# Determination of Hemoglobin Aıc Content by an Automated Hemoglobin Analyzer, HLC-723G7, Myanmar

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**ABSTRACT.** The HLC-723G7, an automated hemoglobin analyzer, is fully automatic and results are reproducible. Results are obtained within 90 seconds and printed out. The analyzer has been tested on a total of 120 blood samples from different patient and subject populations. The results obtained were described and discussed. We suggest use of this machine in the management of diabetes and in future research work related to diabetes. Hb Aıc determination can now be performed at a reasonable cost rapidly, reliable and accurately in Myanmar, an important step towards control of the complications of diabetes and monitoring of diabetic patients under various treatment regimes.

**Key words : automated hemoglobin analyzer, HLC-723G7 — glycosylated hemoglobin (Hb Aıc) — diabetes mellitus — hemoglobinopathies — Myanmar**

Glycosylated hemoglobin (Hb Aıc) content is one of the most important laboratory parameters in the management of patients with diabetes mellitus, particularly in case monitoring and assessment.[1] In Myanmar, where both insulin dependent and non-insulin dependent diabetes mellitus are common, Hb Aıc can be determined only in a few selected cases due to various constraints. Economic and laboratory factors are the main reasons. Recently a set-up for Hb Aıc determination has been established in the Pathology Research Division, Department of Medical Research (Lower Myanmar). A preliminary report on the results and findings after the establishment of this laboratory set-up for Hb Aıc are presented here.

## MATERIALS AND METHODS

An automated hemoglobin analyzer, HLC-723G7 (Tosoh Corporation, Tokyo, Japan) was provided to the Pathology Research Division, Department of Medical Research (Lower Myanmar). It was fully installed and calibrated according to the instruction manual of the manufacturer, Tosoh Corporation.[2] Calibrator and control reagents were used for

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calibration and fresh whole blood samples from apparently healthy persons (n=65) and from patients of different clinical entities (n=55) were used for test runs. Hb Aıc content results for one sample were printed out as the percentage of total hemoglobin content in 90 seconds.

After installation and calibration, Hb Aıc content was determined in verious subjects and patients in ongoing projects, some of which are being done in collaboration with Japanese scientists. Subject and patient populations include blood donors, pregnant mothers, normal control subjects from stroke studies and cardiovascular studies, diabetic patients, stroke patients, children with dengue hemorrhagic fever or hemolytic anemias, and patients with different forms of thalassemia or hemoglobinopathies.

Samples which had extremely unexpected Hb Aıc and Hb F content or questionable Hb peaks underwent isoelectric focusing (IEF ; pH range : 6-9) of their hemolysates to confirm the presence of Hbs including abnormal Hb.[3)]

## RESULTS AND FINDINGS

The retention times of Hb F, labile Hb Aıc, stable Hb Aıc (Hb Aıc) and Hb A shown on chromatograms were about 0.5, 0.6, 0.7 and 1.0 minutes, respectively. The time required for the examination of one sample is only 90 seconds (Fig 1). The Hb content is estimated from areas and printed out with the chromatogram.

The Hb Aıc content of the subjects determined here ranged from 1.0% to 12.2%. The content of subjects, excluding most diabetic patients, whose serum glucose level was not controlled by drugs, exercise or diet treatments, and $\alpha$-thalassemic patients with Hb H disease, was within a range from 3.5 to 5.8, which is the normal range in Japan (Table 1 and Fig 2). The Hb

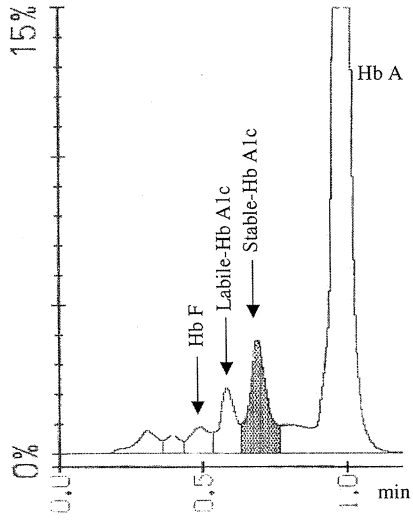


Fig 1. A chromatogram of a blood sample from normal blood donor or the normal volunteer. The Hb F, labile Hb Aıc and stable Hb Aıc (Hb Aıc) contents were 0.5%, 1.8% and 4.1%, respectively. The normal range of Hb Aıc in Japan is 3.5-5.8%.

