Report on HbA1c Proficiency Testing in Asia in 2012

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In 2010, the Japan Diabetes Society decided to introduce the National Glycohemoglobin Standardization Program (NGSP) values into clinical practice. Accordingly, NGSP Certification of Japanese manufacturers of HbA1c-related diagnostic reagents and instruments was initiated in February, 2012, through an NGSP network laboratory, the Asian Secondary Reference Laboratory (ASRL) #1. Traceability to the NGSP reference system can be endorsed by manufacturer certification, as well as by the College of American Pathologists (CAP) survey. Nevertheless, only a few manufacturers participate in the CAP survey in Japan. Thus, proficiency testing (PT) was proposed and executed by ASRL #1. Single-donor whole-blood samples were used for the PT. The participated measurement systems were NGSP certified. Twenty-two laboratories obtained certification through ASRL #1; 2 through the Secondary Reference Laboratory (SRL) #8; and 9 through the SRL #9. The combination plots of the bias data in this PT and in the NGSP certification performed in March and May in 2012 were consistent with each other: mean NGSP values at each level agreed well with the target value. In conclusion, PT using whole blood is useful in endorsing NGSP certification.

Key Words: HbA1c, National Glycohemoglobin Standardization Program, Proficiency testing, Asian Secondary Reference Laboratory

The Japan Diabetes Society (JDS) decided to use National Glycohemoglobin Standardization Program (NGSP) values, as well as JDS HbA1c values, in clinical practice for international clinical harmonization of HbA1c [1]. Accordingly, NGSP certification of Japanese manufacturers of diagnostic reagents and instruments related to HbA1c was initiated in February, 2012, through NGSP Network Laboratory, the Asian Secondary Reference Laboratory (ASRL) #1 [2]. As a result, 21 routine methods were certified in 2012 and 42 in 2013. Moreover, traceability to the NGSP reference system can now be endorsed by both manufacturer certification and the College of American Pathologists (CAP) survey. The CAP survey is useful for monitoring between-certification proficiency of certified methods. However, only a few manufacturers participate in the CAP survey in Japan. Thus, proficiency testing (PT) similar to the CAP survey targeting NGSP-certified manufacturers and laboratories in Asia has been proposed and executed by ASRL #1 in collaboration with the Japan Reference Measurement Institute (JRMI). The committee for the PT was organized by the Reference Material Institute for Clinical Chemistry Standards (ReCCS) in collaboration with JRMI.

Five PT samples were prepared: PT samples 1, 2, 4, and 5 were single-donor whole-blood aliquots, and PT sample 3 was a mixture of PT samples 1 and 4, with plasma replaced by physiological saline solution. PT samples were drawn from two Japanese individuals in Japan and two diabetes patients in USA with informed consent and with the approval of the Ethics Committee of ReCCS. PT sample 3 for A1c GEAR in Table 1 shows abnormally high HbA1c values because only whole blood can be applied to A1c GEAR; therefore, in the mean value obtained by immunoassay, the A1c GEAR result of this sample was excluded in Table 2.

