

External Quality Assessment in Isolation and Identification of Bacteria

Wijit Wonglumsom*, Chaniya Leepiyasakulchai and Wacharee Tiyasuttipan

ABSTRACT

External quality assessment for evaluation of individual competency in isolation and identification of bacteria for clinical microbiological laboratory was studied. Lyophilized or freeze-dried bacteria were prepared and a panel of lyophilized bacteria was shipped once a year. The first set consisted of two mixed cultures and one pure culture. Forty-three laboratories participated in this first set. The second set provided three ampoules of lyophilized mixed bacteria. Thirty-eight laboratories participated in this second set. Fifteen (34.9%) laboratories and 21 (55.3%) laboratories were correctly reported for the first and second proficiency sets, respectively. Of 33 laboratories participated in both trials, fifteen (46%) laboratories showed performance improvement. A meeting concerning on quality assurance and good laboratory practices in clinical microbiology was also provided for participating laboratories before shipment of the second panel. This pilot scheme showed a high possibility of establishing the External quality assessment scheme in bacterial isolation and identification for continuous improvement of quality assurance.

Key words: external quality assessment, bacterial identification

INTRODUCTION

External quality assessment (EQA) is a valuable tool for a laboratory to recognize analytical inadequacy and to stimulate the development of quality assurance in many countries. EQA schemes provide helpful information to individual participants involving their performance and also provide an independent check of laboratory results to establish inter-laboratory comparability permitting a comparison of quality between laboratories. Additionally, EQA programs permit comparison of different methods and instruments for the same test and thus assessment of reliability (Bartlett *et al.*, 1994, Goldie, 2001). The schemes are generally

organized by the national professional associations, professionals on behalf of a government or commercial companies. EQA programs require operating costs for provision of materials, mailing expenses and clerical time. Therefore, the programs need financial support by participants, government, or in some other ways (IFCC, 1996). Quality assurance of clinical laboratory services is emphasized in ISO 15189 and CLIA'88 (Sharp and Elder, 2004) and also required for hospital accreditation in Thailand. The Faculty of Medical Technology, Mahidol University is one of a few EQA providers in Thailand that provides EQA schemes in many disciplines of medical technology including in chemistry, tumor markers and immunology, except in microbiology.

An EQA Scheme in microbiology usually uses freeze-dried bacteria for evaluating a laboratory performance. Freeze-drying or lyophilization technique is commonly used in preservation of microorganisms for long-term maintenance. The technique involves a removal of water from frozen bacterial suspensions by sublimation and by desorption under a vacuum condition (Siberry *et al.*, 2001). A suspending fluid has a great effect on viability of freeze-drying cultures after reconstitution. It protects bacteria from mechanical and chemical damages during freezing, drying and storage (Milanovic *et al.*, 2001). In our previous study, skim milk showed a good performance in term of survival rates and structural qualities of the products (Wonglumsom *et al.*, 2004) and the optimal storage temperature of freeze-dried bacteria was at 4°C (Wonglumsom *et al.*, 2006).

The objective of this study is to perform external quality assessment for evaluating personnel competency concerning in isolation and identification of in-house freeze-dried bacteria both in the form of pure cultures and mixed cultures. The improvement of individual performance between two trials in clinical microbiological laboratories is also evaluated.

MATERIALS AND METHODS

Preparation of freeze-dried bacteria

Seventeen bacterial strains from the collection of our stock culture were cultured on blood agar or chocolate agar plates and were incubated in proper conditions at 37°C for 18 hours. Bacterial cells were harvested and suspended in sterile distilled water for adjusting cell concentration by comparing the turbidity with McFarland no.3. The suitable ratios of bacterial mixtures according to previous study (Wonglumsom *et al.*, 2004) were performed as follows; a ratio of 2:1 for a mixture of *Salmonella* Typhi and *Escherichia coli*, and a mixture of

