TECO DIAGNOSTICS
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HEMOGLOBIN A1C (HBA1C) TURBIDIMETRIC REAGENT SET

TC MATRIX 160

INTENDED USE

The Teco HbA1c assay is used in clinical laboratories for the quantitative in vitro measurement of HbA1c (hemoglobin fraction) in human whole blood on auto analyzers.

SUMMARY AND EXPLANATION OF TEST[1,2,3]

The HbA1c assay is used as an aid in the monitoring of long-term blood glucose control and compliance in individuals with diabetes mellitus.

The Teco HbA1c assay is used for the measurement of the concentration of HbA1c relative to the concentration of the total hemoglobin (THb).
An increase in the percentage of HbA1c has been found in individuals diagnosed with diabetes. Uncontrolled diabetes can lead to acute complications of hyperglycemia and ketosis. In addition, long-term complications such as cardiovascular disease, retinopathy, nephropathy, and neuropathy can occur. According to several studies, including the findings of diabetes Control and Complications Trial (DCCT), these complications can be prevented by long-term control of diabetes.

Therefore, measurement of HbA1c percentage can be invaluable in the monitoring of long-term glycemic control of diabetic patients.
The Teco HbA1c assay has no cross reactivity with labile HbA1c since the antibody used in this assay is specific for the ketoamine form of HbA1c. Stable HbA1c does not increase or decrease in response to rapid changes in physiological factors and therefore allows the measurement of individuals' average blood glucose levels over several months.

PRINCIPLE[4,5,6]

Hemoglobin A1c is an important test recommended by the American Diabetes Association (ADA) and its usefulness was clarified by the United Kingdom Prospective Diabetes Study (UKPDS) and Diabetes Control and Complications Trial (DCCT). Currently, the HbA1c test is recommended for patients with diabetes every 2-3 months as part of the patient diabetes management program. Glycohemoglobin is produced by non-enzymatic addition of glucose to amino groups in hemoglobin. HbA1c refers to glucose modified hemoglobin A (HbA) specifically at N-terminal valine residues of hemoglobin beta chains. HbA1c test is used both as an index of mean glycemia and as a measure of risk for the development of diabetes complications. Therefore, the HbA1c test is a good indicator of glycemic control in the preceding 2-3 months.

This method utilizes the interaction of antigen and antibody to directly determine the HbA1c in whole blood. Total hemoglobin and HbA1c have the same unspecific absorption rate to latex particles. When mouse antihuman HbA1c monoclonal antibody is added (R2), latex-HbA1c- mouse anti human HbA1c antibody complex is formed. Agglutination is formed when goat antimouse IgG polyclonal antibody interacts with the monoclonal antibody. The amount of agglutination is proportional to the amount of HbA1c absorbed on to the surface of latex particles. The amount of agglutination is measured as absorbance.

TEST PARAMETERS

Method : Immunoturbidimetric
Wavelength : 660 nm (Sub: Optional 800nm)
Linearity :15%

REAGENTS

Lyse Reagent: Stabilizers, Buffers, lysing agent, water
R1: Latex <0.15%, Buffer stabilizers
R2: Mouse anti-human HbA1c monoclonal antibody <0.06mg/ml, goat anti- mouse IgG polyclonal antibody <0.09mg/dl, buffer, stabilizers.

REAGENT PREPARATION

Reagents are ready for use.

STORAGE AND STABILITY[7]

1. Reagents are stable at 2-8°C until the expiration date stated on the label which is only for closed vials.
2. Once opened vials are stable for 30 days at 2-8°C in optimum conditions. On board stability is strongly related to auto analyzers' cooling specification and carry-over values
3. Reagent stability and storage data have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.

SPECIMEN COLLECTION AND PREPARATION

The assay is formulated for use with human whole blood samples. Venous whole blood samples collected with EDTA anticoagulant can be used. It is recommended that samples be used within 7 days of collection when stored and refrigerated.

Prior to testing, whole blood samples should be mixed by gentle inversion to re-suspend settled erythrocytes.

Auto analyzer usage: Samples should be tested by stat mode (Emergency mode) to avoid precipitation.

INSTRUMENTS

Refer to specific instrument application for suggested settings.

PRECAUTIONS

1. This reagent is for *in vitro* diagnostic use only.
2. Not for internal or external use in humans or animals.

MATERIALS PROVIDED

Refer to “Reagents”

MATERIALS REQUIRED BUT NOT PROVIDED

Pipettes, Test Tubes, Hemoglobin A1c calibrator set, control set.

PREPARATION OF HEMOLYSATE

1. Whole blood samples are taken to room temperature,
2. Blood samples are mixed in order to mix erythrocytes homogeneously,
3. Using a calibrated pipette, transfer 1000 uL Lyse solution to the sample cup,
4. 40 uL of homogenized blood sample is transferred to the sample cup with Lyse added,
5. Hemolysate is mixed thoroughly, incubated for 5 minutes at room temperature,
6. Hemolysate is ready for use for HbA1c.

