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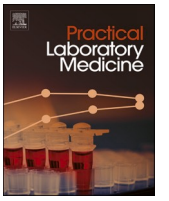

Model of implementing proficiency testing in Vietnam, a developing country

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ABSTRACT

Backgrounds and aims: The aim of this study is to provide a good approach for a quantitative EQA scheme assigned value with limited resources.
Materials and methods: Twelve lyophilized EQA items were distributed to participants in 2021 from North to Southeast Vietnam to measure the concentration of nine parameters, including glucose, urea, creatinine, cholesterol, triglyceride, uric acid, AST, ALT, and GGT. The consensus value of the expert group and all participants were calculated and statistically compared to choose the most appropriate consensus value.
Results: Fifty-nine laboratories attended the EQA scheme, including an expert group using automatic biochemistry analyzers (AAs) and all participants with auto and semi-auto biochemistry (SAA) analyzers. Consensus values of six per nine parameters were different between the two groups for at least two EQA items, including glucose, creatinine, cholesterol, uric acid, AST, and ALT. The coefficients of variation of glucose, urea, creatinine, triglycerides, uric acid, and GGT in the expert group were significantly lower than those in all the participants.
Conclusion: Using the consensus values of expert groups as the assigned values of the EQA program is a relevant strategy to increase testing quality in developing countries with limited resources, such as Vietnam.

1. Introduction

The external quality assessment (EQA) scheme is an essential component of a laboratory's quality management system, and EQA is one of the important criteria for clinical laboratory accreditation requirements in the clinical laboratory [1]. An EQA involves testing the identical control sample in more than one laboratory for results comparison [2]. In this context, the control sample must be analyzed under the same conditions as the patient's samples to ensure the quality of the testing system. Essential roles of EQA, in addition to monitoring and documenting the analytical quality, are identifying inferior performance, detecting analytical errors, and taking corrective actions. In addition, participation in EQA evaluates the performance of the individual laboratory regarding the different methods and instruments. In EQA schemes, the quality of samples, including homogeneity and stability, is crucial.

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Furthermore, the assigned value of all parameters in the sample is a crucial aspect. According to ISO 13528, to calculate the assigned value, there are three main approaches [3]:

(1) From a single reference laboratory that uses the primary reference method.
(2) Consensus value from expert laboratories' results.
(3) Consensus value from participants' results.

The first approach is the best choice to give an accurate result, regardless of the uncertainty of the method in the participating laboratory. Nevertheless, it is expensive and unavailable in developing countries such as Vietnam and other countries because of the lack of reference labs. In the second approach, the expert laboratories conform to national criteria, accredited international standards, and continuous, reliable performance in EQA schemes that use routine methods. In developing countries, the laboratories classified from different rankings attend the EQA program, which may cause bias in the assigned value. Moreover, the lab quality varies from the lab with low-tech infrastructure to the modern lab with an automation system accredited by ISO 15189, CAP, or JCI. Therefore, the consensus value from them is a big question. In Vietnam, the Quality Control Center for Medical Laboratory of University of Medicine and Pharmacy at Ho Chi Minh City (UMP) is the agency responsible for implementing EQA in laboratories extending from the Southeast region to Central Highlands provinces. At the national level, the Prime Minister signs the document that requires harmonizing the routine tests [4]. The EQA is one of six pillars in the temple of laboratory standardization and plays a crucial role in harmonization by ensuring the evaluation and monitoring of the comparability of test results across different laboratories and over time [5,6].

In this study, we evaluated the assigned value of the clinical chemistry EQA program from consensus value expert laboratories or participants to determine the best choice for testing harmonization in developing countries such as Vietnam.