Staphylococcus aureus and *Pseudomonas aeruginosa*; 100:1 for a mixture of *Neisseria meningitides* and *Corynebacterium diphtheriae*, and a mixture of *Streptococcus pneumoniae* and *Corynebacterium xerosis*; 1:1 for *Acinetobacter lwoffii* and *Corynebacterium diphtheriae* mixture; 50:1 for *Haemophilus influenzae* and *Corynebacterium xerosis* mixture. After suspending the bacterial suspensions in 10% skim milk (final concentration), 0.1 ml of pure or mixed cultures suspension was placed into a 0.5-ml ampoule with a gauze cap. After freezing at -80°C for at least 24 hours, the ampoules were freeze-dried for 5 hours and then sealed by a gas-sealing torch. All freeze-dried samples were stored at 4°C (for not longer than four weeks) before using as EQA materials in our scheme. For the first set that containing three EQA samples, pure culture used was *Streptococcus pyogenes* (M2) and mixed cultures were *S. Typhi* and *E. coli* (M1), *N. meningitides* and *C. diphtheriae* (T1), *A. lwoffii* and *C. diphtheriae* (T2), *H. influenzae* and *C. xerosis* (T3), *S. aureus* and *P. aeruginosa* (T4), and *S. pneumoniae* and *C. xerosis* (T5). Sample coded M1 and M2 were sent to all participated laboratories while each laboratory had received only one number of code T. For the second panel, three EQA samples of mixed cultures were used included *Listeria monocytogenes* and *Edwardsiella tarda* (A), *Acinetobacter baumannii* and *Aeromonas hydrophila* (B), and *Salmonella* Paratyphi A and *Enterococcus faecalis* (C).

External quality assessment scheme

The program entitled “the External Quality Assessment Scheme for Microbiology” (EQASM) was initially established and run for two years, 2004 to 2005 with no fee for participation. A set of freeze-dried bacteria was packed and sent by mail once a year. The participated microbiological laboratories located in various regions of Thailand. The scheme required a report of bacteria identification and identification

techniques used for isolation and identification of unknown bacteria from the participants. After data analysis and performance evaluation were investigated, an EQASM report was sent to each participant. The performance score (p-score) indicated semi-quantitative variation between results reported from a participant and the intended results from the scheme. P-score was calculated with some modification according to the guidance of International Federation of Clinical Chemistry (IFCC, 1996) and it was the expression of an individual EQA result as the deviation from the target value, divided by the standard deviation (as shown in a formula following).

$$P - score = \frac{\text{Total score} - \text{Participant score}}{\text{Standard deviation}}$$

P-score was classified into five levels: 0.00-0.50 as an excellent, 0.51-1.00 as very good, 1.01-1.50 as good, 1.51-2.00 as fair, and >2.00 as need improvement. The intended results of unknown bacteria and the score of participating reports including signified result = 2, probable result = 1, and undesirable result = 0 were also presented in the EQASM report. The identity of participants was confidential and known only to a few persons involved in the evaluation of the scheme. On June of 2005, one-day training concerning on quality assurance in clinical

microbiology, good laboratory practices of microscopic examination and cultivation, and quality assurance in susceptibility testing was provided for participants before a shipment of the second set of freeze-dried samples. The meeting was also organized for discussion on problems encountered regarding the first EQASM trial and on statistical analysis used in EQA.

RESULTS AND DISCUSSION

In the first EQASM trial, EQA materials were sent to fifty-five laboratories, while the reports of forty-three laboratories were obtained. These laboratories were located in various regions of Thailand including, ones from the North, the Northeast, the South, the East, and the Middle and in Bangkok. From the total score of 10 for three unknown samples, one pure culture and two mixed cultures, the scores of participating reports were ranged from 4 to 10. Thirty-five percent of laboratories achieved a perfect score of 10 and their performance scores were also 0.00 showing an excellent performance (Figure 1). The lowest report score of 4 found in 21% of participating laboratories.

