Proficiency Testing of HbA1c: A 4-Year Experience in Taiwan and the Asian Pacific Region

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BACKGROUND: The correlation between hemoglobin A1c (HbA1c) and risk for complications in diabetic patientsheightens the need to measure HbA1c with accuracy. We evaluated the current performance for measuring HbA1c in the Asian and Pacific region by examining data submitted by laboratories participating in the Taiwan proficiency-testing program.

METHODS: Five fresh-pooled blood samples were sent to participating laboratories twice each year. The results were evaluated against target values assigned by the National Glycohemoglobin Standardization Program network laboratories; a passing criterion of ±7% of the target value was used. Measurement uncertainty at HbA1c concentrations of 7.0% and 8.0% were determined.

RESULTS: A total of 276 laboratories from 11 countries took part in the HbA1c survey. At the HbA1c concentrations tested method-specific interlaboratory imprecision (CVs) were 1.1%–13.9% in 2005, 1.3%–10.1% in 2006, 1.2%–8.2% in 2007, and 1.1%–6.1% in 2008. Differences between target values and median values from the commonly used methods ranged from −0.24% to −0.22% HbA1c in 2008. In 2005 83% of laboratories passed the survey, and in 2008 93% passed. At 7.0% HbA1c, measurement uncertainty was on average 0.49% HbA1c.

CONCLUSIONS: The use of accuracy-based proficiency testing with stringent quality criteria has improved the performance of HbA1c testing in the Asian and Pacific laboratories during the 4 years of assessment.

Hemoglobin A1c (HbA1c)6 measurements obtained for a period of 2–3 months provide an important index for assessing glycemic control in patients with diabetes. Results from the Diabetes Control and Complications Trial (DCCT) in patients with type 1 diabetes (1) and the United Kingdom Prospective Diabetes Study in patients with type 2 diabetes (2, 3) indicate that HbA1c is directly related to the risk of development and progression of diabetic complications. The National Glycohemoglobin Standardization Program (NGSP) was established in 1996 to standardize HbA1c testing, sothat clinical laboratory results would be traceable to the clinical studies and outcomes from DCCT and the United Kingdom Prospective Diabetes Study (4, 5). Guidelines for the management of diabetes rely closely on the measurement of HbA1c (6–8). Maintenance of HbA1c at <7.0% has been recommended for the effective treatment of diabetes (9). In addition, recent studies have suggested that HbA1c can be used for the screening and diagnosis of diabetes (10–12). Thus, precise and accurate measurement of HbA1c is critical and essential for proper diabetes care.

Proficiency testing (PT) that is accuracy based is important for improving and maintaining the quality of laboratory test results. Both imprecision and bias influence PT performance. Studies have suggested that maintaining a CV of less than one-third of the PT limit will guarantee passing PT events, given a bias less than one-fifth of the PT limit (13, 14).

The Taiwan Society of Laboratory Medicine (TSLM) began an HbA1c proficiency testing survey program in 2004 and has used NGSP-assigned target values to assess accuracy since 2005. In this study, we aimed to evaluate the current performance of HbA1c testing in Taiwan and the Asian Pacific region by examining data submitted by laboratories participating in the TSLM HbA1c survey.

The Laboratory Management Committee of the Asian Pacific Federation for Clinical Biochemistry started the HbA1c project and invited its members to participate in the TSLM HbA1c survey free of charge in 2005. The HbA1c PT consists of 5 samples per survey shipped twice a year to each laboratory. For those laboratories outside the Taiwan area, only 1 survey per

6 Nonstandard abbreviations: HbA1c, hemoglobin A1c; DCCT, Diabetes Control and Complications Trial; NGSP, National Glycohemoglobin Standardization Program; PT, proficiency testing; TSLM, Taiwan Society of Laboratory Medicine; SRLs, secondary reference laboratories; CAP, College of American Pathologists; MU, measurement uncertainty.
year was performed owing to the limited budget of the Asian Pacific Federation for Clinical Biochemistry. The TSLM began a process of accuracy grading based on NGSP-assigned target values for each survey sample in 2005. The survey samples were prepared from pooled human fresh whole blood from healthy and diabetic individuals. The blood samples were negative for HIV antibodies, hepatitis B surface antigen, and hepatitis C antibody. Sample deterioration was minimized by shipment on cold packs within 5 days of collection.

