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

Szu-Hui Chiang,1, BSc, Mei-Ling Fan,1, BSc, Kwang-Jen Hsiao,1, 2, PhD

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.

Key words: External Quality Assurance, Glucose-6-Phosphate Dehydrogenase, Newborn Screening

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.4-6 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.5-7 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...
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
conducted since January 1988.\textsuperscript{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.\textsuperscript{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

\textit{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.\textsuperscript{13}
The quality control (QC) materials with different G6PD
activity used for quantitative assay were prepared as
described previously.\textsuperscript{11} Briefly, the G6PD activity of
the red blood cells (RBC) was assayed.\textsuperscript{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, 10 QC specimens
were randomly picked for each survey and distributed to
each neonatal screening laboratory 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.

\textbf{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\textsuperscript{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\textsuperscript{13} to set the
reference values before the QC specimens were sent 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.

\textbf{Results}

\textit{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
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
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