External Quality Assurance Programme for Newborn Screening of Glucose-6-Phosphate Dehydrogenase Deficiency

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Abstract

Introduction: The nationwide neonatal screening for glucose-6-phosphate dehydrogenase (G6PD) deficiency in Taiwan was started on 1 July 1987. A network of G6PD referral hospitals distributed all around Taiwan was organised for follow-up, confirmatory testing, medical care and genetic counselling. To assess the reliability of confirmatory and screening tests, an external quality assurance (QA) programme for G6PD assay was developed. Materials and Methods: Lyophilised quality control (QC) materials and dried blood spots were prepared from erythrocytes and whole blood for confirmatory and screening tests, respectively. The external QA surveys were carried out every 1 to 2 months. The QA results were evaluated and compared to the consensus result and reference value. The test results were submitted through internet by participating laboratories and the summary reports were published on a webpage (http://www.g6pd.tw) within 2 weeks. Results: Twenty-one referral laboratories in Taiwan and 16 screening laboratories in Germany, Lebanon, Mainland China, Philippines, Thailand, Taiwan, Turkey, and Vietnam have been participating in the QA programme. From 1988 to 2007, 144 QA surveys for confirmatory testing were sent to referral laboratories. Among the 2,622 reports received, 292 (11.1%) were found to be abnormal. Interlaboratory coefficient of variation (CV) for the confirmatory test has reached below 10% in recent years. The significant improvement in interlaboratory CV was found to be correlated with the preventive site visits to the referral laboratories since November 2004. From 1999 to 2007, 52 external QA surveys for the screening test were performed. Among 504 reports received, 97 (19.2%) were found to be abnormal. From the 5040 blood spots tested by the screening laboratories, 95 false negative (1.9%) and 187 false positive (3.7%) results were reported. Conclusions: The external QA programme has been useful for monitoring the performance of the referral hospitals and screening laboratories and helpful for the participating laboratories to improve their test quality.

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Introduction

Glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49) deficiency is the most common enzymopathic disease in Southeast Asia and other tropical areas worldwide.[1-3] This X-linked genetic disorder (MIM 305900) has been found to be an important cause of neonatal jaundice and acute haemolytic anaemia in the southern Chinese population in Taiwan.[4,5] In order to reduce the complications of G6PD deficiency, such as kernicterus, permanent neurological damage and death, nationwide neonatal screening for G6PD deficiency was started on 1 July 1987 after a pilot project conducted between November 1984 and June 1987 had demonstrated the practicality and the efficiency of neonatal screening of G6PD deficiency in Taiwan.[6,7] The effective collection rate has reached more than 99% of all newborns in Taiwan since 1996 and the overall incidence rate of G6PD deficiency was found to be about 2%.[8-10] The screening programme in Taiwan consists of 3 screening centres and 22 referral hospitals. The referral hospitals, which were located all around Taiwan including

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the outlying islands, were organised to provide confirmatory testing, medical care and genetic counselling for the screen positive cases. In order to assess the reliability of the confirmatory test performed by the referral laboratories, an external quality assurance (QA) programme for the determination of G6PD activity in erythrocytes has been carried out since January 1988.[11,12] In 1999, an external QA survey for the G6PD screening test was incorporated into this QA programme to assess the reliability of the G6PD screening test for the neonatal screening centres.[12] This report presents the results of the external QA programme for the G6PD confirmatory and screening test for the past 20 and 9 years, respectively.

Materials and Methods

External QA Programme for Quantitative Assay of G6PD Activity in Erythrocyte

Standardised procedures for quantitative analysis of G6PD activity in erythrocyte and methods for the calibration of spectrophotometer and micropipette were distributed to all participating laboratories. Erythrocyte G6PD activity was recommended to be determined kinetically at 37°C using the reagent kit (Cat. No. 345) produced by Sigma Chemical Co. (St. Louis, MO, USA) and Trinity Biotech (Co. Wicklow, Ireland) with maleimide as the inhibitor.[13] The quality control (QC) materials with different G6PD activity used for quantitative assay were prepared as described previously.[11] Briefly, the G6PD activity of the red blood cells (RBC) was assayed.[13] The RBC with normal and deficient G6PD activity were lysed and then mixed with each other in different proportions to prepare QC samples with different G6PD activities. These haemolysates were then dispensed into glass bottles and lyophilised.