ReCCS prepared and shipped the test samples. PT samples were stored in a refrigerator (2-8°C) until shipment, and at each laboratory, they were removed from the container immediately
| Method            | Participating laboratory                     | Measurement system | PT sample |
|-------------------|-----------------------------------------------|--------------------|-----------|
|                   |                                               |                    | 1 | 2 | 3 | 4 | 5 |
| Immunoassay       | Kyowa Medex Co., Ltd.                         | Determiner HbA1c/DM-JACK | 4.755 | 5.485 | 7.895 | 12.530 | 12.560 |
| Immunoassay       | Kyowa Medex Co., Ltd.                         | Determiner HbA1c/JCA-BM9130 | 4.805 | 5.500 | 7.940 | 12.565 | 12.625 |
| Immunoassay       | Kyowa Medex Co., Ltd.                         | Determiner L HbA1c/DM-JACK | 4.795 | 5.520 | 8.005 | 12.540 | 12.625 |
| Immunoassay       | Kyowa Medex Co., Ltd.                         | Determiner L HbA1c/JCA-BM9130 | 4.795 | 5.480 | 7.985 | 12.485 | 12.570 |
| Immunoassay       | TFB, INC.                                     | Rapidia Auto HbA1c/L/Hitachi 7170s | 4.570 | 5.400 | 7.660 | 12.200 | 12.420 |
| Immunoassay       | ROHM Co., Ltd.                                | Banalyst Ace HbA1c/Banalyst Ace | 4.700 | 5.500 | 7.900 | High* | High* |
| Immunoassay       | ROHM Co., Ltd.                                | Spotchem Banalyst HbA1c/ | 4.800 | 5.600 | 8.000 | High* | High* |
|                   |                                               | Spotchem Banalyst SI-3610 |         |      |      |      |      |
| Immunoassay       | Wako Pure Chemical Industries, Ltd.           | Autokit HbA1c/Hitachi 7170S | 4.790 | 5.440 | 8.075 | 12.585 | 12.535 |
| Immunoassay       | SAKAE Corporation                             | A1c GEAR | 4.650 | 5.300 | 8.650 | 12.050 | 12.500 |
| Immunoassay       | Kotobiken Medical Laboratories, Inc.          | Determiner L HbA1c/JCA-BM9130 | 4.830 | 5.360 | 7.660 | 12.205 | 11.965 |
| Immunoassay       | Siemens Healthcare Diagnostics                | DCA2000+/DCA Vantage | 4.950 | 5.600 | 8.150 | 13.050 | 13.300 |
| Immunoassay       | Siemens Healthcare Diagnostics                | Dimension Rxl MAX | 5.400 | 6.065 | 8.440 | 13.190 | 13.180 |
| Immunoassay       | Ortho Clinical Diagnostics                    | Vitros d%A1c/Vitros 5,1FS | 4.970 | 5.475 | 8.125 | 12.735 | 12.780 |
| Immunoassay       | Ortho Clinical Diagnostics                    | Vitros d%A1c/Vitros 5600 | 4.980 | 5.475 | 8.110 | 12.605 | 12.585 |
| Immunoassay       | Roche Diagnostics                             | TQ HbA1c Gen.3/cobas c501 | 4.650 | 5.475 | 8.275 | 12.495 | 12.590 |
| Immunoassay       | Roche Diagnostics                             | TQ HbA1c Gen.2/cobas c501 | 5.055 | 5.645 | 8.135 | 12.280 | 12.335 |
| Immunoassay       | Boditech Med. Inc.                            | i-ChromaTM | 4.450 | 5.600 | 8.050 | 12.150 | 12.450 |
| Enzymatic assay   | Kyowa Medex Co., Ltd.                         | MetaboLead HbA1c/JCA-BM9130 | 4.850 | 5.440 | 7.910 | 12.880 | 13.065 |
| Enzymatic assay   | SEKISUI MEDICAL CO., LTD.                     | Norudia N HbA1c/Hitachi 7170S | 4.855 | 5.715 | 7.690 | 12.730 | 13.420 |
| Enzymatic assay   | Hitachi Chemical Co., Ltd.                    | Seratetum A1C | 4.780 | 5.465 | 8.085 | 12.555 | 12.855 |
| Enzymatic assay   | NIHON KOHDEN CORPORATION                      | BM Test HbA1c/JCA-BM6010 | 4.870 | 5.445 | 8.140 | 13.205 | 13.315 |
| Ion-exchange HPLC | Kotobiken Medical Laboratories, Inc.          | HLC-723 G8 | 4.740 | 5.460 | 7.920 | 12.690 | 12.840 |
| Ion-exchange HPLC | SRL, Inc.                                     | ADAMS A1c HA-8160 | 4.900 | 5.650 | 8.200 | 12.800 | 13.100 |
| Ion-exchange HPLC | Bio-Rad Laboratories                           | Variant II Turbo | 4.635 | 5.535 | 7.875 | 12.530 | 12.675 |
| Ion-exchange HPLC | Bio-Rad Laboratories                           | D-10 | 4.800 | 5.600 | 7.950 | 12.500 | 12.600 |
| Ion-exchange HPLC | Tosoh Corporation                             | HLC-723 G7 | 4.710 | 5.510 | 7.975 | 12.735 | 12.885 |
| Ion-exchange HPLC | Tosoh Corporation                             | HLC-723 G8 | 4.680 | 5.500 | 7.965 | 12.805 | 12.865 |
| Ion-exchange HPLC | Tosoh Corporation                             | HLC-723 G9 | 4.700 | 5.525 | 7.995 | 12.855 | 12.910 |
| Ion-exchange HPLC | Tosoh Corporation                             | HLC-723 GX | 4.695 | 5.495 | 7.980 | 12.835 | 12.890 |
| Ion-exchange HPLC | Korea Association of Health Promotion         | HLC-723 G8 | 4.740 | 5.585 | 8.100 | 13.035 | 13.120 |
| Ion-exchange HPLC | Seoul National University Bundang Hospital    | Variant II Turbo | 4.600 | 5.600 | 8.050 | 13.100 | 13.400 |
| Ion-exchange HPLC | NEODIN MEDICAL INSTITUTE                      | HLC-723 G8 | 4.900 | 5.750 | 8.250 | 13.150 | 13.250 |
| Ion-exchange HPLC | Chung-Ang University Hospital                 | Variant II Turbo | 4.450 | 5.300 | 7.650 | 12.500 | 12.600 |

*out of measurement range; †abnormally high values (whole blood only can be applied) for sample 3 (mixture of samples 1 and 4, replacing the plasma with physiological saline solution).

