

External quality assessment program for detection of glucose-6-phosphate dehydrogenase deficiency in the Guangxi region

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Abstract. The aim of this study is to improve the quality of testing for glucose-6-phosphate dehydrogenase (G6PD) deficiency through evaluation and analysis of the laboratory tests for G6PD activity. External quality assessment (EQA) was carried out twice per year with five samples each from 2014 to 2016. Samples were used for quantitative and qualitative assays. Quantitative results were collected, qualitative results were determined with reference values, and information about methods, reagents and instruments from participating laboratories within the required time. Laboratory performance scores, coefficient of variation (CV), and the rates of false negative and positive results were calculated. As a result, a total of 2,834 cases of negative quality control (QC) samples and 2,451 cases of positive QC samples were assessed, where the rates of false negative and false positive results were 1.31% (37/2,834) and 1.34% (33/2,451), respectively. Quantitative results indicated an increasing trend in testing quality, which were consistent with conclusions based on the comparison of EQA full-score and acceptable ratio in six assessments. The 2nd assay in 2016 had the best full-score ratio of 68.9% (135/196) and best acceptable ratio of 84.2% (165/196). There was a decreasing trend in the average CV of six reagents produced in China, and the range of average CV increased to 14.6-23.6% in 2016. The average CV of low level and high level samples was 22.5% and 15.3%, respectively, demonstrating that samples with low G6PD activity have greater interlaboratory CV values. In conclusion, laboratories improved their testing quality and provided better diagnostic service for G6PD deficiency in areas with high incidence after participation in the EQA program in the Guangxi region.

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common enzymopathic disease and is a single-gene disorder (1,2). Previous studies indicated that there is a high frequency of this inherited blood disease among the population of southern China, and is primarily distributed in Guangxi, Guangdong, Yunnan, and the Sichuan region (3). A recent study showed that the incidence was 10.75% in the Guangxi region (4). G6PD deficiency is an incurable disease. However, population prevention, individual prevention and careful monitoring of neonates with this deficiency can prevent complications such as acute hemolytic anemia, jaundice, and kernicterus (5-7). It is imperative that large-scale screening for G6PD deficiency is performed, especially in pregnant women and newborns in areas with high incidence (8,9).

A Guangxi external quality assessment (EQA) G6PD program was carried out in 2014. In this study, a 3-year EQA was carried out from 2014 to 2016 to describe the problems related to G6PD testing in the Guangxi region. Herein, we provide recommendations for corrective actions that can help reduce the error rate and decrease the interlaboratory coefficient of variation (CV), thus, ensuring consistent results between laboratories.

Materials and methods

Preparation of quality control samples for EQA. Anticoagulated whole blood from healthy donors [human immunodeficiency virus (HIV) antibodies, hepatitis C virus (HCV) antibodies, and hepatitis B surface antigen (HBsAg) were negative] was mixed and centrifuged to separate red blood cells (RBCs) and plasma. RBCs were washed three times with normal saline and stored at -80°C, followed by repeated freezing and thawing at 2-8°C to obtain hemolysates. After isolation of fibrinogen based on a methodo-logy using magnetic beads and filtration with 200-mesh screen filters, plasma was mixed with hemolysates as hemostroma and various doses of the G6PD analytical reagent to prepare high level and low level quality control (QC) samples. A total volume of 0.25 ml of these samples were then dispensed into vials with a Hamilton dispenser and lyophilized with an LGJ-25C freeze dryer (Four-Ring Science Instrument Plant Beijing Co., Ltd, Beijing, China).

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Table I. Results of qualitative assays on G6PD from 2014 to 2016 in the Guangxi region.

Period	Assay	Negative samples		Positive samples	
		n	False negative (%)	n	False positive (%)
2014	1st assay	456	8 (1.75)	304	8 (2.63)
	2nd assay	310	8 (2.58)	465	8 (1.72)
2015	1st assay	540	15 (2.78)	360	10 (2.78)
	2nd assay	364	5 (1.37)	546	4 (0.73)
2016	1st assay	576	1 (0.17)	384	2 (0.52)
	2nd assay	588	0 (0.00)	392	1 (0.26)
Total		2,834	37 (1.31)	2,451	33 (1.34)

G6PD, glucose-6-phosphate dehydrogenase.

Assessment of uniformity and stability of lyophilized QC samples. Ten samples were randomly chosen at each level for assessing the uniformity of dispensation and lyophilization. G6PD activity was determined monthly for 12 months to assess the stability of QC samples at 2–8°C. The detection of G6PD activity and hemoglobin (Hb) concentration were performed with a Beckman AU5800 chemistry analyzer (Beckman Coulter, Inc., Brea, CA, USA) and corresponding reagent kits (Changchun Huili Biotech Co., Ltd., China).