Reported methods included: (a) Bio-Rad D-10, (b) Bio-Rad Variant II, (c) Fujirebio immunoturbidimetry, (d) Primus affinity chromatography, (e) Roche Cobas Integra immunoassay, (f) Tosoh G7 Standard analysis mode, (g) Tosoh G7 Variant analysis mode, and (h) Tosoh A1c 2.2 Plus. Methods (a), (b), and (f)–(h) are automated ion-exchange HPLC assays. All methods except method (c) are NGSP certified as traceable to the DCCT reference method.

Participants’ results were evaluated according to several different accuracy-based grading schemes, the criteria of TSLM (±7% of the NGSP target), the College of American Pathologists (CAP; 2008 criterion ±12% of the NGSP-target), and Australia (±0.5% HbA1c if <10.0% HbA1c, or ±5% of the NGSP-target if ≥10.0% HbA1c). Acceptable performance was deemed as at least 80% of PT samples within the acceptable limits. We also analyzed the results obtained from 4 consecutive years, including method-specific median, mean, interlaboratory imprecision (CV), and bias for methods with participant numbers ≥10. The observed trueness (bias) for each method was assessed from the difference between the method-specific mean and the NGSP target value. Measurement uncertainty (MU) for each method was calculated from the square root of the sum of the squares of method-specific bias and method-specific SD, with a coverage factor of 2.

<table>
<thead>
<tr>
<th>Method</th>
<th>Labs</th>
<th>Mean</th>
<th>SD</th>
<th>CV%</th>
<th>Bias</th>
<th>Bias%</th>
<th>MU, *% HbA1c</th>
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<td></td>
<td></td>
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<td>5.17</td>
<td>−0.06</td>
<td>−0.9</td>
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<td>Primus</td>
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<td>1.95</td>
<td>0.15</td>
<td>2.1</td>
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* MU (expanded uncertainty), calculated from the square root of the sum of the squares of method-specific bias and method-specific SD, with a coverage factor of 2.
Laboratories participating in the HbA1c Survey in Taiwan and the Asian Pacific region included hospital-based laboratories, freestanding facilities, and physician office laboratories. Each year there were 208–276 laboratories in Taiwan and 13–15 laboratories outside Taiwan enrolled in this program. The participants located outside Taiwan included Australia (n = 1), Hong Kong (n = 1), Indonesia (n = 2), India (n = 2), Korea (n = 1), Malaysia (n = 3), Pakistan (n = 2), Singapore (n = 4), Thailand (n = 1), and Vietnam (n = 1). All participants reported results as percentage HbA1c. Ion-exchange HPLC methods were used by 57% of laboratories in 2005; this percentage increased to 73% in 2008. Immunoassay methods were used in 26% of laboratories in 2005 and 16% in 2008. The remaining participants used affinity HPLC.

The method-specific interlaboratory imprecision values (CVs) ranged from 1.3% to 11.4% in 2005, 1.4% to 9.0% in 2006, 1.9% to 7.4% in 2007, and 1.6% to 6.1% in 2008 at HbA1c concentrations of 6.0%–8.0%. In 2008, the CVs of HPLC-based methods were 1.1%–4.1% at HbA1c concentrations of 4.8%–9.1%, whereas the interlaboratory CVs for immunoassays were 3.8%–6.1%. There were 4 methods (Primus and Tosoh G7 Standard, G7 Variant, and A1c 2.2 Plus) having interlaboratory CVs less than one-third of the PT limit (2.3%) at HbA1c concentrations ≥5.0%. This interlaboratory CV is consistent with the CAP survey results, which showed CVs of 1.5%–3.5% for HPLC methods and 3.5%–8.0% for immunoassays (16).

Several expert groups in laboratory medicine recommended within-laboratory and between-laboratory imprecision goals of 3% and 5%, respectively (6, 17). Overall assessment revealed that the imprecision for all HPLC methods was good and fulfilled the quality goal. An optimal within-laboratory imprecision goal of 2% was advocated recently (18). The comparable between-laboratory CV is estimated as 3.3%. However, only about one-half of analytical methods used in this survey achieved this quality goal of imprecision at all concentrations tested in 2008, and all of the methods that achieved this goal were HPLC based.