Abbreviations: NGSP, National Glycohemoglobin Standardization Program; PT, proficiency testing; ASRL, Asian Secondary Reference Laboratory; ReCCS, Reference Material Institute for Clinical Chemistry Standards.
on receipt and then stored in a refrigerator (2-8°C) until use. Analyses were completed within two days of receipt when less than 14 days passed after each sample collection.

The participated measurement systems were NGSP certified (according to manufacturer's methods and laboratory certification) by ASRL #1, SRL #8, and SRL #9. Each PT sample was measured in duplicate. Measured HbA1c values were reported to 2 decimal places. Mean values (calculated to 3 decimal places) of the duplicate values for each PT sample were reported as final values, which were compared with target values determined by ASRL #1. The obtained values were consistent with those of SRL #8 and SRL #9. An overall test of coincidence by 2 least squares linear regression lines was performed using Microsoft Office Excel 2007.

A total of 33 laboratories (29 Japanese and four overseas laboratories) participated in the PT. Among these facilities, 22 were certified by NGSP through ASRL #1, two through SRL #8, and nine through SRL #9. Furthermore, 17 participating laboratories used immunoassay, four used enzymatic assay, and 12 used HPLC. PT samples 4 and 5 yielded HbA1c values over 12 NGSP %, which exceeded measurement ranges for some measurement instruments; therefore, these values were excluded for those systems. Target values of PT samples 1 through 5 were determined by ASRL #1 to be 4.86 NGSP%, 5.64 NGSP%, 8.00 NGSP%, 12.68 NGSP%, and 12.78 NGSP%, respectively. The comparison of values as measured by instruments and the NGSP/ASRL target values are shown in Table 1.

The regression line comparing the relationship between total mean values and target values was $Y = 1.013 \times (the \ target \ value) + 0.141$. That is, the total mean values were higher than the target values. Furthermore, the values of CV of PT samples 1 through 5 were 3.72%, 2.54%, 2.23%, 2.41%, and 2.71%, respectively. PT sample 3 (containing red cells and saline) did not show any difference compared with other hemolyzed blood samples. CV values were 2.51-4.51% by immunoassay, 1.05-2.53% by enzymatic assay, and 1.73-2.63% by HPLC (Table 2).

Compatibility with JDS criteria (less than or equal to a relative bias of $\pm 5.0\%$ by mean of duplicate measurement) was checked by comparing the measured values of test samples 1 through 3 for the certification of 5% to 10.0% HbA1c. The results indicate that 26 out of 28 methods in test sample 1, 26 out of 28 in test sample 2, and 26 out of 27 in test sample 3 were compatible with JDS criteria [1].

Fig. 1. Observed HbA1c values of each participating laboratory compared to the NGSP/ASRL target (dashed line) in 2012 based on ASRL PT data. Closed circles show within $\pm 5\%$ (dotted line) of relative bias. Open circles show within $\pm 7\%$ (dashed line) of relative bias. Open squares show biases of all manufacturers versus ASRL target PT samples. Abbreviations: NGSP, National Glycohemoglobin Standardization Program; ASRL, Asian Secondary Reference Laboratory; PT, proficiency testing.

Table 2. Comparison of three methods: immunoassay, enzymatic assay, and HPLC

| Sample | 1      | 2      | 3      | 4      | 5      |
|--------|--------|--------|--------|--------|--------|
| Target value | 4.863  | 5.643  | 7.997  | 12.678 | 12.779 |
| Method | Immunoassay (17 measurement systems) | | | | |
| Mean   | 4.823  | 5.525  | 8.025  | 12.511 | 12.601 |
| SD     | 0.217  | 0.165  | 0.199  | 0.317  | 0.317  |
| CV (%) | 4.51   | 2.99   | 2.47   | 2.53   | 2.51   |
| Method | Enzymatic assay (4 measurement systems) | | | | |
| Mean   | 4.855  | 5.552  | 7.997  | 12.843 | 13.164 |
| SD     | 0.051  | 0.140  | 0.198  | 0.239  | 0.220  |
| CV (%) | 1.05   | 2.53   | 2.48   | 1.86   | 1.67   |
| Method | HPLC (12 measurement systems) | | | | |
| Mean   | 4.713  | 5.543  | 7.993  | 12.795 | 12.928 |
| SD     | 0.124  | 0.110  | 0.155  | 0.222  | 0.250  |
| CV (%) | 2.63   | 1.99   | 1.94   | 1.73   | 1.93   |

Abbreviations: NGSP, National Glycohemoglobin Standardization Program.
clusion, the PT is useful to monitor NGSP-certified methods. Mean values at each level correspond well with the target value, including point-of-care testing.

Authors’ Disclosures of Potential Conflicts of Interest

No potential conflicts of interests relevant to this article were reported.

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