External Quality Assurance Program for Neonatal Screening of Glucose-6-Phosphate Dehydrogenase Deficiency

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Introduction

- Glucose-6-Phosphate Dehydrogenase (G6PD, OMIM:305900) deficiency is most common hemolytic disease in human.
- The nationwide neonatal screening of G6PD deficiency in Taiwan was started on July 1, 1987. ***(Southeast Asian J Trop Med Public Health 1999;30:Suppl 2:72-4)***
  - The effective collection rate has reached > 99% of all newborns since 1996.
  - The overall incidence rate of G6PD deficiency is about 2%.
  - A network of referral hospitals distributed all around Taiwan was organized to provide confirmatory test, medical care and genetic counseling.
- To assess the reliability and assure the quality of the confirmatory and screening tests, an external quality assurance (EQA) program for G6PD assay has been developed.
  - Blood quantitative test (since 1988)
  - Dried blood screening test (since 1999)

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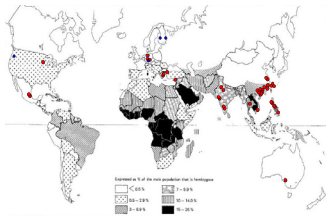
PMF EQA Program for Neonatal G6PD Screening Tests

Materials and Methods

- The QC materials were prepared from **human whole blood** by spotting on to Guthrie cards (Whatman 903).
- Periodically (~ 2 month), 10 EQA specimens were distributed to each participant by speed post
- Reports were requested to be returned within **3** days online (**7** days for overseas screening centers).
- The results of each screening center were compared with:
  - Results of all the participants
  - ***quantitative reference value*** (determined by QA Center)
- Survey Summary report available online within 15 days.

<http://g6pd.qap.tw/MS/>

Neonatal Screening Laboratories Participating in G6PD EQA Program



G6PD Deficiency Worldwide Distribution, *WHO working group. Bull WHO 1989;67:601*

- Neonatal Screening Lab **(n = 46)** :
  Australia, China (14), Germany (3), Greece (2), India (6), Lebanon, Mexico (2), Philippines (5), Switzerland, Taiwan (3), Thailand, Turkey, Unite States, Vietnam (5)
- Reagent Manufacturer **(n = 4)**

EQA Results of G6PD Screening Test at Different Ranges of G6PD Activity (1999 ~ 2015)

G6PD Activities* (U/gHb)	Specimens	Positive (P)	Negative (N)	False P	False N
0.1 ~ 2.9	4,955	4,901	54	0	**54 (1.1%)**
3.0 ~ 4.3	1,450	1,318	132	0	**132 (9.1%)**
4.4 ~ 6.0	1,292	147	1,145	147 (11.4%)	0
6.1 ~ 26.8	12,173	191	11,982	191 (1.5%)	0
Total	19,870	6,557	13,313	338 (1.7%)	**186 (0.9%)**

* EQA Reference Lab cut off value = 4.4 (U/gHb)

☛ **Most errors were found around 4.4 U/gHb (cut-off range)**

Reagent Kits of G6PD Screening Test Used by the Laboratories

Reagent Kit	n	Cut-off Value used in 2015
Bio-Rad	2	2.0
Guangzhou Fenghua	3	2.6
GSP Neonatal G6PD	1	20.5
Laboratory Prepared (Qualitative)	5	–
Laboratory Prepared (Quantitative)	4	2.0, 2.2, 2.6, 4.0, 6.2, 10
Micky	1	4.5
Labsystems Diagnostics	3	2.0, 2.6, 3.5
Perkin Elmer (ND-1000)	27	2.0, 2.1, 2.2, 2.5, 2.6, 2.9
R&D Diagnostics (OSMMR-2000D)	1	2.5
Spotcheck	4	40, 41
Trinity Biotech 203-A (Qualitative)	1	–
Zentech	1	2.0

☛ **The EQA program might be helpful for the screening laboratories to adjust their cut-off values.**

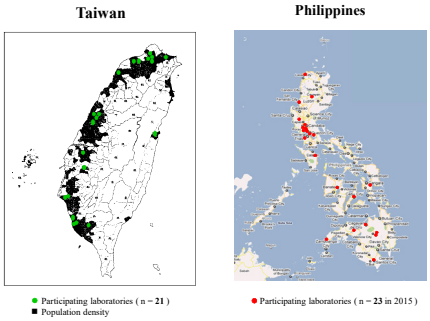
PMF EQA Program for G6PD Confirmatory Test

Materials and Methods

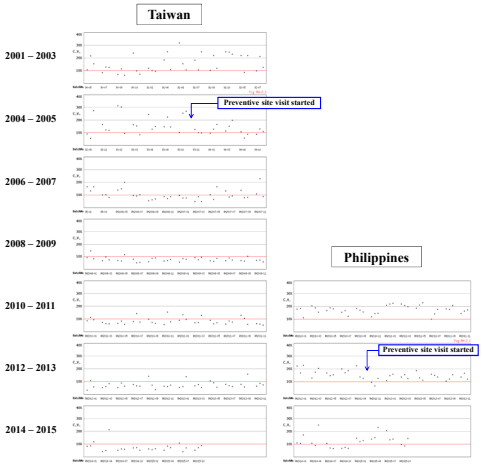
<http://g6pd.qap.tw/MS_Ph/>

- The QC materials were prepared from **human red blood cells (no extra G6PD added)**.
- Periodically (~ 3 month), 3 RBC lyophilized survey samples were sent to each participant on dry ice.
- Reports were requested to be returned within 7 days online.
- The assigned values for ***z*** scores statistical analysis are the median of all the measurement results using the same reagent kit.
- In case an **unsatisfactory** report was identified, **immediately**, troubleshooting proceeded with either telephone calls and/or visiting the confirmatory hospital laboratory.
- Survey Summary report available online within 15 days.
- All of the participants in Taiwan and Philippines are using the same kinetic methods at 37 degree C.
- All of the participants in the same country used the same reagent kit.
  - Taiwan: Trinity Biotech (previously Sigma)
  - Philippines : AMP Diagnostics (Austria)