Differences between NGSP-assigned target values and method-specific median values at the low, medium, and high HbA1c concentrations from 7 NGSP-certified methods were within 0.4%, 0.2%, and 0.5% HbA1c of NGSP targets, respectively, in 2006; within 0.2%, 0.3%, and 0.6% HbA1c in 2007, and within 0.2%, 0.2%, and 0.3% HbA1c in 2008. The variability and bias trends at 7.0%–8.0% HbA1c during the 4

Fig. 1. The pass rates of the HbA1c survey for methods with N ≥10 in 2005–2008. G7 STD, G7 Standard.
years are shown in Fig. 1 in the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol55/issue10. Three methods (Primus and Tosoh G7 Standard and Variant modes) had systematic error less than one-fifth of the PT limit (1.4%), calculated from the regression lines between NGSP-target values vs method-specific medians during the 4 years at the concentration of 7.0% HbA1c. The difference plots against NGSP-assigned target values are shown in online Supplemental Fig. 2. A larger median positive bias at high concentrations (8.0%–11.2%) of HbA1c was found during 2005–2007 in most methods. This positive bias in high-concentration survey samples was also reported in CAP 2005–2007 in most methods. This positive bias in high-concentration survey samples was also reported in CAP surveys (16). The cause of the bias is unclear. However, the difference plots indicate that the bias has resolved over time.

The performance of HbA1c assessed from 2007 and 2008 data for samples with NGSP-assigned target values of 7.03% HbA1c and 7.96% HbA1c, respectively, is shown in Table 1. The range of the method-specific bias was −0.07% to 0.26% HbA1c and −0.17% to 0.19% HbA1c at the levels of 7.03% HbA1c and 7.96% HbA1c, respectively. Method-specific MU ranged from 0.37% to 0.73% HbA1c in 2007 and 0.38% to 0.96% HbA1c in 2008. National Academy of Clinical Biochemistry guidelines suggest interpreting changes of more than 0.5% HbA1c to be reporting units for treatment (19). Our data on mean MU (0.49% HbA1c) conform to the 0.5% HbA1c change criterion. This change criterion of a 0.5% HbA1c reporting unit corresponds to results within ±7% of the target value in the PT scheme being adequate to meet clinical needs.

Acceptable performance for each method during the 4-year study period is shown in Fig. 1. The overall pass rates for the laboratories were 83%, 91%, 90%, and 93% in 2005, 2006, 2007, and 2008, respectively. Based on the CAP 2008 survey criterion, 90%–98% of laboratories performed adequately during the 4 years. Based on the Australian survey criterion, the overall pass rates were improved from 79% in 2005% to 93% in 2008 (online Supplemental Fig. 3).

Studies have shown that decreases in HbA1c reduced the risk of complications in diabetes (3, 7). Stringent analytical quality goals for HbA1c would provide improved precision and accuracy, facilitating better assessment of patient glycemic control. Use of pooled fresh human blood as PT survey samples was recommended to accurately assess the performance of HbA1c testing by avoiding potential sample matrix effects during shipping and processing. The NGSP SRL network is anchored by the DCCT reference method, which has proven to be consistent during a 25-year period (20). The network is also monitored against the IFCC definitive reference method, and the uncertainty (2 SD) of values assigned by the NGSP network has been shown to be 0.10% HbA1c (21). Thus, the contribution of the uncertainty of the NGSP value assignments to the total uncertainty of the Taiwan Proficiency Survey results is negligible. With the NGSP-assigned target values, the accuracy-based HbA1c survey enables laboratories to evaluate harmonization among methods and traceability to the DCCT reference method (6).

The improvement of interlaboratory CVs and bias and pass rate during the 4 years suggests that the PT program played a role in this improvement. The majority of the participants using NGSP-certified methods showed an acceptable HbA1c testing performance. Nevertheless, the deficiencies found in some laboratories confirm the need for continuing quality improvement for HbA1c measurement.

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