Design of external quality assessment. EQA (10) was performed twice per year (referred to as the 1st and 2nd assessments) with five QC samples each from 2014 to 2016, among which 2–3 vials were low level QC samples (1.0–2.5 U/gHb or 100–800 U/l), while the others were high level QC samples (7.0–17.0 U/gHb or 1700–2,400 U/l). Samples were then sequentially numbered and sent to participating laboratories. The ranges of G6PD activity for the different levels of QC samples were determined according to a cut-off value. A sample was considered to have G6PD deficiency if the value was <4.8 U/gHb or 1,300 U/l.

Treatment of QC samples. Lyophilized samples in vials were re-dissolved in 0.25 ml of distilled water and allowed to stand for 30 min. Samples were then mixed well by repeated inversion and processed according to the reagent instructions.

Implementation of external quality assessment. Data, including quantitative and qualitative results were requested to be returned via the internet within 7 days of completion. Quantitative data were filled correctly with units of U/gHb or U/l, and qualitative data were expressed as positive (+) or negative (–) according to laboratory testing cut-off values. Negative meant normal whereas positive meant deficiency of G6PD. Data were analyzed based on the methods of detection and reagents used. Outliers, which are more than three standard deviations from the mean of a set, were eliminated after three repeated calculations. The median was considered as the reference value. Quantitative results that fell within two standard deviations from the reference value were permitted.

Evaluation of results. Data from participating laboratories were collected and analyzed with Microsoft Excel 2007 (Microsoft Corp., Redmond, WA, USA). For the quantitative survey, data within the permitted range was marked 20 points and considered acceptable if the total score was over 80, while for the qualitative survey, outcomes corresponding with the expected results were marked 20 scores and the overall coincidence rates of samples were calculated.

Results

Results of confirmatory tests on lyophilized QC samples. There were no significant differences in G6PD activity of samples after being dispensed, lyophilized, and re-dissolved ($P > 0.05$). Unused QC samples were stable for up to 1 year at 2–8°C and opened samples were stable for 3 days.

Collection of reports from participating laboratories. A total of 166 laboratories participated in the EQA program in 2014, while 152 and 155 effective reports were received for the 1st and 2nd assessments, respectively, within the stated period following the distribution of QC samples. For surveys in 2015 and 2016, 185 and 196 laboratories were involved, while 180, 182, 192 and 196, effective reports were received, respectively. All participating laboratories performed the detection of G6PD activity with biochemistry analyzers and domestic reagents by the rate method (11). Among them, 25 laboratories determined the values of G6PD activity and hemoglobin concentration, while other laboratories analyzed only G6PD activity.

Results of qualitative assays. As shown in Table I, a total of 2,834 cases of negative QC samples and 2,451 cases of positive QC samples were assessed from 2014 to 2016, where the coincidence rate of negative and positive were 98.69% (2,797/2,834) and 98.65% (2,418/2,451), respectively. The rate of false negative and false positive results were 1.31% (37/2,834) and 1.34% (33/2,451), respectively. The best coincidence rate was found in 2016, where the rate of false negative and false positive results were 0% and 0.26% (1/392), respectively, on the 2nd assessment.

Table II. Results of the EQA program for G6PD testing from 2014 to 2016 in the Guangxi region.

Period	Assay	Number of laboratories	Results of EQA					
			100 scores	Full-score ratio (%)	80 scores	Acceptable ratio (%)	<80 scores	Reject ratio (%)
2014	1st assay	152	78	51.3	25	67.8	49	32.2
	2nd assay	155	84	54.2	24	69.7	47	30.3
2015	1st assay	180	76	42.2	49	69.4	55	30.6
	2nd assay	182	115	63.2	35	82.4	32	17.6
2016	1st assay	192	113	58.9	31	75.0	48	25.0
	2nd assay	196	135	68.9	30	84.2	31	15.8

EQA, external quality assessment. G6PD, glucose-6-phosphate dehydrogenase.

Table III. Evaluation of the testing quality of different reagents.

Reagents	2014			2015			2016			Total		
	N	Acceptable ratio (%)	Average CV (%)	N	Acceptable ratio (%)	Average CV (%)	N	Acceptable ratio (%)	Average CV (%)	N	Acceptable ratio (%)	Average CV (%)
A	16	82.3	33.7	23	80.6	24.6	25	89.3	18.8	64	84.1	25.7
B	60	75.2	26.0	84	71.9	27.3	83	80.2	23.6	227	75.8	25.6
C	39	93.2	20.4	41	92.6	15.4	41	94.1	14.6	121	93.3	16.8
D	16	75.8	22.9	14	76.9	16.7	16	89.8	20.2	46	80.8	19.9
E	-	-	-	13	76.2	15.7	16	84.2	15.4	29	80.2	15.6
F	-	-	-	12	63.6	28.1	10	79.4	22.2	22	71.5	25.2

N is the number of participating laboratories. CV, coefficient of variation.

Table IV. Average CV of QC samples with different concentrations.