Geographical Distribution of the Participants in EQA Program for G6PD Confirmatory Test

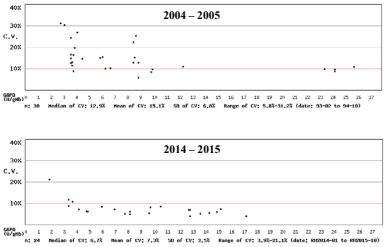


Long Term Observation of Inter Lab C.V. *vs* Survey



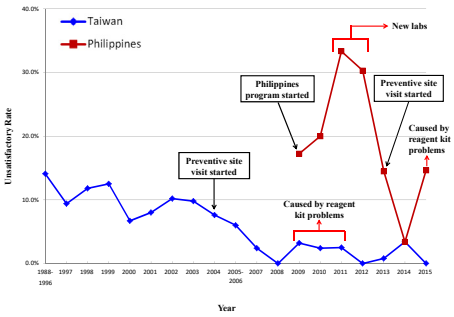
☛ **Preventive site visit was helpful for the confirmatory laboratories to prevent their problem before EQA survey.**

Comparison of Interlaboratory C.V. between the Decades in Taiwan

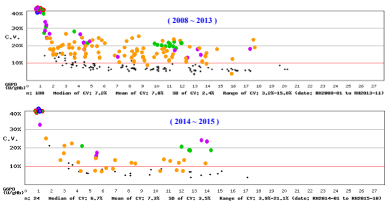


☛ **This EQA program effectively improved the interlaboratory CV from 10 ~ 30% to < 10% .**

Unsatisfactory Rate in G6PD EQA Surveys



Comparison of Interlaboratory C.V. between CAP/RCPAQAP and PMF EQA Survey



G6PD Method = Trinity reagent kit, U/g Hb, 37°C

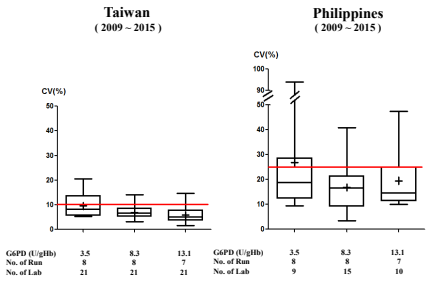
No. of Participants (CAP Program) = 32 ~ 50
No. of Participants (QAP Program) = 22 ~ 26
No. of Participants (This Program) = 20 ~ 22
No. of Participants (Philippines AMP kit Program) = 17
● ● : CV over 50%

● CAP Program
● QAP Program
● Taiwan EQA Program
● Philippines EQA Program

☛ **The interlaboratory CV of EQA program in Taiwan (most of CV< 10%) is better than in other EQA programs.**

☛ **The interlaboratory CV of EQA program in Philippines (10% ~ 20%) is lower than in CAP and RCPA EQA programs.**

Between-Run Imprecision of Each Laboratory in Taiwan and Philippines (Intermediate Measurement Precision)



☛ **Most participants within laboratory long term (6 years) CVs were < 10% in Taiwan and < 25% in Philippines.**

☛ **The prepared EQA samples are stable for at least 6 years at -70℃.**

Conclusions and Discussions

- G6PD Screening Tests
  - There are 50 worldwide Labs participating PMF EQA survey in 2015.
  - From 1999 to 2015, 0.9% false negative and 1.7% false positive results were found in the EQA surveys. (mainly caused by different cut-off values)
- G6PD Confirmatory Tests
  - Taiwan : 1988 to 2015. (21 participants in 2015)
    - < 5% unsatisfactory reports since 2007
    - Interlaboratory CV reduced to < 10% since 2007
    - Within laboratory long term (6 years) CV < 10% (median ~ 6%)
  - Philippine: 2009 to 2015. (23 participants in 2015).
    - < 15% unsatisfactory reports were found in 2015
    - Interlaboratory C.V. were between 6.6% and 25.0% (1.5 ~ 20.5 U/gHb)
    - Within laboratory long term (6 years) CV < 25% (median ~ 20%)
- Interlaboratory C.V. in Taiwan and Philippines is lower than those found in CAP and RCAP EQA programs for G6PD quantitative test using the same method.
- These G6PD EQA programs have been useful for monitoring the performance and to improve the laboratory test quality of the referral and screening laboratories.
- The EQA program might be helpful for the screening laboratories to adjust their cut-off values.

Acknowledge *: These works were partially supported by grants from Health and Welfare Surcharge of Tobacco Products, Taiwan, R.O.C.*