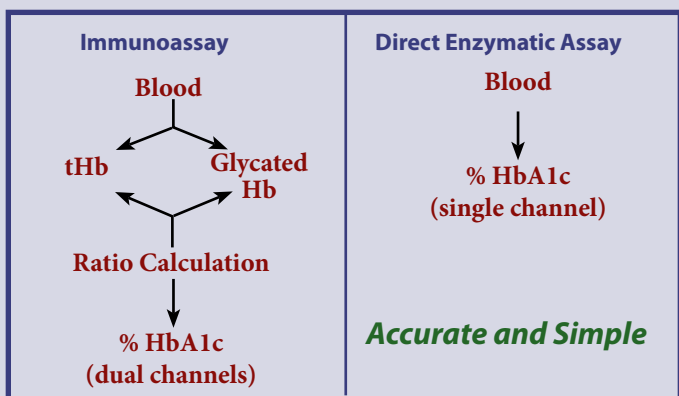


Direct Enzymatic HbA1c Assay™



Advantages:

- Two reagents, liquid stable
- Single channel
- Cost Effective
- No need for total hemoglobin measurement
- Applicable to most analyzers
- Excellent correlation with HPLC and immunoassays
- No interferences from hemoglobin variants
- On-board blood lysis possible



Direct Enzymatic HbA1c Assay™

1. Introduction

Hemoglobin A1c (HbA1c) is an important test recommended by the American Diabetes Association (ADA) and other diabetes organizations worldwide, for management of patients with diabetes mellitus. Currently, there are several methods available for measuring HbA1c in blood, the HPLC, immunoassay and enzymatic assay methods. A majority of the assays require dual channel testing such that separate tests are required, for glycated hemoglobin (GHb) and for total hemoglobin (THb). The final %HbA1c value is expressed as a ratio of the specific GHb in relation to the THb found in the whole blood sample. This type of dual channel testing is usually achieved by running both assays in parallel on chemistry analyzers and utilizing a calculation program to present final data in % HbA1c.

Recent innovation has yielded a Direct Enzymatic HbA1c Assay™ which uses a single channel test and reports %HbA1c values directly, without the need for a separate THb test or a calculation step.

2. Assay Principle

Oxidizing agents in the lysis buffer react with the whole blood sample to eliminate low molecular weight and high molecular weight signal interfering substances. After lysis, the whole blood samples are subjected to extensive proteolytic digestion. This process releases amino acids, including glycated valines, from the hemoglobin beta chains. The

glycated valines serve as substrates for a specific recombinant fructosyl valine oxidase (FVO) enzyme. The recombinant FVO specifically cleaves N-terminal valines and produces hydrogen peroxide in the presence of selective agents. This, in turn, is measured using a horseradish peroxidase (POD) catalyzed reaction and a suitable chromagen. The signal produced in the reaction is used to directly report the %HbA1c of the sample using a suitable linear calibration curve expressed in %HbA1c. The Direct Enzymatic HbA1c Assay principle is depicted in Figure 1 below.

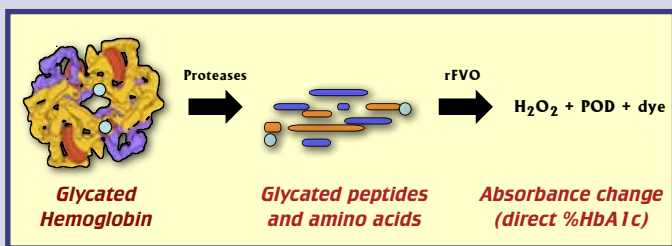


Figure 1

3. Assay Advantages

The Direct Enzymatic HbA1c Assay™ has all the advantages of both the HPLC and immunoassays methods in the areas of accuracy, specificity, applicability to chemistry analyzers and yet is cost effective, simpler and has less interferences. The direct enzymatic HbA1c test uses two ready-to-use liquid stable reagents. Since it does not require a separate measurement of total hemoglobin content in the samples, the Direct Enzymatic HbA1c Assay™ only needs a single channel to perform the test on chemistry analyzers in comparison with some immunoassays that require a separate measurement of total hemoglobin and need two channels for the test on chemistry analyzers as shown in the Figure 2 below.