QC samples	Concentration	Unit	Number of laboratories	Average CV (%)
Low level	1.0-2.0	U/gHb	182	27.7
	2.0-2.5	U/gHb	129	16.4
	100-400	U/l	1,507	26.3
	400-800	U/l	1,167	19.9
				(22.5)
High level	7.0-10.0	U/gHb	200	15.4
	10.0-17.0	U/gHb	111	14.2
	1,700-2,400	U/l	1,481	16.4
				(15.3)

Cut-off: 4.8 U/gHb and 1,300 U/l. CV, coefficient of variation; QC, quality control; Hb, hemoglobin.

Results of quantitative assays. We compared the full-score ratio and acceptable ratio of the EQA program for G6PD testing performed in 2014, 2015 and 2016. Table II indicates that the 2nd assay in 2016 had the best results for full-score ratio (68.9%, 135/196) and acceptable ratio (84.2%, 165/196),

while the 1st assay in 2015 had the lowest full-score ratio of 42.2% (76/180) and acceptable ratio of 69.4% (125/180), which were consistent with the results of the qualitative assay. Table III shows an increasing trend in the testing quality of six reagents produced in China, as the range of average CVs of different reagents increased to 14.6-23.6% in 2016, which was significantly lower than that in 2014 and 2015. Reagent B was the most commonly used, and was adopted in 227 laboratories. Reagent C had the best results of acceptable ratio (93.3%) and average CV (16.8%). Table IV demonstrates that samples with low G6PD activity had greater interlaboratory CV values. In this study, the average CV of low level (1.0-2.5 U/gHb or 100-800 U/l) and high level (7.0-17.0 U/gHb or 1700-2400 U/l) QC samples was 22.5 and 15.3%, respectively.

Discussion

This EQA program plays an instrumental role in monitoring and promoting the performance of G6PD testing in the Guangxi region. The coincidence rate of screening tests of QC samples for G6PD deficiency was relatively good. About 3% of reports were found to have false negative or positive results. False results were mainly attributed to clerical error or systematic error that made measurements consistently greater or lower than the true values.

Data from the six EQA quantitative assays from 2014 to 2016 indicated a gradual improvement in the quality of G6PD tests. It was found that the acceptable ratio of the 1st assay in 2015 was lower than that of 2014, and this correlated with the additional reagents E and F used in tests and increasing CV. Further investigation showed that this problem was principally attributed to improper procedure (incorrect handling of QC materials and reagents) and changes of operating process. Measurements were consistently higher than the true values since labeled values on the instructions were taken as the calibration value for the use of RANDOX QC materials. The results were lower than others obtained from tests with the same reagent because of the use of whole blood instead of hematocrit, as was required for dilution and detection. Additionally, G6PD reagents were not used with the corresponding Hb reagents for the measurement of G6PD in Hb. For example, detecting G6PD activity with reagents provided by A while measuring Hb concentrations with reagents provided by other manufacturers would lead to large bias in the results. With the implementation of standard procedures, summary reports have been published on the website of the Guangxi Center for Clinical Laboratory (<http://www.gxcl.com>) as a warning and prevention to other laboratories. There was a decrease of the average CV and an increase of the acceptable ratio of all reagents in 2016.

Measurements of samples with different concentrations demonstrate that low level QC samples ranging from 1.0-2.0 U/gHb and 100-500 U/l had large interlaboratory CV values (27.7% and 26.3%, respectively) while high level QC samples ranging from 7.0-17.0 U/gHb and >1,300 U/l had small CV values (the average CV was 15.3%). This conclusion is consistent with recently published EQA data from the Taiwan region, which showed that the CV of samples with low G6PD activity were greater than those with high G6PD activity (12).

QC samples with critical G6PD activity were not evaluated in the present EQA program in the Guangxi region. Two critical values for G6PD activity in QC samples for EQA programs were reported by the College of American pathologists (CAP) in 2006 and 2007. The average values were 3.86 and 4.18 U/gHb (values ranged from 2.3-5.3 U/gHb and 1.8-6.7 U/gHb, respectively). The qualitative results did not meet the requirements of a coincidence rate >90%, and there was no comment on critical value samples from the CAP. Regarding assays in the Taiwan region, almost all interlaboratory CVs were controlled within 10% of QC samples with critical G6PD activity ranging from 3.0-4.3 U/gHb and 4.4-6.0 U/gHb (13). This has contributed to longstanding external quality assurance.

At present, a total of 197 laboratories have participated in the EQA program for G6PD testing in the Guangxi region, among which 95 implemented internal quality control, while 102 did not establish and execute internal quality control properly. Therefore, it is imperative for laboratories to strictly implement internal quality control (14). Participation in the

EQA program for G6PD testing is helpful for minimizing laboratory errors and improving testing quality, thus providing better diagnostic service for G6PD deficiency in areas with high incidence of the disease.

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