

## Intended Use

For the quantitative determination of Hemoglobin A1c (HbA1c) in human blood using the Mindray BS-200 analyzer. 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 *in vitro* diagnostic use only.

## Summary and Explanation of Test

Throughout the circulatory life of the red cell, Hemoglobin A1c is formed continuously by the adduction of glucose to the N-terminal of the hemoglobin beta chain. This process, which is non-enzymatic, reflects the average exposure of hemoglobin to glucose over an extended period. In a classical study, Trivelli et al<sup>1</sup> showed Hemoglobin A1c in diabetic subjects to be elevated 2-3 fold over the levels found in normal individuals. Several investigators have recommended that Hemoglobin A1c serve as an indicator of metabolic control of the diabetic, since Hemoglobin A1c levels approach normal values for diabetics in metabolic control.<sup>2,3,4</sup>

Hemoglobin A1c has been defined operationally as the "fast fraction" hemoglobins (HbA<sub>1a</sub>, A<sub>1b</sub>, A<sub>1c</sub>) that elute first during column chromatography with cation-exchange resins. The non-glycosylated hemoglobin, which consists of the bulk of the hemoglobin has been designated HbA<sub>0</sub>. The present procedure utilizes an antigen and antibody reaction to directly determine the concentration of the HbA1c.

## Principle

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 anti-mouse 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. The HbA1c value is obtained from a calibration curve.

## Reagents

R1: Latex 0.13%, Buffer, stabilizer.

R2: Mouse anti-human HbA1c monoclonal antibody 0.05mg/ml, goat anti-mouse IgG polyclonal antibody 0.08mg/dl, Buffer, stabilizers.

Hemolysis reagent: water and stabilizers.

## Reagent Storage

Store all reagents refrigerated at 2-8°C.

## Reagent Preparation

R1, R2 and Hemolysis reagents are supplied as ready to use liquids. Mix gently before use.

## Reagent Deterioration

Alterations in the physical appearance of the reagents or values of control materials outside of the manufacturer's acceptable range may be an indication of reagent instability.

## 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.

## Specimen Collection and Preparation

Special preparation of the patient is unnecessary. Fasting specimens are not required. No special additives or preservatives other than anticoagulants

are required. Collect venous blood with EDTA using aseptic technique. All human specimens should be regarded as potentially biohazardous. Therefore, universal precautions should be used in specimen handling (gloves, lab garments, avoid aerosol production, etc.).

To determine HbA1c, a hemolysate is prepared by the analyzer using the on-board lyse application:

1. Well mixed whole blood is placed directly onto sample carousel or into sample cups. An inaccurate result could be produced from an unmixed whole blood sample. Note: If plastic or glass tubes are used, verify appropriate size.
2. Erythrocytes in a whole blood sample will settle out over time. Analysis of sample should be initiated as soon as possible after samples have been placed onto analyzer.

## Storage and Stability

1. All reagents are stable to the expiration date stated on the labels. Do not use the reagents past their expiration date.
2. R1 and R2 are stable for at least one month after opening stored at 2-8°C.
3. Hemoglobin A1c in whole blood collected with EDTA is stable for one week at 2-8°C.<sup>5</sup>

## Interferences

1. Bilirubin to 50mg/dL, ascorbic acid to 50mg/dL, triglycerides to 2000mg/dL, carbamylated Hb to 7.5mmol/L and acetylated Hb to 5.0mmol/L do not interfere in this assay.
2. 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.<sup>6, 7, 8, 9</sup>
3. It has been reported that elevated levels of HbF may lead to underestimation of HA1c.<sup>10</sup> Also, it has been reported that labile intermediates (Schiff base) are not detected and do not interfere with HbA1c determination by immunoassay.<sup>5</sup>
4. It has been determined that Hemoglobin variants HbA2, HbC and HbS do not interfere with this method.
5. Other very rare variants of hemoglobin (e.g. HbE) have not been assessed.

## Materials Provided

Refer to "Reagents"

## Materials Required but not Provided

1. Mindray BS-200 Analyzer.
2. BS-200 Operation manual.
3. Pipettes
4. Hemoglobin A1c calibrator set and control set.

## Test Parameters (On-Board Lyse)

Test:	HbA1c	R1:	180
No.:	021	R2:	60
Full Name:	HbA1c	Sample Volume:	5
Standard No.:		R1 Blank:	
Reac. Type:	End-point	Mixed Rgt. Blank:	
Pri. Wave:	670nm	Linearity Range:	2.0 – 15.8
Sec. Wave:	0	Linearity Limit:	
Direction:	Increase	Substrate Limit:	
Reac. Time:	1 / 24	Factor:	
Incuba. Time:	19	<input type="checkbox"/> Prozone check	
Unit:	%	q1: q2: q3: q4:	
Precision:	0.1	PC: Abs:	
<b>Pretreatment</b>			
Sample Vol. Pretreated (ul): 4		Pretreat. Reagent Vol. (ul) 200	

# Hemoglobin A1c Reagent Set

## Calibration Parameters

Rule:	Spline	Calibrator 1:	Deionized Water
Sensitivity:		Calibrator 2:	A1c1*
Replicates:	1	Calibrator 3:	A1c2*
Interval (day):		Calibrator 4:	A1c3*
Difference Limit:		Calibrator 5:	A1c4*
SD:		Calibrator 6:	
Blank Response:		<input type="checkbox"/> Dilute Calibrators	<div>Details</div>
Error Limit:		<input type="checkbox"/> Highlight Calibrators	
Coefficient:	0	Check "Dilute Calibrator". Click the "Details" button. Check first box and enter values as described below.	