2. Methods

2.1. Materials

The EQA samples originated from the serum of donors screened for HIV ½, HBsAg, HCV Ab, malaria, and syphilis at the Blood Transfusion Hematology Hospital in Ho Chi Minh City. Approximately 200 ml of human serum was separated from a 450 ml blood bag without an anticoagulant. The EQA scheme includes nine parameters: glucose, cholesterol, triglyceride, urea, creatinine, uric acid, ALT, AST, and GGT. These parameters have been the most laboratories enrolled in the biochemistry EQA [7]. These parameters in the serum were determined by a Beckman Coulter AU 480. The concentration of these parameters was adjusted to a predetermined value by spiking directly pure materials supplied from Sigma (D-(+)- glucose,99.5% (GC); glycerol – ACS reagent, 99.5%; urea powder bioreagent for molecular biology; creatinine anhydrous (≥98%); uric acid crystalline (≥99%); glutamic-pyruvic transaminase from porcine heart (200 units); glutamic-oxaloacetic transaminase from porcine heart (1000 units); glutamyltranspeptidase from the equine kidney (100 units). Glassware including measuring cylinders, flasks, conical flasks, and vials was washed and rinsed with distilled water and dried at 130 °C for 30 min for sterilization and keep in an aseptic condition before use. During this period, all steps were prepared in the clean room at a temperature of 16–20 °C, and steps of serum distribution into vials were finished in the biological safety cabinet level 2. The whole procedure took about three to 4 h. The samples were then pipetted into 2 ml amber glass vials. These vials were lyophilized and then assessed for homogeneity and stability according to ISO 13528 (fixed with an aluminum plastic cap). For homogeneity assessment, 10 vials were chosen randomly. Then, these vials were reconstituted according to a guideline inserted in the package. Each vial was analyzed for nine parameters in replicate. The general average ($\overline{x}$), the between-sample standard deviation (Ss) was calculated, if Ss was equal to or less than 0.3 times the standard deviation for proficiency assessment (σpt), the samples in this batch were homogeneous. For stability assessment, 3 vials were chosen randomly on the first day after the closing date, nine parameters in each vials were analyzed in replicate. The average ($\overline{y}$) was calculated, the samples considered stability as the absolute difference between $\overline{x}$ and $\overline{y}$ equal to or less than 0.3 times σpt [3]. The homogeneity and stability assessments were performed by Beckman Coulter AU480 with reagents and calibrator supplied by the instrument manufacturer. All samples were stored in the refrigerator at 2–8 °C. The levels of the sample parameters fall within the medical decision points and analytical range to check the competence of participants [8]. To challenge them, the parameter concentrations in the samples were selected to represent pathological conditions such as diabetes, chronic kidney disease, metabolic lipid disorders, liver dysfunction, and gout [2]. In 12 different samples, each sample was made from one identical donor's serum. Four different samples are prepared and shipped to each participant every four months. All storage EQA samples were kept in the refrigerator at 2–8 °C in a central location as well as at testing sites.

The vials were packed in 3 layers for transportation: the first was the carton box with the packed insert, the next was a plastic box, and the last was a Styrofoam box with eight ice packs. The time to dispatch the EQA sample to participants ranged from one to three days. At the predetermined time point, the sample will be dissolved in 2 ml of distilled water, kept for 15 min in a temperature room, and gently swirled for 5 min before testing. The results were collected over twelve months. Every two months, 2 different samples with various levels of parameters were measured under the same conditions as the patient's samples.

2.2. Methods

This study included two groups: the first was an expert group that included laboratories with continuous, reliable performance in EQA schemes that are accredited according to international standards such as ISO 15189:2012, JCI, or CAP and ranked level 4 or

