred. For other jurisdictions, reporting must be made in accordance with regulatory requirements. Reporting serious incidents helps provide more information about the safety of the diagnostic medical device.

PROCEDURE

Quality control

Use SCLAVO Diagnostics Int.: Low HbA1c Control B35182584 and High B35182585 at least once a day. Perform control analysis even after each calibration. **Controls after reconstitution must undergo the process of haemolysis**. The values obtained must be contained within the acceptability range.

ANALYTICAL TECHNIQUE

For automatic procedures, please refer to the user manual and application notes of the Konelab-Indiko®® analyzer. All applications not explicitly approved by Sclavo Diagnostics cannot be guaranteed in terms of performance and must therefore be evaluated by the user.

Calibration

For calibration use the HbA1C Sclavo Diagnostics B35182583 Calibrator. The calibrator values may vary from batch to batch as indicated on the instructions for use. Calibration is stable for up to 14 days. A new calibration is necessary in case of batch change or if a check is out of the acceptability range.

Traceability

HbA1c is traceable to the reference standards of the International Federation of Clinical Chemistry (IFCC) and NGSP (National Glycohemoglobin Standardization Program).

SAMPLE

Type of sample and storage

Blood obtained by normal medical technique can be used. No specific patient preparation is required. Only samples with the following substances may be used:

- EDTA dipotassium (K2- EDTA)
- Lithium heparin (Li-Heparin)

Other additives have not been tested for use with this kit.

Do not use samples: heat inactivated, mixed in pools, with obvious microbial contamination.

Fresh / Not frozen samples

- Do not spin
- Mix samples thoroughly before use

Frozen samples

- Defrost samples for a minimum of 30 minutes
- Thoroughly mix thawed samples by inversion
- Visually inspect the sample. If stratification is observed, continue mixing until they are visibly homogeneous.

Sample storage and stability

Whole blood samples are stable:

- 6 hours at room temperature
- 5 days at 2-8 ° C or 14 days at -20 ° C

Avoid more than one freeze/thaw cycle

The lysate (Hemolysate) is stable:

- 6 hours at room temperature
- 10 days at 2-8 ° C

If stored on board the instrument, the test must be performed within two hours.

Precautions

All human specimens should be treated as potentially infected specimens.

PREPARATION OF THE REAGENT

Reagents are ready-to-use liquids. After opening, the reagents are stable until their expiry date if maintained under the conditions indicated in "Storage and stability**".**

Automation

The kit can be used with all automatic analyzers that can meet the operating conditions of the reagent while maintaining the volumetric ratios R1 / R2 / C. Validated applications are available for Sclavo Konelab® - Indiko® and CHEMILAB instruments. Applications not approved by Sclavo Diagnostics do not guarantee the performance of the reagent and must therefore be approved under the responsibility of the user.

MANUAL METHOD

The kit, in Open format, can be used manually through the use of spectrophotometer or photometer with the following parameters:

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Reaction conditions

Wavelength: 600 nm (600-660)
Temperature: 37°C

	U.M.	Blank	Calibr. Serum	Sample
Reagent A	µL	360	10	360
Mix gently and incubate at 37° for 5 minutes, add:				
Reagent B	µL	120	10	120

Mix well and read the absorbance after 5 minutes. The reaction volumes can be varied proportionately, without altering the results.

Calculation of results

The results are calculated automatically by the analyzer using the calibration curve. The unit of measurement used is % of HbA1c. For alternative units, the following formulas shall be used:

% HbA1c a IFCC mmol / mol = [% HbA1c x 10,93] - 23.50
IFCC mmol / mol a % IFCC mmol / mol = [mmol / mol x 0,09148] + 2,152

REFERENCE RANGES

For monitoring diabetic patients, it is recommended that glycemic targets be identified according to the recommendations of the American Diabetes Association (ADA) summarized in the following table:

Value HbA1c	Glycemic Objective
64 mmol/mol (< 8%)	Not very stringent "Diabetic patient with poor glycemic control"
53 mmol/mol (< 7%)	General (non-pregnant adults) "Diabetic patient with good glycemic control"
48 mmol/mol (< 6.5%)	Very stringent "Normal non-diabetic patient"

As recommended by ADA, patients in the range of 5.7 - 6.4 % HbA1c (39 - 46 mmol/mol) may be placed in the category with increased risk of diabetes.