TABLE 1. Hb Aıc content seen in various study population

| Patient/Subject | No. of cases | Age range (years) | Hb Aıc range (%) | Type of sample | Remarks (n) |
|---|---|---|---|---|---|
| Blood donors (Fig 1) | 18 | 19-51 | 3.6-4.5 | Fresh | hemolysate |
| Pregnant mothers | 11 | 29-39 | 3.6-4.7 | Fresh | hemolysate |
| Stroke (control*[a]) | 11 | 23-70 | 2.5-5.3 | Fresh | whole blood |
| Stroke (Patient) | 11 | 27-73 | 3.2-6.8 | Fresh | whole blood |
| CVD (control*[a]) | 6 | 42-57 | 4.8-5.7 | Fresh | whole blood |
| CVD (Patient) | 6 | 47-63 | 4.5-6.7 | Fresh | whole blood |
| Diabetics | 11 | 50-70 | 5.2-12.2 | Fresh | under treatment |
| DHF children | 3 | 1.5-5 | 4.1-4.7 | Old | hemolysate |
| Thalassemia major | 4 | 1-2.5 | 1.6-3.5 | Fresh | hemolysate |
| Thalassemia traits (Fig 2, 3) | 13 | 14-33 | 1.0-9.9 | Old | AT(7)*[b], BT(6) |
| Hemoglobinopathy (Fig 4) | 7 | 47 | 3.8-4.8 | Fresh | HbE(5) ; u/k(2) |
| Volunteers (Fig 1) | 15 | 26-70 | 4.1-5.5 | Fresh | whole blood |
| Others*[c] | 4 | u/k | 2.5-3.8 | Old | hemolysate |
| Total | 120 | 1-70 | 1.0-12.2 | — | — |

Old : old hemolysate using $CCl_4$ ; a questionable abnormal Hb band was seen in a chromatogram
*[a] : control means under control or under treatment.
*[b] : an unexpectedly high Hb Aıc peak appeared in a chromatogram of a patient diagnosed with Hb H disease (Fig 3).
*[c] : u/k=unknown
CVD=cardiovascluar disease
DHF=dengue hemorrhagic fever
AT=$\alpha$-thalassemia
BT=$\beta$-thalassemia

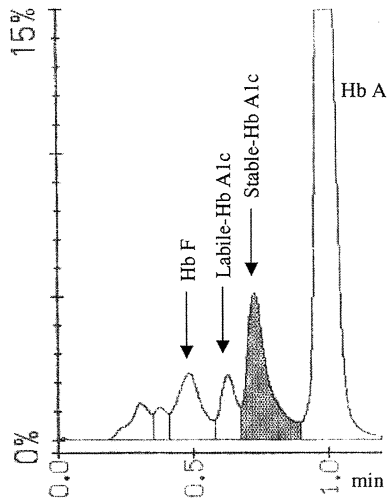


Fig 2. A chromatogram obtained from a blood sample of a patient with $\alpha$-thalassemia-1, The stable Hb Aıc content was 5.1%, while the contents of Hb F and labile Hb Aıc were 2.6% and 1.6%, respectively. That from a well-controlled diabetic patient was similar.