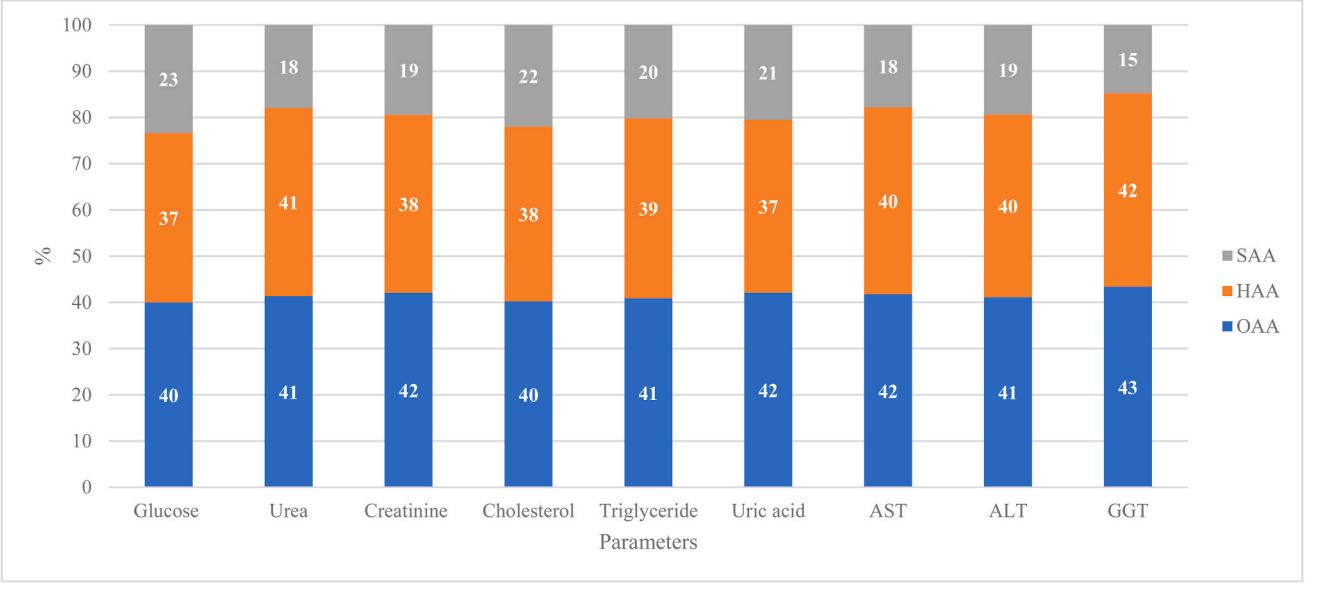

Fig. 1. The average percentage of the homogenous systems (HAA), the heterogeneous system (OAA), and semi-auto biochemistry analyzer (SAA) in all participant laboratories.