CHARACTERISTICS/PERFORMANCE

Measuring range

The method ensures a correct fit of the data in the range between the minimum detectable concentration and the maximum concentration of the calibrator. Samples above the measuring range **MUST NOT BE DILUTED**. These samples should be tested with alternative methods.

Accuracy

The accuracy of analytical results was determined in accordance with CLSI EP15-A2 protocol by analyzing the control samples. The data obtained with Konelab – Indiko series are shown in the table below (95% confidence interval):

Level	Replicates	Value	Mean (%)	SD	Recovery%
Low	15	6,00	5,98	0,14	99.7 %
High	15	11,10	13,07	0,10	117.7 %

Specificity

Hemoglobin (Hb) derivatives

Labile fractions of Hb, acetylated Hb and carbamylated do not interfere in this test. Two human whole blood samples with HbA1c concentrations of 6-7% and 8-9%, were tested in the presence of physiological concentrations of Sodium Cyanate, Acetylsalicylate, Glucose and Urea and no differences were detected compared to the reference samples (considering as acceptable a standard deviation < 10%).

Hemoglobin variants

The following variants do not interfere with the test: Hbs, HbC, HbD, HbA2, HbE. High levels of HbF can lead to an underestimation of HbA1c. Other variants have not been tested in dosage. However, caution should be exercised when interpreting HbA1c results from patients with elevated levels of Hb variants.

Limits of the procedure

- Hemoglobinopathies may interfere with the analysis of glycated hemoglobin. Common hemoglobin variants have been tested in this assay (see Specificity section).
- It's possible that other substances and / or factors not tested may interfere with the assay. (Refer to the interference section)

It has been reported that results may be inconsistent in patients who have the following conditions: opiate addiction, lead-poisoning, alcoholism, ingest large doses of aspirin.
- This test is not intended for:
- The diagnosis of diabetes
- It should not be used to replace daily home testing of urine and blood glucose levels.
- Samples from patients with total hemoglobin <8 g/dL may be partially negative
- Analysis of samples from patients with conditions causing shortened red blood cells survival time such as hemolytic anemia, or other hemolytic diseases, significant acute or chronic blood loss or pregnancy.

Interference

The influence on the analytical response of the following potentially interfering substances has been tested up to the following concentrations:
Bilirubin 38 mg/dL, Ascorbic acid 50 mg/dL, Triglycerides 1000 mg/dL, Rheumatoid Factor 600 IU/mL, Total Protein 14 g/dL. Higher concentrations have not been tested. Caution should be exercised when analysing samples from patients who have received murine monoclonal antibody preparations for diagnosis or therapy as they may contain anti-mouse antibodies (HAMA). Samples containing HAMA may give unexpected values during the test both in this assay and in all those using mouse antibodies.

Precision

The accuracy of the analytical results was determined in terms of repeatability and total accuracy according to the CLSI EP15-A2 protocol, analyzing commercial control sera. The data obtained are shown in the following table (95% confidence interval determinations.

Within-run precision – Repeatability				
Level	**Replicated**	**Media (%)**	**DS**	**CV%**
Low	20	5.95	0.19	3.2
High	20	12.15	0.18	1.47
Total accuracy (Within-lab precision)				
Level	**Replicated**	**Media (%)**	**DS**	**CV%**
Low	20	5.97	0.14	2.31
High	20	12.21	0.15	1.24

Sensitivity Limit

The minimum measurable quantity is 1% HbA1c (0.056 A)

Method comparison

The test method was compared with another commercially available method according to CLSI EP09-A2-IR protocol, analyzing 40 samples. The correlation data between the two methods are shown in the table below.

Parameter	Estimation
Intercepts	-0.047
Incline	0.989
Correlation Coeff. (R)	0.995

Symbols used in IFU and Packaging	
IVD In vitro diagnostic medical device vitro	Manufacturer
REF Catalogue Number	Instruction for use
LOT Lot Number	Temperature limitation
Expiration date	

REFERENCES

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REVISION	DATE	CHANGE
Rev.A	01/2023	New Issue for IVDR Regulation (UE) 2017/746 compliance

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