In the second year, from fifty-one laboratories received EQA materials, thirty-eight laboratories were participated by sending their

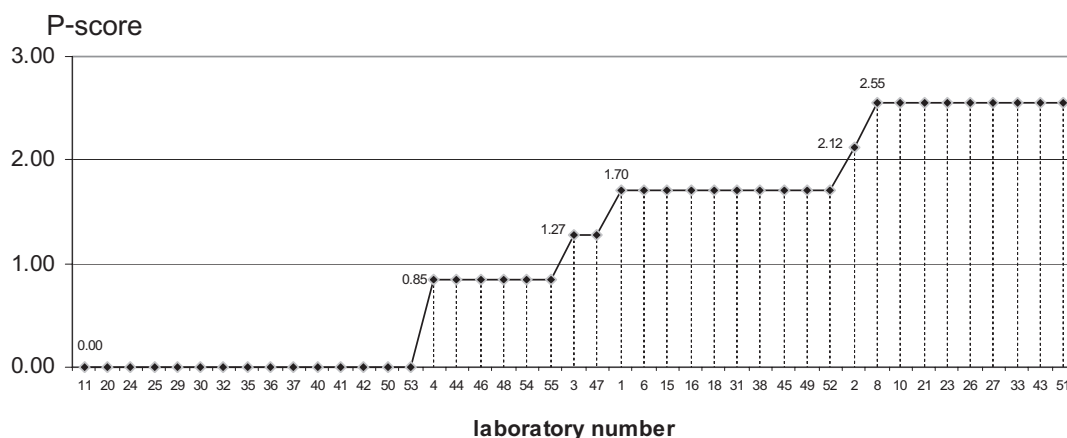


Figure 1 P-scores of participants in the first year of EQASM program.

reports back to the program. From the total score of 6 for three mixed cultures, the scores of participating reports were ranged from 0 to 6. Sixty-one percent of laboratories achieved a perfect score of 6 while 3 from 36 participants (8.3%) got a score of 0. Their performance scores were shown in Figure 2. A comparison of p-score from participating laboratories was analyzed as shown in Table 1. The better performance was found in the second year over in the first year of the scheme. In the second year, up to 79% of laboratories achieved very good or excellent competency while only 16% laboratories showed their performance of fair or need improvement. In the first year 49% of laboratories have very good or excellent competency and, almost equally, 47% of laboratories showed their performance of fair or need improvement.

Thirty-three laboratories were jointed in both trials of the scheme and their p-scores in comparison of two periods were demonstrated in Figure 3. Forty-six percent of laboratories showed improvement of the scores and 27% laboratories consistently showed excellent performance (Figure 4). Regarding laboratory techniques, all or almost participants used Gram staining and a conventional method using biochemical tests for bacterial identification. A serological technique

was generally used for confirmation or serogrouping of certain bacteria including *Streptococcus pyogenes* (code M2) *Salmonella* Typhi (code M1), *Haemophilus influenzae* (code T4), and *Salmonella* Paratyphi A (code C). Most participants used the serological technique for EQA sample code M1 and code C, while a few laboratories used for grouping of bacteria code M2 and code T4. Interestingly, a using of rapid identification techniques including commercial rapid identification kits or an automated system showed slightly increasing in the second year.

Ninety-one percent of participants could identify *Streptococcus pyogenes* and most laboratories also were able to correctly report *Salmonella* Typhi, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Corynebacterium* species. The ability for identification of *Acinetobacter baumannii*, *Salmonella* Paratyphi A and *Listeria monocytogenes* was found in 84%, 79% and 68% of participants, respectively. Some laboratories could not identify *Neisseria meningitides*, *Haemophilus influenzae* and *Streptococcus pneumoniae*. The results were similar to the report of Bartlett *et al.* (1994) that participants experienced with difficulty in identification of these miscellaneous and fastidious bacteria. Errors

