QUALITY ASSURANCE PROGRAM FOR NEONATAL SCREENING OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY

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Abstract. The nationwide neonatal screening of glucose-6-phosphate dehydrogenase (G6PD) deficiency in Taiwan was started on July 1, 1987. The effective collection rate has reached more than 96% of all newborns since 1993 and the overall incidence rate of G6PD deficiency was about 2%. This screening program has 3 screening centers and 18 referral hospitals, distributed around Taiwan including outlying islands. To assess the reliability of the confirmatory and screening tests, an external quality assurance (QA) program for G6PD assay was developed. For quantitative assay of G6PD activity, lyophilized quality control materials with different G6PD activities were prepared from red blood cells. For G6PD screening, quality control materials with different G6PD activities were prepared from whole blood and spotted onto the blood collecting filter paper. Periodically (1-2 months), the QC materials were sent to each of the referral hospitals and screening centers by speed post delivery. The external QA results were evaluated and compared to the median of all the reports and the reference values determined by our laboratory. Whenever an analytical system error was detected in any participating laboratory, troubleshooting was carried out either by contacting by phone or visiting in person. Twenty-one referral laboratories and 8 screening centers (3 in Taiwan, 2 in Mainland China, and one each in the Philippines, Thailand and Lebanon) participated in the QA program. Three to 5 QC specimens were sent to every participating referral laboratory for each quantitative survey. From January 1988 to June 2001, 104 QA surveys were carried out and 1,891 reports were received. Two hundred and thirty-nine (12.6%, 239/1,891) QA reports had abnormal results, attributed to clerical (13%, 31/239), experimental (17.2%, 41/239), and instrumental errors (46.4%, 111/239). Most of the experimental and instrumental errors were found in those laboratories that did not execute internal QA properly. For the screening QA program, 10 QC blood spots were delivered to every participating screening center for each survey. From March 1999 to June 2001, 15 screening surveys were performed with 111 reports received. One false negative (1/1,110) and 14 (14/1,110) false positive results were found in four (3.6%, 4/111) of the screening QA reports. The external quality assurance program proved useful for monitoring the performance of G6PD tests in referral hospitals and screening centers, and provided guidance for correcting analytical errors.

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49) deficiency is the most common enzymopathic disease in Southeast Asia (WHO, 1989; Luzatto *et al*, 2001). This X-linked genetic disorder (MIM 305900) has been found to be an important cause of neonatal jaundice and acute hemolytic anemia in the southern Chinese population in Taiwan (Chen *et al*, 1986; Yu *et al*, 1992). In order to reduce the complications of G6PD deficiency such as kernicterus, permanent neurological damage, and death, the nationwide neonatal screening of G6PD deficiency was started on July 1, 1987 after a pilot project (1984.11 to 1987.6) had demonstrated the practicality and the efficiency of neonatal screening of G6PD deficiency in Taiwan (Hsiao and Wuu, 1989; Hsiao, 1992). The effective collection rate has reached more than 96% of all newborns in Taiwan since 1993 and the overall incidence rate of G6PD deficiency was found to be about 2% (Chaing *et al*, 1999). The screening program in Taiwan has 3 screening centers and 18 referral hospitals. The referral hospitals, which are distributed all around Taiwan including outlying islands, were organized to provide confirmatory tests, medical care and genetic counseling on follow up of the positively screened cases. In order to assess the reliability of the confirmatory test performed by the referral hospitals, an external quality assurance (QA) program for the determination of G6PD activity in erythrocytes has been carried out since January 1988. In 1999, an external QA survey for the G6PD screening test was incorporated into

this QA program to assess the reliability of the G6PD screening test for the neonatal screening centers. This report presents the results of the external QA survey for the G6PD confirmatory and screening test for the past 13 and 3 years, respectively.