Aıc content of the diabetic patients under treatment and uncontrolled, on the contrary, was relatively high and that of patients with Hb H disease, which is a severe type of $\alpha$-thalassemia, was unexpectedly high (Fig 3). The Hb Aıc content of patients with the heterozygote for Hb E was relatively low, but there was a small peak suspected to be an abnormal

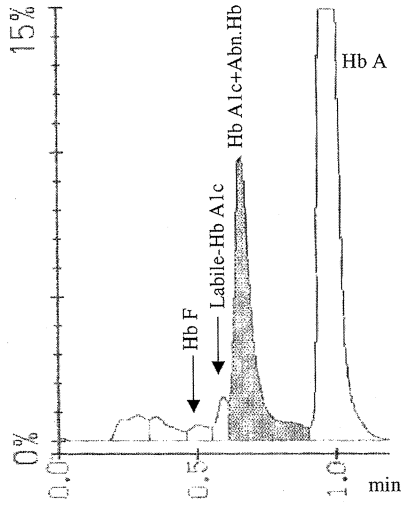


Fig 3. A chromatogram of a blood sample from a non-diabetic patient diagnosed with Hb H disease, in whom the Hb Aıc level was at an extremely high level of 9.9%. However, since no abnormal Hb or Hb H peak was observed at any retention time, the Hb peak eluted at the same position as Hb Aıc was considered to include Hb H.

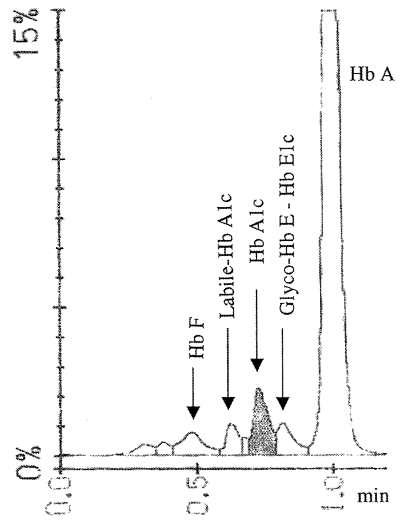


Fig 4. A chromatogram of a blood sample from a patient having Hb E showed an unexpectedly low Hb Aıc level, 2.5%. However, as Hb E is characterized by electrophoretically slow-moving behavior, the Hb peak appearing between the Hb Aıc and Hb A peaks was considered to be a glycosylated Hb E (Hb Eıc), with a value of 1.8%. Therefore, the glycosylated Hb level of this patient with Hb E was estimated to be 4.3%.

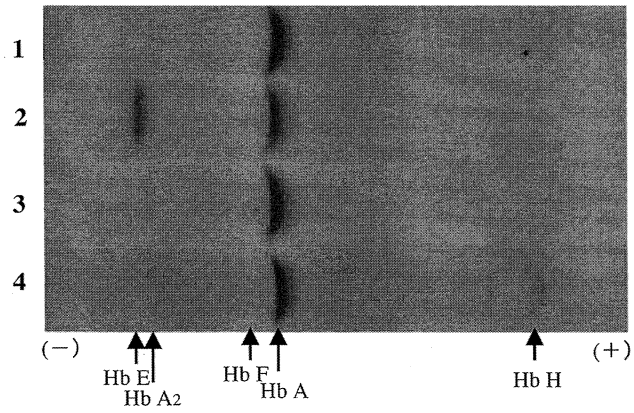


Fig 5. IEF of the hemolysates prepared from patients with various kinds of diseases on polyacrylamide gel (pH range 6-9). The Hb E and Hb H bands were isoelectrofocussed around the Hb $A_2$ zone and the anodic site to Hb A, respectively. 1 : Normal control. 2 : Hb E heterozygote. 3 : Normal. 4 : Hb H disease.

peak between the Hb Aıc and Hb A peaks (Fig 4). It seemed to be glycosylated Hb E (Hb Eıc), because as Hb E moves electrophoretically slower than Hb A, Hb Eıc is considered to be eluted later than Hb Aıc. The suspected presence of abnormal Hbs ; e.g., Hb H and Hb E, that are the most common abnormal Hbs in Myanmar and neighboring countries,[4-6] was confirmed by application of the IEF method to their hemolysates (Fig 5). The Hb Aıc level of patients with hyperglycemia or uncontrolled serum glucose levels may by higher than 5.8%, while that of non-diabetic patients with Hb H is not considered to go beyond 5.8%, as seen in Fig 3. Based on these results, it was concluded that the Hb H peak is eluted at a position to the Hb Aıc peak and the Hb Eıc peak is between the Hb Aıc and Hb A peaks. Therefore, it is difficult to dteremine the accurate glycosylated Hb content of a patient with Hb H or Hb E disease accurately with only the HLC-723G7. To make a decision requires the data from a complete blood cell count, Hb analysis, Hb biosynthesis, and red cell morphology.