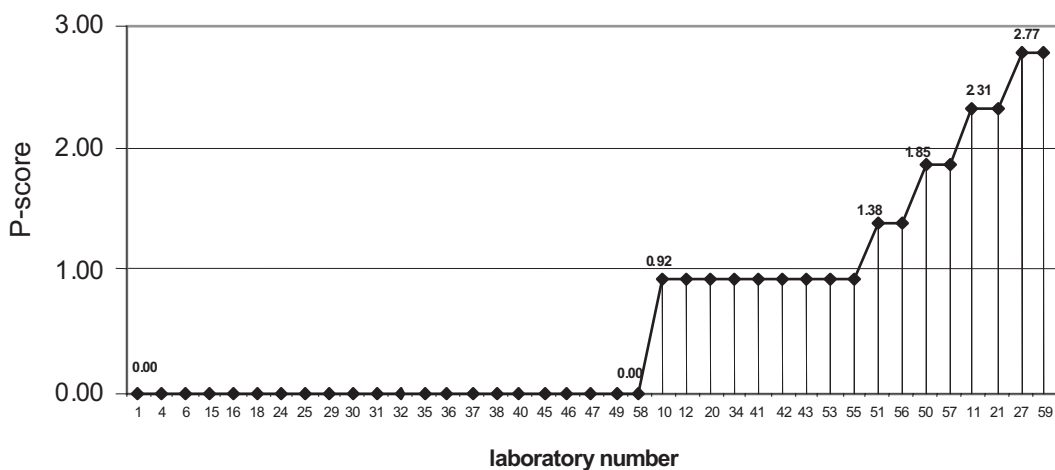


Figure 2 P-scores of participants in the second year of EQASM program.

for proficiency tests of *Haemophilus influenzae* could be a result of using improper media and susceptibility testing materials (Snell *et al.*, 1986). In our study most laboratories could precisely identify a pure culture of *Streptococcus pyogenes*, while their accuracy rates were decreased when

identifying freeze-dried samples of mixed cultures. The problems of distribution of samples by transportation for a long distances, such as to the Northern parts of Thailand, might affect the quality of EQA materials (Pritchard, 1987) and storage temperature could cause greater changes in

Table 1 P-score classification of participants attending in the first and second year of EQASM program.

P-score	No. in first year	No. in second year
0.00-0.50 (excellent)	15 (34.9%)	21 (55.3%)
0.51-1.00 (very good)	6 (13.9%)	9 (23.7%)
1.01-1.50 (good)	2 (4.6%)	2 (5.3%)
1.51-2.00 (fair)	10 (23.3%)	2 (5.3%)
>2.00 (need improvement)	10 (23.3%)	4 (10.5%)
Total numbers of laboratories	43	38

Note: the number in parenthesis is represented to percent of laboratories classified by P-score.

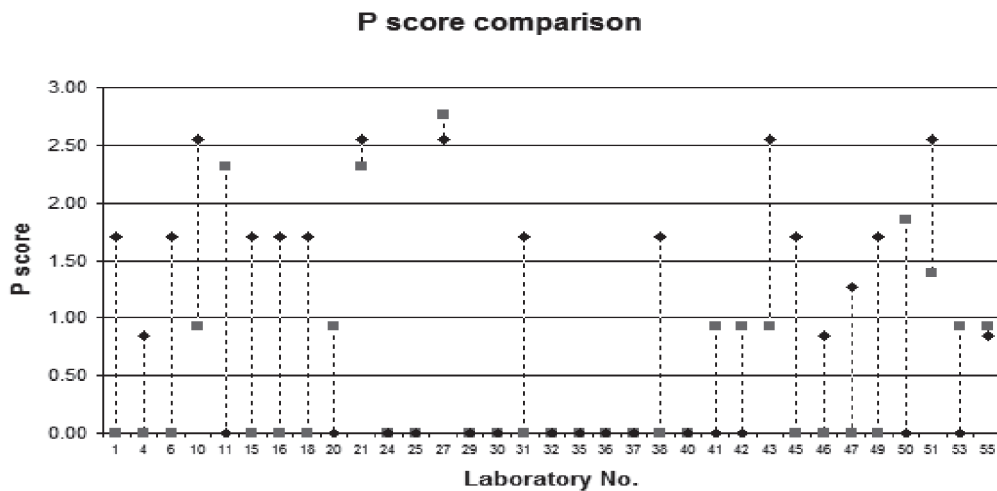


Figure 3 P-score comparison of each participant in the first year (u) and second year (n) of EQASM program.

