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Direct Enzymatic HbA1c Assay

Configuration

The Diazyme Direct Enzymatic HbA1c reagent is provided in bulk and in the following kit configurations:

| Instrument | Catalog No. | Kit size |
|------------------------------------------|-------------|----------------------------------------------------------------------------------------------------|
| Universal OlympusAU400 Hitachi 917 | DZ168A-K | Lysis Buffer 1 x 30 mL R1a: 1 x 16.8 mL R1b: 1 x 7.2 mL R2: 1 x 10 mL Cal: DZ168A-CAL* |
| | DZ168A-KY2 | Lysis Buffer 2 x 60 mL R1a: 2 x 33 mL R1b 2 x 14.1 mL R2: 2 x 19 mL Cal: DZ168A-CAL* |
| | DZ168A-K01 | Lysis Buffer 1 x 30 mL R1a: 1 x 15.4 mL R1b 1 x 6.6 mL R2: 1 x 8.0 mL Cal: DZ168A-CAL* |
| Beckman CX/LX | DZ168A-KB1 | Lysis Buffer 1 x 20 mL R1a: 1 x 15 mL R1b 1 x 5 mL R2: 1 x 5.5 mL Cal: DZ168A-CAL* |
| Cobas Mira On-Board Lysis | DZ168A-K | Lysis Buffer 1 x 30 mL R1a: 1 x 16.8 mL R1b: 1 x 7.2 mL R2: 1 x 10 mL Cal: DZ168A-SL4* |
| | DZ168A-KY2 | Lysis Buffer 2 x 60 mL R1a: 2 x 33 mL R1b 2 x 14.1 mL R2: 2 x 19 mL Cal: DZ168A-SL4* |

* Note: Calibrators Sold Separately

Intended Use

Diazyme Direct Enzymatic Hemoglobin A1c (glycated hemoglobin A1c; A1c; HbA1c) reagents are intended for use in the quantitative determination of stable HbA1c in human whole blood samples. Measurement of hemoglobin A1c is a valuable indicator for long-term diabetic control. For *in-vitro* diagnostic use only.

Clinical Significance

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.¹⁻³ Therefore, the HbA1c test is a good indicator of glycemic control in the preceding 2-3 months.

Assay Principle

Direct Enzymatic HbA1c test is an enzymatic assay in which lysed whole blood samples are subjected to extensive protease digestion with *Bacillus sp* protease. This process releases amino acids including glycated valines from the hemoglobin beta chains. Glycated valines then serve as substrates for specific recombinant fructosyl valine oxidase (FVO) enzyme, produced in *E. coli*. The recombinant FVO specifically cleaves N-terminal valines and produces hydrogen peroxide. This, in turn, is measured using a horseradish peroxidase (POD) catalyzed reaction and a suitable chromagen.

The HbA1c concentration is expressed directly as %HbA1c by use of a suitable calibration curve in which the calibrators have values for each level in %HbA1c.

Reagent Composition

| | Concentration |
|---------------------|---------------|
| <u>Lysis Buffer</u> | |
| CHES, pH 8.7 | 100 mM |
| Triton-X-100 | 1 % |
| SDS | 0.45 % |
| Redox Agents | 0.5 mM |
| <u>Reagent R1a</u> | |
| MES pH 7.0 | 5 mM |
| Proteases | 4 KU/mL |
| Triton-X-100 | 0.5% |
| Redox agents | >10µM |
| <u>Reagent R1b</u> | |
| MES pH 6.3 | 1 mM |
| Redox agent | <3 mM |
| <u>Reagent R2</u> | |
| Tris pH 8.0 | 15 mM |
| FVO enzyme | >10 U/mL |
| POD | 90 U/mL |
| Chromagen | 0.8 mM |

Materials Required but not Provided

- 1) HbA1c calibrator set (DZ168A-CAL): Intended for use only with Direct Enzymatic HbA1c Assay reagents (DZ168A).
- 2) HbA1c calibrator set (DZ168A-SL4): Intended for use only with Direct Enzymatic HbA1c Assay reagents (DZ168A) on the Cobas Mira on-board lysis application.