Concentration	Diluted Sample Vol.	Dilution Vol.	Samp Vol.
**	4.0	200	5

**\*\* Enter concentration of Calibrator.**

## Limitations

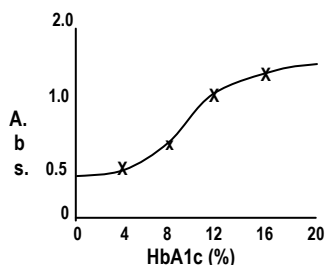
1. This assay should not be used for the diagnosis of diabetes mellitus.
2. Patient specimens should always be assayed using a calibration curve.
3. It has been reported that results may be inconsistent in patients who have the following conditions: opiate addiction, lead-poisoning, alcoholism, ingest large doses of aspirin.<sup>6, 7, 8, 9</sup>
4. It has been reported that elevated levels of HbF may lead to underestimation of HA1c and, that uremia does not interfere with HbA1c determination by immunoassay.<sup>10</sup> It has been reported that labile intermediates (Schiff base) are not detected and therefore, do not interfere with HbA1c determination by immunoassay.<sup>5</sup>
5. It has been determined that Hemoglobin variants HbA2, HbC and HbS do not interfere with this method.
6. Other very rare variants of hemoglobin (e.g. HbE) have not been assessed.
7. Specimens with elevated Erythrocyte Sedimentation Rate (ESR) may generate inaccurate results.

## Quality Control

The reliability of test results should be monitored whenever patient samples are assayed using a standard and quality control materials analyzed in the same manner employed for the unknowns. We suggest the use of commercially available Hemoglobin A1c controls with an assayed range. If controls do not fall into the assayed range patient values from that run should not be reported. The run should be repeated, making sure that all mixing and handling instructions are strictly followed. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements. Linearity of the assay should be verified with a commercial linearity check set, or dilutions of a high specimen, at least every six months.

## Calculations / Results

HbA1c results for the unknowns and controls are determined using the prepared calibration curve. An example curve is illustrated below:



## Expected Values<sup>11</sup>

Recommended Values: less than 6% for a non-diabetic, less than 7% for glycemic control of a person with diabetes.

Each laboratory should establish its own expected values. In using Hemoglobin A1c to monitor diabetic patients, results should be interpreted individually. That is, the patient should be monitored against him or herself. There is a 3-4 week time lag before Hemoglobin A1c reflects changes in blood glucose level.

## Performance

1. Linearity: The Hemoglobin A1c assay range is 2.0%-15.8%.
2. Accuracy: Studies comparing HbA1c Reagent method used on the Mindray BS200 and a similar analyzer yielded the following results:

Method	HbA1c
N	32
Mean HbA1c (%)	8.52
Range (%)	3.2 – 15.8
Standard Deviation (%)	2.61
Regression Analysis	$Y=1.035x + 0.25$
Correlation Coefficient	0.987

3. Precision:  
Within-Day precision was determined following a modification of NCCLS document EP5-T2<sup>17</sup> using the Mindray BS200. Within-Day precision studies produced the following results:

Sample	Low	Mid	High
N	20	20	20
Mean HbA1c (%)	5.6	11.7	15.5
Standard Deviation	0.13	0.23	0.19
Coefficient of Variation (%)	2.3	2.0	1.2

Day to Day: The between day precision was established by assaying blood samples following NCCLS protocol EP5 on a Hitachi 717.

Sample	Low	Mid	High
N	20	20	20
Mean HbA1c (%)	5.95	8.34	12.15
Standard Deviation	0.190	0.093	0.179
Coefficient of Variation (%)	3.19 %	1.12 %	1.47 %

\*NOTE: Day to Day data does not reflect Mindray BS-200 performance.

4. Sensitivity: Sensitivity was investigated by reading the change in absorbance at 660nm for a saline sample and a whole blood sample with a known concentration. Ten replicates of each sample were performed. The results of this investigation indicated that, on the analyzer used (Hitachi 717), the HbA1c reagent showed little or no drift on the zero sample. Under the reaction conditions described, a 0.056 absorbance change is approximately equivalent to 1.0% HbA1c.

## References

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12. NCCLS document "Evaluation of Precision Performance of Clinical Chemistry Devices", 2<sup>nd</sup> Ed. (1992).