The Hb Aıc content in various study populations is shown in Table 1.

## DISCUSSION

The automated hemoglobin analyzer, HLC-723G7, has been well known in Japan[7,8] and worldwide, but particularly in Asian and Southeast Asian nations during the last few years for its accurate determination of Hb Aıc and Hb $A_2$ content. Whole blood or hemolysate is used and elution time and total area exposed to individual hemoglobin are recorded and printed out as the percentage of total hemoglobin (Fig 1).

The machine has been used effectively for the determination of Hb Aıc in 120 samples since its successful installtation and establishment. The minimal and maximal Hb Aıc values obtained have been 1% and 12.2%, respectively, varying with the subject population under study (Table 1).

Among apparently healthy volunteers and blood donors, the Hb A$_{1c}$ level has ranged from 3.6% to 5.5% (Table 1). They have had no detectable abnormal hemoglobin or thalassemia. Therefore, this value can be taken as the normal Hb A$_{1c}$ level in the Myanmar population. In a previous study of diabetic patients in Mandalay, Myanmar, the whole glycosylated hemoglobin concentration (Hb A$_1$ = labile Hb A$_{1c}$ + stable Hb A$_{1c}$) content for the control subjects was $2.2 \pm 0.5\%$ and that for the diabetic patients was $6.4 \pm 3.6\%$.[9,10] In that study the phenol-sulphuric acid method was used. In Japan the normal Hb A$_{1c}$ value ranges from 3.5% to 5.8%.

The Hb A$_{1c}$ level tends to be lower in patients with the $\alpha$-thalassemia syndromes, $\alpha$-thalassemia-2 and $\alpha$-thalassemia-1. Many cases of $\beta$-thalassemia and $\alpha$-thalassemia-1 have Hb A$_{1c}$ content within the normal range (3.4-5.2%) (Fig 2). Hb A$_{1c}$ content is sometimes much reduced in thalassemia major cases, ranging from 1% to 3.5%. No significant reduction in glycosylated Hb content was observed in Hb E carriers (Fig 4), because Hb E$_{1c}$ is eluted between the Hb A$_{1c}$ and Hb A peaks, being estimated as Hb A$_{1c}$ plus Hb E$_{1c}$. There was also no significant difference in the Hb A$_{1c}$ value between stroke or cardiovascular disease patients and controls. In diabetics, the value spread over a wide range, 5.2-12.2% (Table 1). In pregnant mothers the value was within a normal range, 3.6-4.7%.

There are many advantages to use of the HLC-723G7 for the determination of Hb A$_{1c}$. Only a very minute amount of blood is required (10 $\mu$l or less is sufficient). Any form of anticoagulant can be used. The hemolysates used can easily be prepared using distilled water or washing buffer provided together with other reagent solutions.[2] The result is readily obtained in 90 seconds and printed out. It is also relatively more economical, less laborious, and more rapid than other methods such as the ion exchange resin chromatography, affinity chromatography or biochemical colorimetric assays.[11-14]

In conclusion, the HLC-723G7 is most suitable for determining Hb A$_{1c}$ in the management of diabetes and in future research work related to diabetes, since it has many advantages over other methods. We have planned some clinical field and laboratory-based research projects on diabetes using this analyzer to begin in the very near future. The common limitations to the determination of Hb A$_{1c}$ content have been overcome by the development of a set-up locally in Department of Medical Research (Lower Myanmar).

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