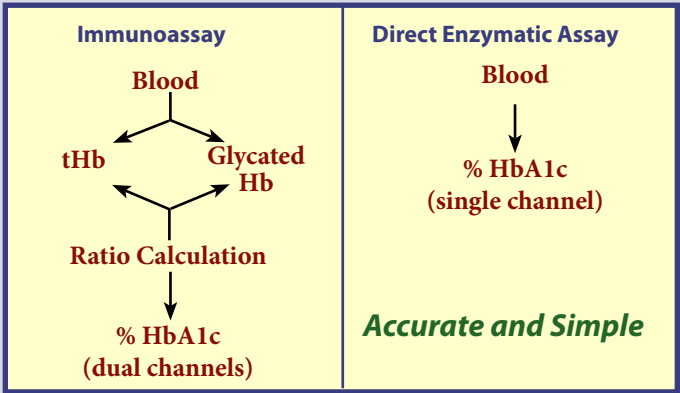


Figure 2

The Direct Enzymatic HbA1c Assay™ procedure is simple and straight forward. After addition of Reagent R1, sample and Reagent R2, the result of %HbA1c will be reported within 2 min as shown in the Figure 3 below.

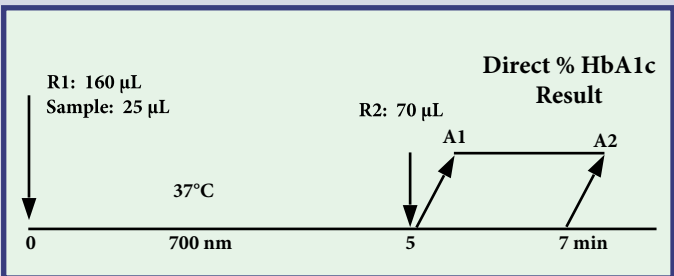


Figure 3

In addition, the reagents do not contain latex particles, and hence do not coat analyzer cuvettes and lines. Most importantly, enzymatic HbA1c assays have the highest specificity among all HbA1c assays as shown in the Tables 1 and 2.

Table 1. Effect of Chemically Modified Hemoglobin Variants

| Modification | Methods Affected | Methods Unaffected |
|---------------------|---------------------------------|--------------------------------------|
| Carbamyl Hb | HPLC, up to 300% errors | Enzymatic HbA1c assay Immunoassay |
| Labile fraction | Some HPLC and some Immunoassays | Enzymatic HbA1c assay |

Table 2. Effects of Genetic Hemoglobin Variants

| Variant | Methods Affected | Methods Unaffected |
|--------------------|---|------------------------------------|
| Hb C | Immunoassay, HPLC assay, up to 40% errors | Enzymatic HbA1c assay |
| Hb S | Immunoassay, HPLC assay, up to 40% errors | Enzymatic HbA1c assay (<10% error) |
| Hb Graz | Immunoassay, HPLC assay, up to 30% errors | Enzymatic HbA1c assay |
| Hb Sherwood Forest | Immunoassay, HPLC assay, up to 30% errors | Enzymatic HbA1c assay |
| Hb D | Immunoassay, HPLC assay, up to 30% errors | Enzymatic HbA1c assay |
| Hb O padova | Immunoassay, HPLC assay, up to 30% errors | Enzymatic HbA1c assay |

As shown in Tables 1 and 2, enzymatic HbA1c assays are not interfered by either chemical or genetically modified hemoglobin variants. Therefore, enzymatic Hb1c tests are reliable tests, and it does not report false results regardless of the patient's

hemoglobin variant types. In summary, the Direct Enzymatic HbA1c Assay™ offers the following advantages over HPLC and Immunoassays:

- Two reagents, liquid stable
- No need for total hemoglobin measurement
- Single channel on analyzers
- Faster, simpler and more cost effective
- No interferences from hemoglobin variants
- On-board blood lysis possible
- Applicable to most analyzers
- Excellent correlation with HPLC and immunoassays

4. Performance Characteristics

Below is a brief summary of the performance characteristics of the Direct Enzymatic HbA1c Assay™.

4a. Assay Precision

Table 3. CV% of Intra Precision

| | Level 1 (% HbA1c) | Level 2 (% HbA1c) |
|------|------------------------------|------------------------------|
| N | 20 | 20 |
| Mean | 5.7% | 10.3% |
| SD | 0.06 | 0.07 |
| CV% | 1.0 | 0.7 |

Table 4. CV% of Inter Precision

| | Level 1 (% HbA1c) | Level 2 (% HbA1c) |
|------|------------------------------|------------------------------|
| N | 80 | 80 |
| Mean | 5.7% | 10.3% |
| SD | 0.10 | 0.18 |
| CV% | 1.8 | 1.8 |

3b. Correlation with HPLC and Immunoassays:

The Direct Enzymatic HbA1c Assay™ has been compared with Tosoh's HPLC assay and Roche's immunoassay with 100 of random patient blood samples. As shown in the figures below, the HbA1c results obtained by Direct Enzymatic HbA1c assay exhibited excellent correlation with those obtained by HPLC method and immunoassay method with correlation coefficient r^2 values ranging from 0.96-0.98 (Figures 4 and 5).