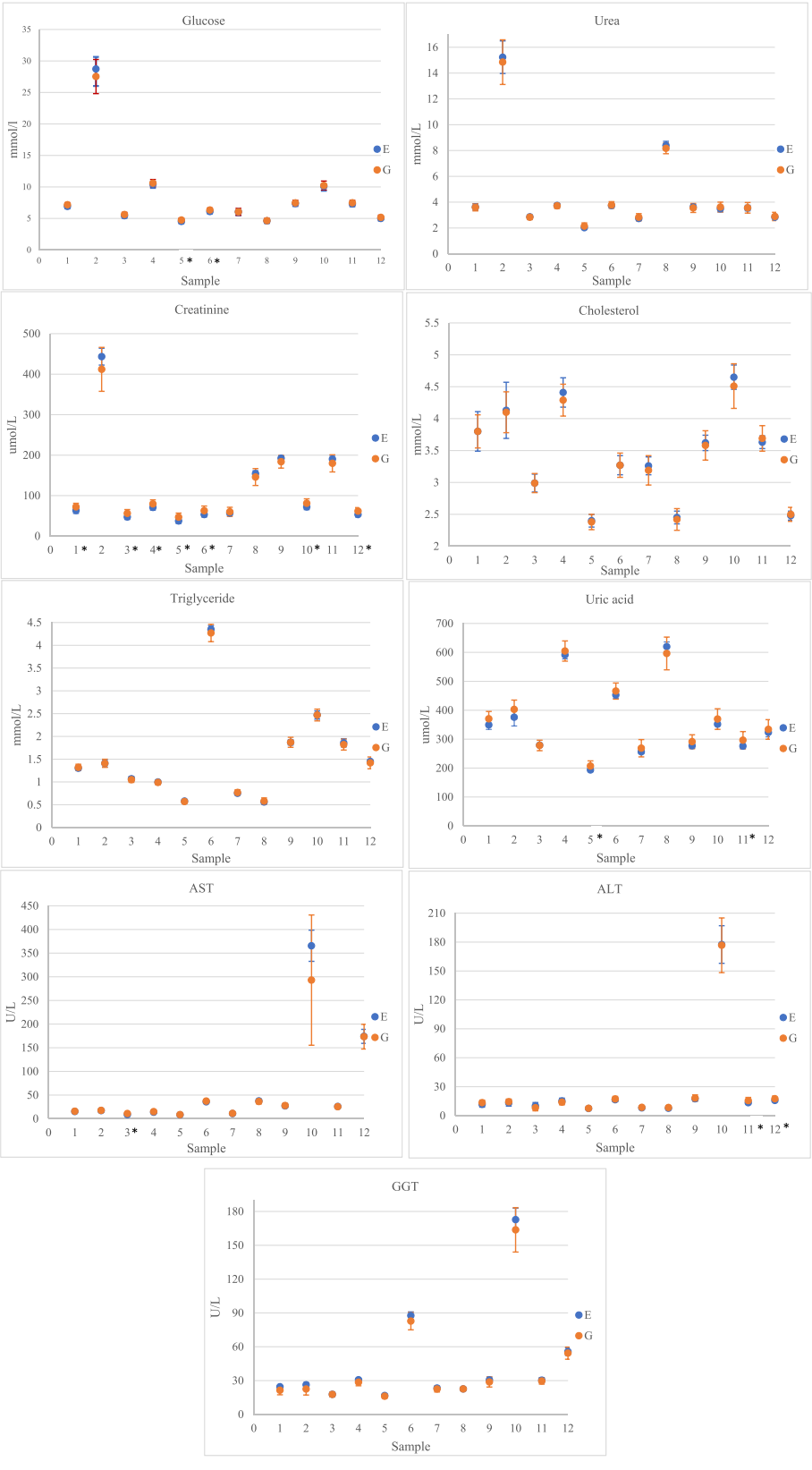

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Fig. 2. The consensus values of 9 parameters (the name of parameter shown in each chart) from the expert group (E) and all participants group (general: G) (12 samples). (*) indicate that the difference between the consensus value between the expert group and all participants significantly, with $p < 0.05$.

higher in national criteria for medical laboratory quality [9]. The second is all participants who registered for this EQA program. The Ethics Committee has approved this study at the UMP. The study was carried out from January to December 2021.

Every two months, the participants submit their results on the website qccump.com. All results were converted to the International System of Units before processing the data. After defining the outlier by the interquartile range, the assigned value was calculated using the consensus value from the whole group (general group - AVg) and the expert's group (AVe). The difference between AVg and AVe was evaluated by *t*-test, and each group's standard deviation and coefficient of variation were calculated. The standard deviation index (SDI) was used to assess the performance of participants [3]:

$$\mathrm{SDI} = \frac{participant\ value - concensus\ expert\ group\ mean}{Concencus\ group\ standard\ deviation}$$

The result of participants is unacceptable when the SDI is out of range [−2;2].

The bias of the consensus value from the expert group to all participants is calculated as follows:

$$\mathrm{Bias}\ (\%) = \frac{concensus\ all\ participants\ mean - concensus\ expert\ group\ mean}{concensus\ expert\ group\ mean}\ \mathrm{x}100$$

To assess the interlaboratory precision of the expert group and all participants, the coefficient of variation (CV%) was calculated:

$$CV(\%) = \frac{SD}{\mathrm{mean}} \times 100$$

The difference between the CV% of the two groups in twelve samples was determined by ANOVA.