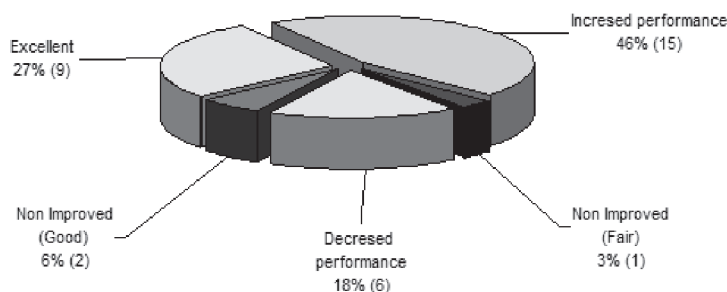


Figure 4 Ratios of laboratory performance in two years of EQASM program.

bacterial viability than did level changes of residual water in the cells (Betty *et al.* 1974). Therefore, improper temperature of transportation by mail and storage temperature at participating laboratory may reduce isolation and identification results.

CONCLUSION

This pilot EQASM scheme using in-house, freeze-dried samples showed a possibility to establish the External Quality Assessment Scheme for evaluating a performance of bacterial isolation and identification. Additionally, implementation with a meeting should improve participating competency.

ACKNOWLEDGEMENTS

We thank Ms. Orawan Rattanapisanwong for analytical support of the research. Financial support of this work was provided by Mahidol University, Bangkok, Thailand.

LITERATURE CITED

- Bartlett, R.C., M. Mazens-Sullivan, J. Z. Tetreault, S. Lobel and J. Nivard. 1994. Evolving approaches to management of quality in clinical microbiology. **Clin. Microbiol. Rev.** 7: 55-88.
- Betty, J., G. Marshall, G. Coote and W. J. Scott. 1974. Some factors affecting the viability of dried bacteria during storage In vacuo. **Appl. Microbiol.** 27: 648-652.
- Goldie, DJ. 2001. Accreditation of external quality assessment schemes in the United Kingdom. **Clin. Chim. Acta.** 309:179-181.
- International Federation of Clinical Chemistry (IFCC). 1996. **Fundamentals for External Quality Assessment (EQA)**. Guidelines for improving analytical quality by establishing and managing EQA schemes. Examples from basic chemistry using limited resources. <http://www.ifcc.org/divisions/EMD/Documents/Fundamentals-for-EQA.pdf> [Accessed 2006-05-23]
- ISO 15189. 2003. Medical laboratories-particular requirements for quality and competency.
- Milanovic, N.T., A. Kodzic, J. Baras and S.D. Brankovic. 2001. The influence of a cryoprotective medium containing glycerol on the lyophilization of lactic acid bacteria. **J. Serb. Chem. Soc.** 66: 435-441.
- Pritchard, R. C. 1987. A medical microbiology quality assurance program. **Malays. J. Pathol.** 9:9-11.
- Sharp, S.E. and B.L. Elder. 2004. Competency assessment in the clinical microbiology laboratory. **Clin. Microbiol. Rev.** 17: 681-694.
- Siberry, G., K.N. Brahmadathan, R. Pandian, M.K. Lalitha, M.C. Steinhoff and T.J. John. 2001. Comparison of different culture media and storage temperatures for the long-Term preservation of *Streptococcus pneumoniae* in the tropics. **Bull. World Health Organization** 79: 43-47.
- Snell, J. J. S., J. V. DeMello and T. J. Phua. 1986. Errors in bacteriological techniques: results from the United Kingdom national external quality assessment scheme for microbiology. **Med. Lab. Sci.** 43: 344-355.
- Wonglumsom, W., N. Waipathom, N. Toksa, W. Tiyasuttipan and C. Leepiyasakulchai. 2006. An evaluation of storage temperature on viability of bacterial mixture after freeze-drying. **J. Lab. Med. & Quality Assurance** 28; S3: 484-489.
- Wonglumsom, W., S. Krutsin, S. Nunpan, W. Tiyasuttipan and C. Leepiyasakulchai. 2004. An Evaluation of Suspending Media in Freeze-Drying of Bacteria. **J. Lab. Med. & Quality Assurance** 26; S3: 504-508.