- 3) Bi-level HbA1c controls (DZ168A-CON): Whole blood hemolysates and stabilizers.
- 4) Direct Enzymatic HbA1c assay blank solution (DZ168A-S0V): Intended for use with Direct Enzymatic HbA1c Assay reagents with instruments that require a zero calibrator.

Reagent Preparation

For analyzers capable of handling 3-reagents, R1a, R1b, R2 are ready to use. For analyzers capable of handling only 2-reagents, Diazyme HbA1c reagents R1a and R1b should be mixed in a 7:3 ratio and allowed to sit at 2-8°C for 24 hours prior to use. To prepare sufficient R1ab mixture, pour the entire contents of R1b bottle into R1a bottle. Mix gently by inversion.

Reagent Stability and Storage

Reagents are stable until their expiration date when stored at 2-8°C. Reconstituted R1ab thus prepared is stable for 4 weeks when stored at 2-8°C. **R1b and R2 reagents are light sensitive.**

Specimen Collection and Handling

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 2 weeks of collection when stored refrigerated.⁴ Prior to testing, whole blood samples should be mixed by gentle inversion to re-suspend settled erythrocytes.

Note: Human specimens and all materials that are in contact with samples should be handled and disposed of according to local and national laws and as if such samples are capable of transmitting infection.

Precautions

- 1) Reagent R1b and R2 are light-sensitive. Store in a dark place.
- 2) Specimens containing human sourced materials should be handled as if potentially infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories (HHS Publication Number [CDC] 93-8395).
- 3) As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
- 4) Avoid ingestion and contact with skin and eyes. See Material Safety Data Sheet.
- 5) Do not use the reagents after the expiration date labeled on the outer box.
- 6) Additional safety information concerning storage and handling of this product is provided within the Material Safety Data Sheet for this product. To obtain an MSDS, please contact our customer service department at 858-455-4768.

Assay Procedure

Whole Blood Bench Top Lysis Procedure

- 1) Dispense 250 µL of Lysis reagent in a sample cup or an Eppendorf microfuge tube.
- 2) Prior to testing, whole blood samples should be mixed by gentle inversion at least 5 times to resuspend settled erythrocytes. **Accuracy of the assay will be affected if whole blood is not**

thoroughly mixed prior to testing. Add 20 µL of fully resuspended whole blood sample. Mix gently with a suitable pipettor without creating foam and incubate at room temperature (25°C) for 10 min to completely lyse the red blood cells. Complete lysis is observed when the mixture becomes a clear dark red solution without any particulate matter. Incubate the samples longer as needed to ensure complete hemolysate preparation. The lysate, thus prepared, is ready for use in the Direct Enzymatic HbA1c assay steps and is stable up to 4 hours at room temperature.

- 3) The calibrators and controls should be treated exactly as patient samples and used per instructions on labeling.
- 4) Direct Enzymatic HbA1c assay reagents are comprised of redox balanced components. **Mixed reagent blank or water as blank sample should not be used in this assay.** For instruments that require a zero calibrator, please order Direct Enzymatic HbA1c Assay blank solution (DZ168A-S0V).

Whole Blood On Board Lysis Procedure (For Cobas Mira Only)

- 1) Load Direct Enzymatic HbA1c assay reagents (Lysis Buffer, R1a, R1b, R2) on assigned positions on Cobas Mira reagent tray.
- 2) Prior to testing, whole blood samples should be mixed by gentle inversion at least 5 times to resuspend settled erythrocytes. **Accuracy of the assay will be affected if whole blood is not thoroughly mixed prior to testing.** Carefully add at least 50 µL of fully resuspended whole blood sample into Cobas Mira sample tube without creating foam or bubble and load on Cobas Mira sample tray. Start to run within 5 minutes from adding the first whole blood sample into sample tube. **Do not test more than 10 samples in one run, due to settlement of the red blood cells.**
- 3) The calibrators and controls should be treated exactly as patient samples and used per instructions on labeling.

Calibration

The Diazyme Direct Enzymatic HbA1c assay requires weekly (168 hours) calibration. Place calibration series on the analyzer in the order of lowest to highest. Enter calibrator lot specific values provided on the specification sheet.