During the enrollment period, information about parameter evaluation, such as methods, reagents, and instruments, was filled out on an enrollment form. In this study, the devices were grouped as semi-automatic analyzers (SAA) and auto analyzers. The auto analyzers have two systems, the homogeneous system (HAA) which refers to reagents and calibrators that were recommended by the instrument supplier, and the heterogeneous system (OAA) which obtains at least one component of the measuring system, reagents, or calibrators from other sources than the instrument's original supplier.

2.3. Statistical analysis

All data were collected and processed by Microsoft Excel in Office 365, including the mean (assigned value), bias (%), and pass rate. Stata 14 was used to perform grouping statistics for the independent sample's *t*-test and ANOVA. A value of $p < 0.05$ was considered statistically significant.

3. Results

The maximum number of participants was 59 in the study, but the expert group ranged from 5 to 11, the other participants ranged from 28 to 49, and the average number of participants ranged from 39 to 55 for different parameters (Table S1). The expert group uses homogeneous systems including four Beckman Coulter systems, three Architect systems, two Advia systems, and two Roche Cobas systems with the same method, and traceability. On the other hand, the rest of the group used automated and semi-automatic analyzers. In the participants' group with the auto analyzer, the percentage of homogeneous systems is similar to that of heterogeneous systems about 40%, the semiauto biochemistry analyzer takes approximately 20% (Fig. 1).

The number of laboratories increased from the 1st sample to the 12th sample in the expert group and all participants (from 49 to 59) (Table S1). Although nine parameters were performed on every sample by each expert laboratory, the number of parameters performed by other participants depended on their demands. Glucose is the most performed parameter; on the other hand, GGT is the least completed parameter in all participants.

Fig. 2 shows the consensus values of the expert group and general group. Seven per twelve consensus values of creatinine in the general group were significantly higher than those in the expert group at the reference range. In the abnormal range, the consensus value of the general group of creatinine is seemingly less than the expert group but insignificant. One to two per twelve consensus values of glucose, cholesterol, uric acid, AST, and ALT in the general group were more significant than those in the expert group. The consensus values of the general group and expert group for urea, triglyceride, and GGT were similar.

The percentage of participants using SAA who obtained unacceptable EQA results is depicted in Fig. 3A. The percentage of participants using HAA and OAA having unacceptable EQA results is substracted respectively by 100% from these values. A high proportion of unacceptable EQA results come from participants using the semi-auto biochemistry analyzer (especially glucose, urea, cholesterol, AST, and ALT). All unacceptable cholesterol EQA results in sample 1 are from participants using SAA. On the other hand, the group using SAA performs well in GGT at samples 4, 5, 7, 8, and 9 with zero unacceptable results (Fig. 3A), but the heterogenous system gave nearly 70% unacceptable results (Fig. 3B). In all EQA samples, the group with homogeneous systems got unacceptable EQA results at least. Generally, in the heterogeneous system, SAA obtains unacceptable results more than in the homogeneous system.