MATERIALS AND METHODS

External QA program for quantitative assay of G6PD activity

Standardized procedures for quantitative analysis of G6PD activity in erythrocyte and methods for the calibration of spectrophotometers and micropipettes 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) with maleimide as the inhibitor (Deutsch, 1978). The quality control (QC) materials with different G6PD activity used for quantitative assay were prepared as described previously (Hsiao and Chiang, 1995). Briefly, the G6PD activity of the red blood cells (RBC) was assayed (Deutsch, 1978), and divided into two separate parts. G6PD activity in one of the portions was subjected to inactivation. Both RBC with normal and inactivated of G6PD activity were lysed and then mixed with each other in different proportions to prepare QC samples with different G6PD activities. These hemolysates were then dispensed into glass bottles and lyophilized.

Periodically (1-2 months), three (1992.7-2001.6) or five (1988.1-1992.6) QC materials were sent to each participant laboratory in dry ice by speed post delivery. The results of G6PD activity analysis were requested to be returned by facsimile within 7 days. The external QA results were evaluated and compared to the median of all reports received and the reference value was determined by our laboratory (Fig 1). The reported result was considered to be erroneous when: 1) more than two-thirds of the G6PD values were outside 80% - 120% of the median; and/or 2) G6PD values were inconsistent with median values. For participants with system errors detected by this QA program, troubleshooting was proceeded either by telephone contact or personal visit.

External QA program for screening test of G6PD deficiency

The QC materials with different G6PD activity were prepared from whole blood and spotted onto blood collection filter paper. In brief, the G6PD activity of whole blood was measured and followed by centrifugation to separate the RBC and plasma. The RBC was washed with normal saline and the G6PD activity of one portion was inactivated. The RBC with normal and inactivated G6PD activity were mixed in different proportions for preparing QC materials with different G6PD activity. These combined RBC was then mixed with plasma (45%) and spotted onto the blood collection filter paper used for neonatal screening.

Periodically (1-2 months), 10 QC specimens were randomly picked for each survey and distributed to each neonatal screening center by speed post delivery. Reports were requested to be returned by fax or e-mail within 3 days for screening centers in Taiwan or 7 days for overseas screening centers. For each QA survey, the G6PD activity of the QC dried blood spots was determined by quantitative assay (Deutsch, 1978) to set the reference values and was checked for G6PD qualitatively by the fluorescent screening method (Beutler and Mitchell, 1968) using the commercial kit (Boehringer Mannheim GmbH Diagnostica, Germany) before the QC specimens were sent out. The results reported by the screening centers were evaluated against the consensus result and compared with the quantitative reference values determined by our laboratory.

RESULTS

External QA program for quantitative assay of G6PD activity

Twenty laboratories have been participating in this QA program for the quantitative assay of G6PD activity. These include clinical laboratories of 9 medical centers, 7 regional hospitals, 3 local hospitals and a pathology institute, which have been providing confirmatory G6PD quantitative assays to the G6PD deficient cases reported by the neonatal screening center. From January 1988 to June 2001, 104 QA surveys of G6PD quantitative test were carried out for the referral laboratories. Every participating laboratory received three (1992.7-2001.6) or five (1988.1-1992.6) QC specimens for each survey. Totally, 1,891 reports were received for these surveys. The reporting rate increased gradually from 81% in 1988 to 100% in 2001 (Table 1). Two hundred and thirty-nine reports (12.6%, 239/1,891) were found to have abnormal QA results, which were attributed mainly to clerk (13%, 31/239), experimental (17.2%, 41/239), and instrumental errors (46.4%, 111/239) (Tables 1, 2). In the recent two years (1999-2001), the error rates decreased to less than 9 % (Table 1). Most of the experimental and instrumental errors were found in those laboratories that did not execute internal QA properly. Some of the referral laboratories were located in rural areas with much less routine workloads on G6PD analysis than those in urban areas. Because of the few samples per run and the commercially