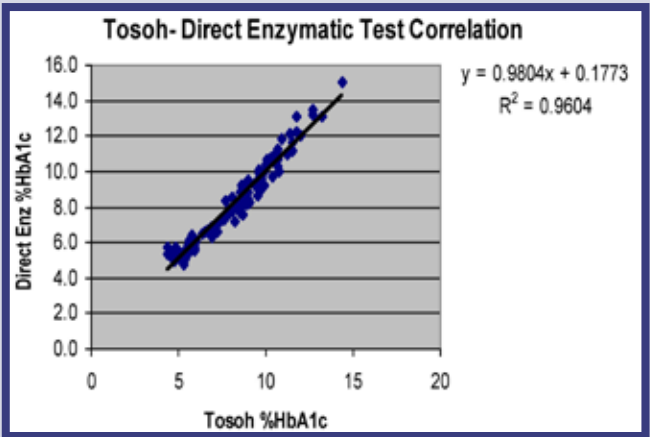


Figure 4. Correlation with HPLC

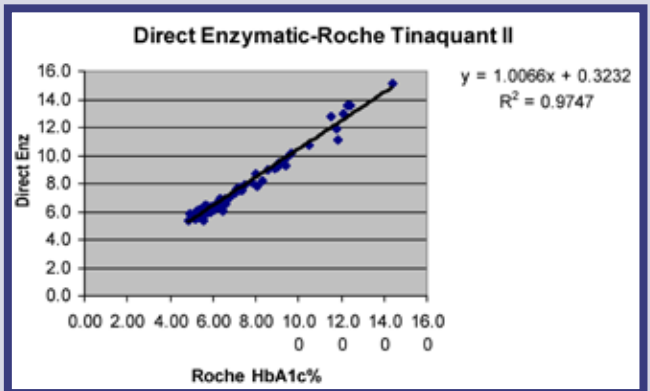


Figure 5. Correlation with Immunoassay

The Direct Enzymatic HbA1c Assay™ has an assay linearity range from 4 to 16% of HbA1c as shown in Table 5 and Figure 6.

Table 5. Assay Linearity of Direct Enzymatic HbA1c Assay™

| Expected %HbA1c | Recovered %HbA1c |
|-----------------|------------------|
| 4.0% | 3.9% |
| 7.0% | 7.2% |
| 10.0% | 10.2% |
| 13.0% | 13.2% |
| 16.0% | 16.2% |
| 21.9% | 21.6% |

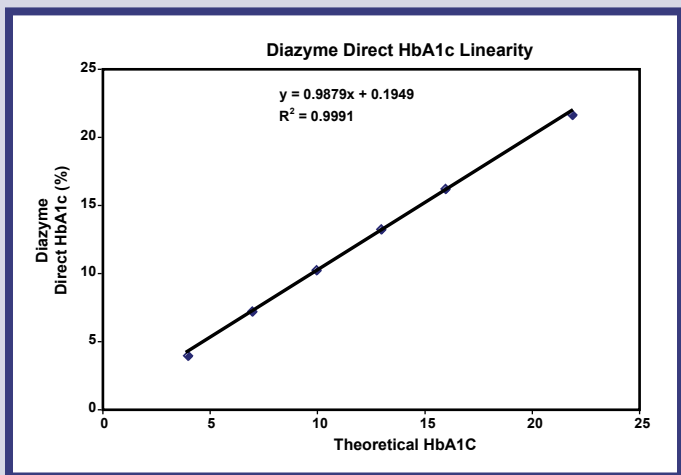


Figure 6

3d. Interferences

Direct Enzymatic HbA1c testing is not adversely affected by HbS, C and E variants in samples. For this study, variants in samples were confirmed by Primus HPLC analysis at the Diabetes Diagnostic lab (University of Missouri). These frozen samples were tested with Direct Enzymatic HbA1c Assay™ and results were compared with those obtained by HPLC and immunoassay methods as shown in Figures 7 and 8.