Periodically, within 1 to 2 months, 3 (July 1992 to December 2007) or 5 (January 1988 to June 1992) QC materials were sent to each participating laboratory on dry ice by speed post delivery. The results of G6PD activity analysis were requested to be returned by facsimile (and internet submission since 2005) within 8 days. The external QA results were evaluated and compared to the median of all reports received and the reference value determined by our laboratory. The reported result was considered to be erroneous when: (i) more than two-thirds of the G6PD values were outside 80% to 120% of the median; and/or (ii) G6PD values were inconsistent with median values. Besides written summary reports, the summary has also been published on the website http://www.g6pd.tw as soon as the report was released since 1995. For participants with system errors detected by this external QA programme, troubleshooting was proceeded either by telephone contact or personal visit from the reference laboratory. In order to further reduce the error rate and improve the interlaboratory CV, a routine preventive site visiting programme to the participating laboratories has been installed since November 2004.

External QA Programme for Neonatal G6PD Screening Test

The QC materials with different G6PD activity were prepared from whole blood and spotted onto newborn screening blood collection filter paper. In brief, the G6PD activity of whole blood obtained from normal and G6PD deficient donors were measured by the quantitative confirmatory test method[13] as mentioned above and followed by centrifugation to separate the RBC and plasma. After washing with normal saline, the RBC with different G6PD activities were mixed in different proportions for preparing QC materials with different G6PD activity. These combined RBC were then mixed with plasma (45%) and spotted onto the blood collection filter paper used for neonatal screening.

Periodically, within 1 to 2 months, 10 QC specimens were randomly picked for each survey and distributed to each neonatal screening laboratory by speed post delivery. Reports were requested to be returned by facsimile (internet submission since 2005) within 4 days for screening centres in Taiwan and 8 days for overseas screening centres. For each QA survey, the G6PD activity of the QC dried blood spots was determined by quantitative assay[13] to set the reference values before the QC specimens were send out. The results reported by the screening centres were evaluated against the consensus result and compared with the quantitative reference values determined by our laboratory. The summary report for each survey was published on website http:/www.g6pd.tw within 2 weeks after the survey samples had been sent.

Results

External QA Programme for Quantitative Assay of G6PD Activity in Erythrocyte

Twenty-one referral laboratories have been participating in this external QA programme for quantitative assay of G6PD activity in erythrocyte. These include 9 medical centres, 7 regional hospitals, 3 local hospitals and 2 independent clinical laboratories, which have been providing confirmatory G6PD quantitative assays to the G6PD screen positive cases reported by neonatal screening centres in Taiwan. From January 1988 to December 2007, 144 QA surveys of G6PD quantitative test were carried out for these referral laboratories. In total, 2662 reports were received for these surveys. The reporting rate increased gradually from 81% in 1988 to 100% since 1996 (data not shown). Two hundred and ninety-two reports (11.1%) were found to have abnormal QA results, which were attributed

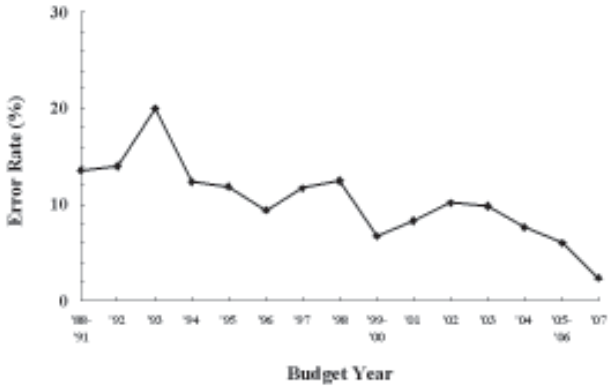

Fig. 1. Error rate found in the external QA survey for quantitative assay of G6PD activity in erythrocyte.

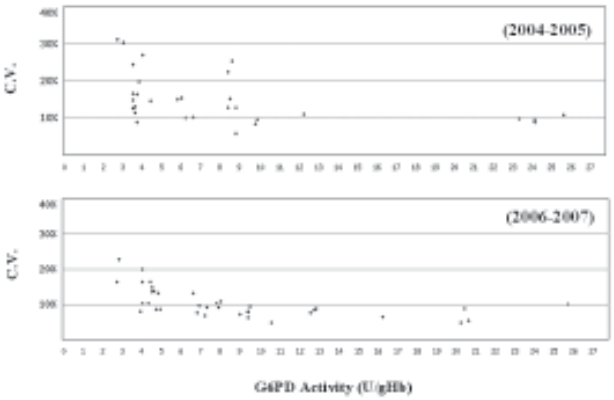

Fig. 2. Interlaboratory CV of the external QA survey for quantitative assay of G6PD activity in erythrocyte at different G6PD activity.
CV: coefficient of variation.