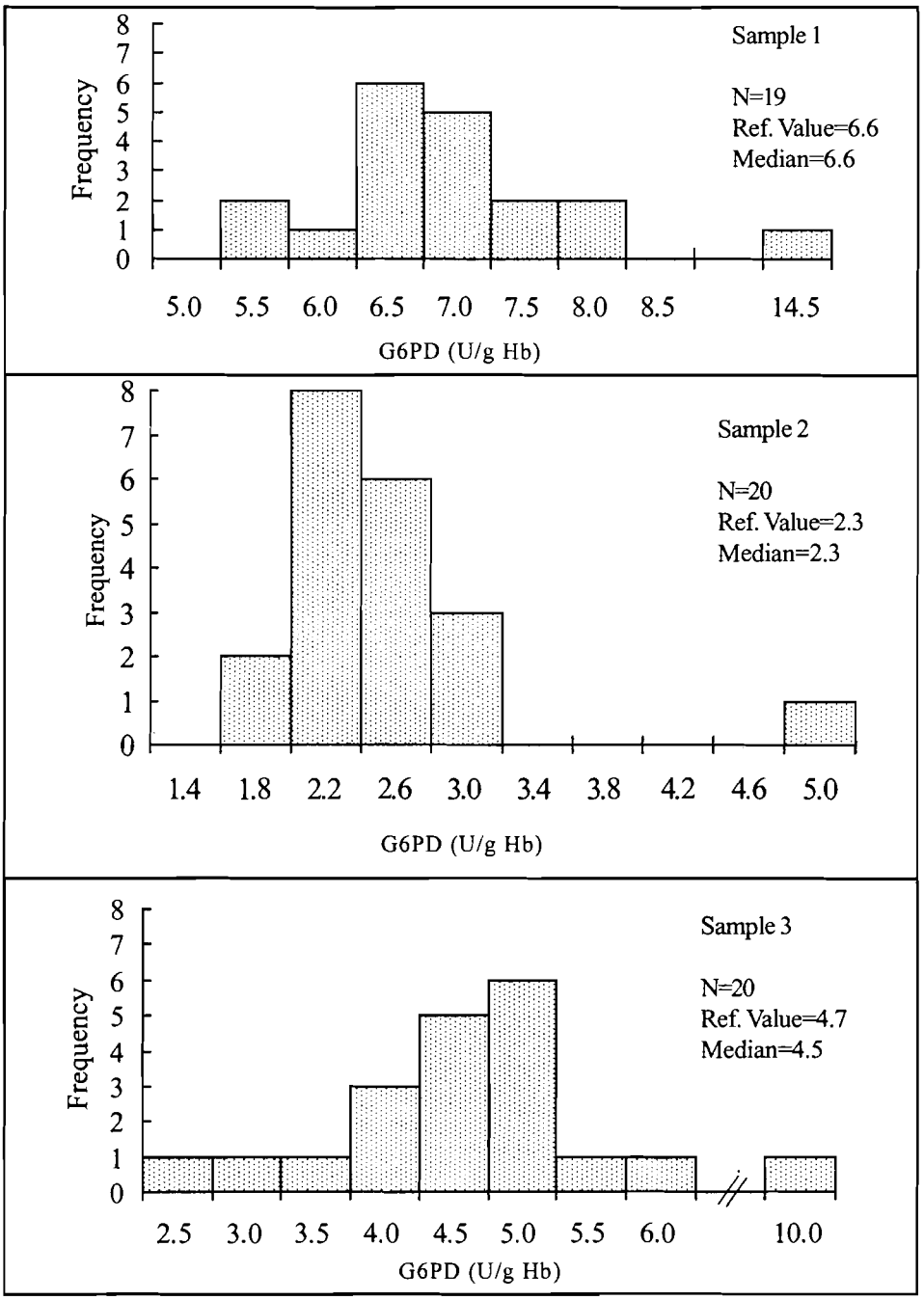

Fig 1. External Quality Control Results - G6PD Lot No. : 89-04 .

available QC materials for G6PD assay were expensive, the internal QC samples were omitted frequently to save the cost in those laboratories. In order to help those laboratories carry out internal QA properly, internal QC materials have been prepared in-house from packed RBC and delivered to 5 low workload laboratories since July 1995. The error rate was reduced in four of these 5 laboratories after receiving the internal QC materials (Table 3). Although the average error rate of the referral laboratory RH-13 remained the same after this internal

QC materials been provided (Table 3), no error was reported by this laboratory in the recent two years (data not shown).