Diazyme Direct Enzymatic HbA1c calibrator sets are intended for use with Hemoglobin A1c enzymatic assay reagents (Cat number: DZ168A). All calibrator vials are stable until their expiration date when stored at 2-8°C. Diazyme HbA1c calibrator set (DZ168A-CAL) is in lyophilized form. Diazyme HbA1c calibrator set for the Cobas Mira On-Board Lysis Application (DZ168A-SL4) includes four levels of calibrator material. Level 0 is in liquid form and ready to use, levels 1-3 are in lyophilized form. Reconstitute lyophilized contents per instructions on labeling and mix gently. Let the vials equilibrate at room temperature for 30 minutes before use. Reconstituted calibrators are stable for 14 days when capped tightly and stored at 2-8°C. The liquid form calibrator zero is stable for 14 days after opening the vial when capped tightly and stored at 2-8°C.

Quality Control

Diazyme Direct Enzymatic HbA1c control set (DZ168A-CON) can be purchased separately. Users should follow the appropriate federal, state and local guidelines concerning the running of external quality controls and handling of bio-hazardous material.

To ensure adequate quality control, level 1 and level 2 controls with known values should be run as unknown samples.

Results

The HbA1c concentration is expressed directly as %HbA1c by use of a suitable calibration curve in which the calibrators have values for each level in %HbA1c. The values reported are aligned with the Diabetes Control and Clinical Trials (DCCT) system and hence reported in the NGSP⁸ format. No calculation step is needed.

The International Federation of Clinical Chemistry (IFCC) values can be calculated by use of published^{10, 11} conversion formula: NGSP = [0.915 x (IFCC)] + 2.15.

Reference Range

Non-diabetic individuals have HbA1c values in the range of 3-6% and controlled diabetic individuals have HbA1c values in the 6-9% range.⁹ Individuals with uncontrolled diabetes can have HbA1c as high as 20%. The American Diabetes Association (ADA) recommends that the primary treatment goal in diabetes should be glucose control equal to that achieved during the DCCT.⁵ Based on DCCT, ADA states HbA1c targets of <7%.⁵⁻⁷ However, each laboratory must establish its own normal range in their country of business taking into account sex, age and ethnicity.

Limitations

- The linearity of the assay is up to 16% HbA1c. Samples with values above 16% should not be diluted and retested. Instead the values should be reported as higher than 16% (>16%).
- The assay is formulated for use with human whole blood samples in EDTA. Total hemoglobin in the sample should be in the range: 9-21 g/dL
- High HbF (>10%) may result in inaccurate HbA1c values.

Performance Characteristics

(Determined on Hitachi 917 chemistry analyzer)

Accuracy

The following HbA1c value data were obtained by comparing Diazyme Direct Enzymatic HbA1c assay to a legally marketed HPLC method.

| | Whole blood application |
|-------------------------|-------------------------|
| <i>n</i> | 44 |
| Slope | 1.0212 |
| Intercept | 0.0135 |
| Correlation coefficient | 0.9874 |
| Range of values | 5% - 13% HbA1c |

Precision

Precision studies were conducted with the Diazyme Direct Enzymatic HbA1c assay reagents. Within-run and total precision studies were done by testing 2 levels of samples per NCCLS EP-5 procedure. Precision data is summarized in the table below:

| | Level 1 (%HbA1c) | Level 2 (%HbA1c) |
|-----------------------|---------------------|---------------------|
| Mean value | 5.7% | 10.3% |
| Within run SD (Swr) | 0.06 | 0.07 |
| Within run CV% | 1.0% | 0.7% |
| Inter assay precision | 0.10 | 0.18 |
| Inter assay precision | 1.8% | 1.8% |

Linearity

Diazyme HbA1c assay has a linear range from 4.0% - 16.0%.

Interference

The assay is not affected by the following interfering substances at the indicated concentrations: ascorbic acid 12 mg/dL, total bilirubin 15mg/dL, bilirubin (conjugated) 13mg/dL, glucose 4000mg/dL, triglyceride 4000mg/dl, uric acid 30 mg/dL, urea 80mg/dL. Stable glycated hemoglobin serves as a substrate for enzymatic reaction used in the Diazyme Direct Enzymatic HbA1c assay. Acetylated, carbamylated and labile HbA1c does not adversely affect the enzymatic reaction used in this assay. Variant hemoglobin S, C and E do not significantly interfere with Diazyme Direct Enzymatic HbA1c assay.

References

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