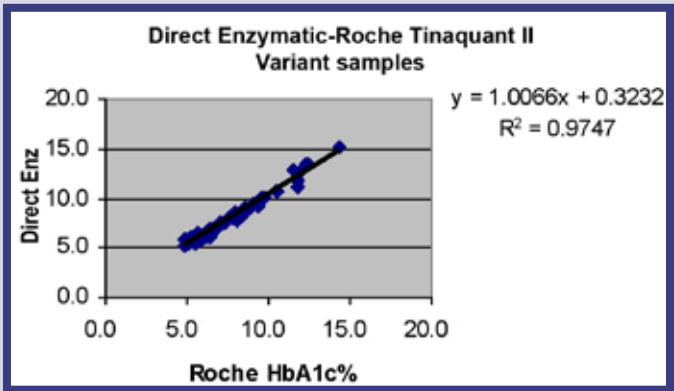


Figure 7

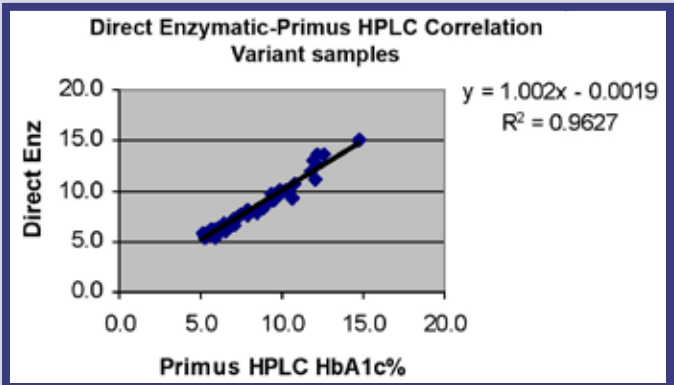


Figure 8

However, several samples were identified to be incorrectly value reported by the TOSOH HPLC method. These samples (shown in table below) were accurately tested with the Direct Enzymatic HbA1c Assay™ reagents and by the Roche Tinaquant II assay reagents.

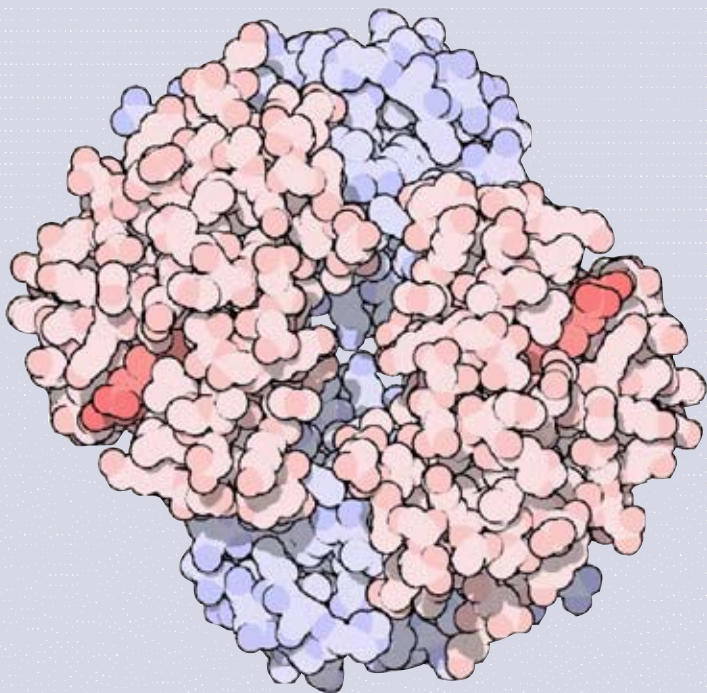
| Sample | TOSOH Test | Roche Tinaquant II | Direct Enzymatic |
|---------------|-------------------|---------------------------|-------------------------|
| 10924315 | 13.4% | 6.4% | 6.9% |
| 10923294 | 10.8% | 6.4% | 6.5% |

In summary, the Direct Enzymatic HbA1c Assay™ is not only simple in assay procedure but also more accurate in assay results. Therefore, the Direct Enzymatic HbA1c Assay™ is a better assay over the immunoassay and the HPLC assay, and is highly recommended for use in all clinical laboratories. Application parameters for most of chemistry analyzers are available.

For more detailed product information, please contact Diazyme Laboratories by email at: support@diazyme.com or by phone at 858-455-4768.



HbA1c Molecule



Please contact us at:

Diazyme Laboratories
12889 Gregg Ct.
Poway, CA 92064

Tel: 001-858-455-4768
Fax: 001-858-455-3701
www.diazyme.com