External QA program for screening test of G6PD deficiency

In addition to the 3 neonatal screening centers in Taiwan, the neonatal screening center in Manila (Philippines), Bangkok (Thailand), Beirut (Lebanon), Guangzhou (China) and Zhanjiang (China) have also been participating in this external QA program. From March 1999 to June 2001, 15 QA surveys for G6PD screening test were performed. The participating centers received 10 QC blood spots for each survey. One hundred and eleven reports were received for these surveys and the reporting rate was 100%. Four reports (3.6%, 4/111) were found to have abnormal QA results, which contained one false negative (1/1,110) and 14 false positive results (14/1,110) (Table 4). These false positive results were

Table 1. External QA results for quantitative assay of G6PD activity.

Period	No. of surveys	Package sent	Report (rate)	Error (rate)
1988.1~1988.6	4	58	47 (81.0%)	5 (10.6%)
1988.7~1989.6	7	117	103 (38.0%)	10 (9.7%)
1989.7~1990.6	11	190	169 (90.0%)	27 (16.0%)
1990.7~1991.6	11	206	184 (89.3%)	25 (13.6%)
1991.7~1992.6	11	215	207 (96.3%)	30 (14.5%)
1992.7~1993.6	10	200	179 (90.0%)	25 (14.0%)
1993.7~1994.6	7	146	140 (95.9%)	28 (20.0%)
1994.7~1995.6	7	132	129 (97.7%)	16 (12.4%)
1995.7~1996.6	6	130	126 (96.9%)	15 (11.9%)
1996.7~1997.6	6	127	127 (100 %)	12 (9.4%)
1997.7~1998.6	6	121	119 (98.3%)	14 (11.7%)
1998.7~1999.6	6	120	120 (100 %)	15 (12.5%)
1999.7~2000.6	6	120	120 (100 %)	10 (8.3%)
2000.7~2001.6	6	120	120 (100 %)	7 (5.8%)
Total	104	2002	1891 (94.5%)	239 (12.6%)

1.1988.1-1992.6: 5 samples/package; 1992.7-2001.6: 3 samples/package
2. Errors were classified as described in the text.

Table 2. Types of error found by external QA for G6PD quantitative test.

Error type	No. of errors (rate) [1]
Clerical error	31 (13.0%)
Procedure error	41 (17.2%)
Instrument error	111 (46.4%)
Other errors	18 (7.5%)
Unknown	38 (15.9%)
Total	239

1. No. of errors / Total No. of errors
2. Labeling error, improper samples storage, bad reagent, etc.

Table 3. The error rate of QA survey before and after receiving internal QC materials prepared from packed RBC.

Referral hospital	No. of errors/ reports (rate) (1988.1~1996.6)	No. of errors/ reports (rate) (1996.7~2001.6)
RH-03	8/61 (13.1%)	2/36 (5.6%)
RH-08	5/59 (8.5%)	2/36 (5.6%)
RH-09	9/53 (17.0%)	1/36 (2.8%)
RH-13	8/59 (13.6%)	5/36 (13.9%)
RH-17	13/36 (36.1%)	5/36 (13.9%)

Table 4. External QA results for G6PD screening test.

Period	No. of surveys	Package sent	Report (rate)	Error (rate)
1999.1~1999.6	3	15	15 (100 %)	0 (0.0 %)
1999.7~2000.6	6	48	48 (100 %)	1 (2.1 %)
2000.7~2001.6	6	48	48 (100 %)	3 (6.3 %)
Total	15	111	111 (100 %)	4 (3.6 %)

1. 10 samples / package, total sample = 1,110
2. One false negative (1/1,110)
3. 14 false positives (14/1,110)

reported by 3 screening centers and all occurred in one QA survey. In this survey, 10 QA specimens with G6PD activity between 5.1 and 6.3 U/gHb (normal cutoff: > 4.3 U/gHb) were mailed to each of the 8 participating centers. The same batch of these QA specimens had been used in a different survey and the results reported were all negative from all the participating centers previously. The systematic false positive results reported by these 3 centers might be because these 3 reporting centers do not have a borderline internal QC material to compare with in their qualitative screening tests. This type of error may be improved by incorporating a quantitative assay of G6PD activity in blood spot screening samples as a backup test for the qualitative screening positive test or using the quantitative assay as the primary screening test.

DISCUSSION

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

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