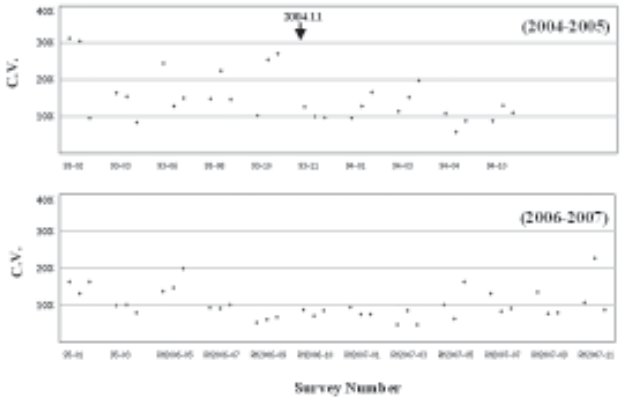

Fig. 3. Interlaboratory CV of the external QA survey for quantitative assay of G6PD activity in erythrocyte. The 2004.11 mark indicates the start of the routine preventive site visiting programme.

mainly to clerical (12.0%), experimental (18.5%), and instrumental errors (44.2%). Since 2006 the error rates have been decreased to less than 6% (Fig. 1). Most of the experimental and instrumental errors were found in those laboratories that did not execute internal QA properly. The average interlaboratory CV has been reduced from 15.1% between 2004 and 2005 to 10.3% between 2006 and 2007 (Fig. 2). Recently (2006-2007), almost all the interlaboratory CVs in the critical G6PD activity range between 6 and 13 U/gHb for the diagnosis of G6PD deficiency had been controlled within 10% (Fig. 2). The significant improvement of interlaboratory CV was found to be correlated with the preventive site visits to the participating laboratories since November 2004 (Fig. 3).

External QA Programme for Neonatal G6PD Screening Test

In addition to the 3 neonatal screening centres in Taiwan, 13 overseas neonatal screening centres/laboratories (6 in Mainland China, 2 in Philippines, and 1 each in Germany, Lebanon, Thailand, Turkey, and Vietnam) have also joined in this external QA programme (Table 1). From March 1999 to December 2007, 52 QA surveys for G6PD screening test were performed. Five hundred and four reports were received for these surveys and the reporting rate was 100%. Ninety-seven reports (19.2%) were found to have abnormal QA results, which contained 95 false negative (1.9%) and 187 false positive results (3.7%) (Table 1). The significant increase in number of errors in year 2003 and 2004 were contributed by those screening centres which changed their testing method during that period of time and as well as by screening centres that had newly joined the QA programme. Most of the false negative and false positive results were found in QC samples with borderline G6PD activities of 3.0-4.3 U/gHb and 4.4-6.0 U/gHb (normal newborn cutoff of the reference method: 4.4 U/gHb), respectively (Table 2). The false negative and false positive decisions in these borderline cases are most likely caused by different cutoff levels used by those screening laboratories. For example, all 6 screening centres using the same PerkinElmer Neonatal G6PD Kit (ND-1000) have reported different cutoffs (1.6, 1.8, 2.0, 2.1, 2.6, and 2.9).

Discussion

The external QA programme with preventive site visits to the referral laboratories has successfully controlled the interlaboratory CV under 10% in the critical G6PD activity range for diagnosis of G6PD deficiency. The well-controlled interlaboratory CV provided a basis for uniform interpretation using the same reference range of confirmatory test data generated from all G6PD referral hospitals nationwide in Taiwan. The screening centres may use the data generated by the external QA programme to compare the decisions made by other screening centres and to adjust the cutoff used in their own screening test accordingly.

The results of this G6PD QA programme revealed the

Table 1. Results of External QA Survey for Neonatal G6PD Screening Test

Period	No. of Laboratory	Report		Specimen		False positive	False negative
		n	error (%)	n	error (%)		
1999	8	39	0 (0.0%)	390	2 (0.5%)	0	2
2000	8	48	0 (0.0%)	480	0 (0.0%)	0	0
2001	8	48	7 (14.6%)	480	23 (4.8%)	23	0
2002	8	47	6 (12.8%)	470	15 (3.2%)	15	0
2003	10	48	17 (35.4%)	480	51 (10.6%)	33	18
2004	12	66	28 (42.4%)	660	92 (13.9%)	72	20
2005	12	48	9 (18.8%)	480	16 (3.3%)	16	0
2006	12	71	19 (26.8%)	710	54 (7.6%)	15	39
2007	16	89	11 (12.4%)	890	29 (3.3%)	13	16
Total		**504**	**97 (19.2%)**	**5040**	**282 (5.6%)**	**187**	**95**