PROCEDURE

Test Name:	HbA1c	R1:	122
Full Name:	HbA1c	R2:	37
Pri. Wave:	630 nm	Sample volume:	2
Sec. Wave:	/	Calibration Type:	Spline
Assay/ Point:	Fixed Time	K Value:	/
Start - End:	14 - 23	Point:	5
Decimal place:	2	Blank Type:	Reagent
Unit:	%	Point 0 (Blank) .:	calibrator/ standard
Linearity Range:	0.2 - 15.00	Point 1 (Cal) Con.:	calibrator/ standard
Correlation Factor:	1.0000 - 0.0000	Point 2 (Cal) Con.:	calibrator/ standard
(For Manual Dilution)	0.019 - 0.000	Point 3 (Cal) Con.:	calibrator/ standard
		Point 4 (Cal) Con.:	calibrator/ standard

For manual dilution sample Volume = 2, Dilution Volume =100

LIMITATIONS

- THE linearity of the assay is up to 15% HbA1c. Samples with values above 15% should not be diluted and retested. Instead the values should be reported as higher than 16% (>16%).
- It has been observed that patients who have alcoholism, high dose of acetyl salicylic acide, opiate and lead poisoning may lead to inconsistency.
- The assay is formulated for use with human whole blood samples in EDTA.
- Elevated levels of HbF may lead to insufficient evaluation of HbA1c and uremia does not interfere with HbA1c determination by immunoassay.

QUALITY CONTROL

The Teco Turbidimetric HbA1c assay requires monthly calibration. Place calibration series on the analyzer in the order of lowest to highest. Enter calibrator lot specific values provided on the specification sheet.

All calibrator vials are stable until their expiration date when stored at 2-8°C. Teco HbA1c calibrator set is in lyophilized form. Teco HbA1c calibrator set for the auto analyzers on-board Lysis Application includes four levels of calibrator material. Levels 1-4 are in lyophilized form. Reconstitute the lyophilized contents according to the directions on each label and mix gently. Let the vials equilibrate at room temperature for 30 minutes before use. Reconstituted calibrators are stable for 30 days when capped tightly and stored at 2-8°C.

Commercially available control material with established values determined by this method may be used.

CONVERSION FORMULA:

NGSP%= [0.09148 x (IFCC)] + 2.152

EXPECTED VALUES[8]

Expected Values : 4.5 - 6.5% (NGSP/DCCT)
Expected Values : 26 - 48 mmol/mol (IFCC)

Levels above 6.5% HbA1c are suitable for the diagnosis of diabetes mellitus according to the data provided by NGSP. Patients with levels between 39-46 mmol/mol (IFCC) or 5.7% - 6.4% HbA1c (NGSP) have a possibility of developing diabetes risk.[9, 10]

It is recommended that each laboratory establish its own normal range. Reference interval has been verified by using CLSI EP28- A3c protocol.

PERFORMANCE

1. The following HbA1c value data were obtained by comparing Teco Direct Turbidimetric HbA1c assay to a legally marketed HPLC method.

Whole blood application	
n	100
Slope	1.001
Intercept	0.027
Correlation Coefficient	0.990
Range of Values	5%-14% HbA1c

2. **Limit of Quantitation** (LoQ) [LoQ values are based on Coefficient of Variation Percentage (CV) 20%][11]: 4%.
   LoQ value has been verified by using CLSI EP17-A protocol.

3. **High Linearity**: The method is linear up to 15.0%.
   Linearity may vary considerably depending on the instrument used.

4. **Precision Studies**:[12]
   Repeatability (Within Run) (Intra-Assay)

Mean Concentration	CV %	n
Low 5.46	1.45	80
High 10.1	1.73	80

   Repeatability (Day to Day) (Inter-Assay)

Mean Concentration	CV %	n
Low 5.46	2.81	80
High 10.1	2.72	80

   Precision Studies data have been verified by using CLSI EP05-A3 protocol.

5. **Interferences:**[3,4,15]
   No significant interactions were observed for Conjugated Bilirubin and Triglycerides up to the interferent concentration given in the table.

Total bilirubin	48 mg/dL
Triglycerides	2000 mg/dL

   Stable glycated hemoglobin serves as a substrate for the Turbidimetric reaction used in Teco Direct Turbidimetric HbA1c assay.

   The acceptable interference limit is set 10% below the highest interference concentration within + 10% recovery of the target.

   Interferences may affect the results due to medication or endogenous substances.

   These performance characteristics have been obtained by using an analyzer. Results may vary if a different instrument or a manual procedure is used.

Warning and precautions

IVD: For in Vitro Diagnostic use only. Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines. Contains sodium azide.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA standards.

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