Table 2. External QA Results of Neonatal G6PD Screening Test at Different Ranges of G6PD

G6PD activity (U/gHb)	No. of specimen	Reported decision		False positive	False negative
		Positive	Negative		
0.1 ~ 1.9	791	791	0	0	0
2.0 ~ 2.9	373	362	11	0	11 (2.9%)
3.0 ~ 4.3	390	306	84	0	84 (21.5%)
4.4 ~ 6.0	954	131	823	131 (13.7%)	0
6.1 ~ 26.8	2532	56	2479	56 (2.2%)	0
Total	**5040**	**1646**	**3394**	**187 (3.7%)**	**95 (1.9%)**

G6PD activity in the QC specimens were determined as described in the text
Cutoff: 4.4 U/gHb

importance of external QA. These external QA programmes for quantitative and qualitative analysis of G6PD activity provide a good system to monitor the performance of the screening and diagnostic services for G6PD deficiency. The external QA programme might also serve as a guide for participating laboratories to improve the quality of their service. Although an external QA programme can help laboratories reduce analytical errors, it is indispensable for every laboratory to establish and to carry out strictly their own internal quality control to achieve better quality of clinical laboratory service.

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REFERENCES

1. Luzzato L, Mehta A, Vulliamy T. Glucose-6-phosphate dehydrogenase deficiency. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. The Metabolic and Molecular Bases of Inherited Disease. 8th ed. New York: McGraw-Hill, 2001.
2. World Health Organization Working Group. Glucose-6-phosphate dehydrogenase deficiency. Bull WHO 1989;67:601-11.
3. Beutler E. Glucose-6-phosphate dehydrogenase deficiency: a historical perspective. Blood 2008;111:16-24.
4. Chen SH, Lin KS, Lee TY, Chen CL. Glucose-6-phosphate dehydrogenase deficiency and neonatal hyperbilirubinemia in Chinese. Acta Paediatr Sinica 1986;27:286-93.
5. Yu MW, Hsiao KJ, Wuu KD, Chen CJ. Association between glucose-6-phosphate dehydrogenase deficiency and neonatal jaundice: interaction with multiple risk factors. Int J Epidemiol 1992;21:947-52.
6. Hsiao KJ, Wuu KD. Neonatal screening for inborn metabolic diseases: the experience in Taiwan. In: Lin HJ, Swaminathan R, Robertshaw AM, editors. Proceedings of the 4th Asian-Pacific Congress of Clinical Biochemistry. Hong Kong: Gardiner-Caldwell, 1989:214-8.
7. Hsiao KJ. Genetic disorders and neonatal screening. In: Myai K, Kanno T, Ishikawa E, editors. Progress in Clinical Biochemistry. Amsterdam: Elsevier Science, 1992:289-92.
8. Hsiao KJ, Chiang SH. Developments of Neonatal Screening in Taiwan. International Conference on Genetic Medicine and Promoting Healthcare for Rare Disorder, Taipei, 2005:255-8.
9. Hsiao KJ. Neonatal Screening of Glucose-6-Phosphate Dehydrogenase Deficiency. 2nd Congress of Asian Society for Pediatric Research, Yokohama, 2006:74.
10. Chiang SH, Wu SJ, Wu KF, Hsiao KJ. Neonatal screening for glucose-6-phosphate dehydrogenase deficiency in Taiwan. Southeast Asian J Trop Med Public Health 1999;30Suppl2:72-4.
11. Hsiao KJ, Chiang SH. Interlaboratory quality assurance program for determination of blood glucose-6-phosphate dehydrogenase activity: experience in Taiwan. In: Ohba Y, Kanno T, Okabe H, editors. Quality Control in the Clinical Laboratory 95. Tokyo: Excerpta Medica, 1995:241-6.
12. Chiang SH, Wu KF, Liu TT, Wu SJ, Hsiao KJ. Quality assurance program for neonatal screening of glucose-6phosphate dehydrogenase deficiency. Southeast Asian J Trop Med Public Health 2003; 34Suppl3:130-4.
13. Deutsch J. Maleimide as an inhibitor in measurement of erythrocyte glucose-6-phosphate dehydrogenase activity. Clin Chem 1978;24,885-9.