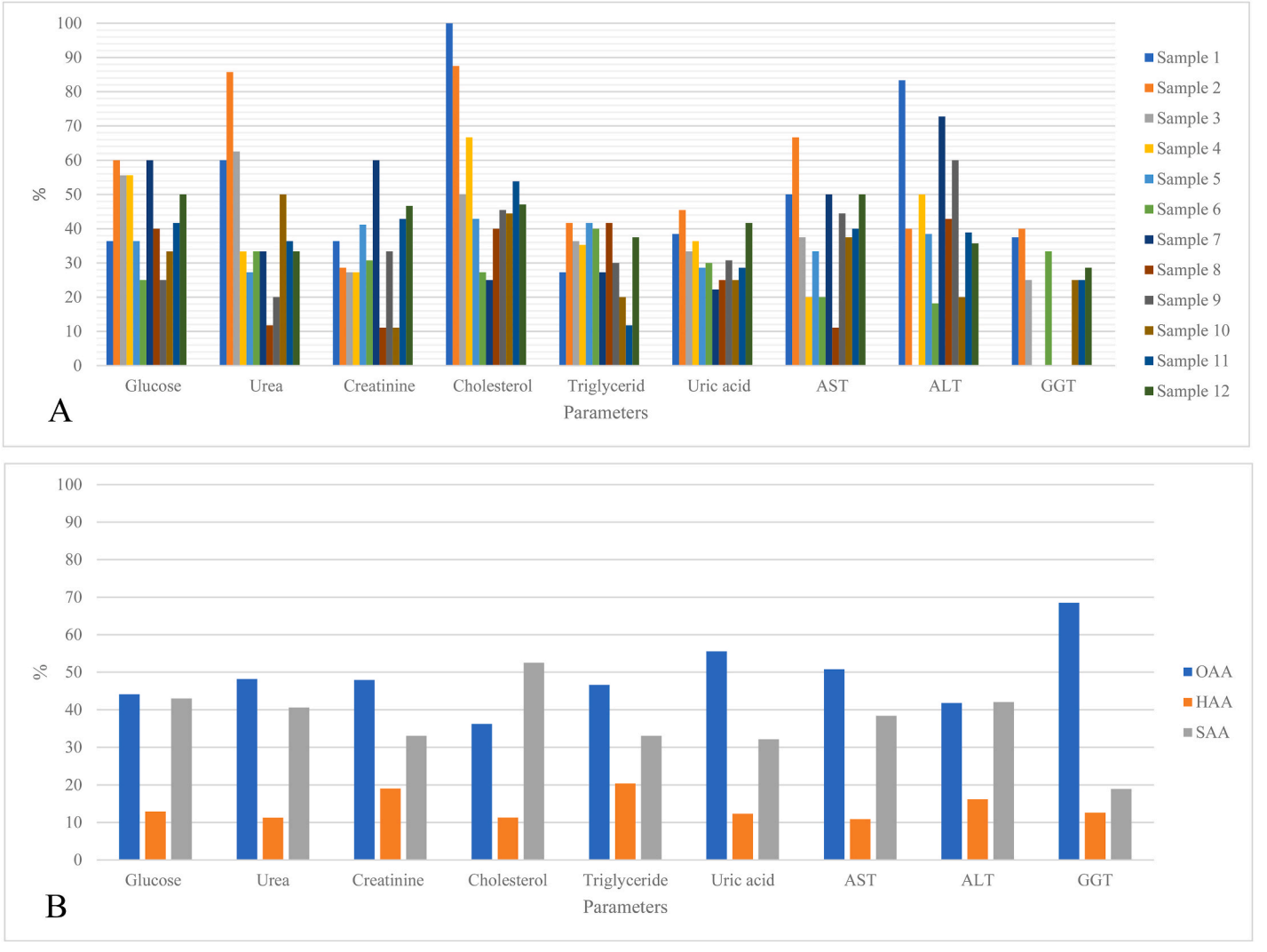

Fig. 3. (3A) The percentage of unacceptable EQA results come from semi-auto biochemistry instruments of nine parameters (12 samples); (3B) The average percentage of unacceptable EQA results come from homogeneous system (HAA), the heterogeneous system (OAA) and semi-auto biochemistry analyzer (SAA).

Table 1
Coefficient of variation (CV%) of the expert group and all participants.

Sample		1	2	3	4	5	6	7	8	9	10	11	12	p
Glucose	E	3.65	6.63	2.25	3.69	3.15	2.98	2.84	3.33	3.28	3.05	3.14	3.72	<0.001
	G	4.64	9.85	6.01	5.51	6.56	5.08	9.07	7.75	5.45	6.88	5.37	6.93	
Urea	E	6.75	8.29	1.83	3.78	4.49	3.29	4.18	3.77	5.63	7.24	5.85	4.62	<0.001
	G	7.80	11.67	6.30	5.39	11.87	6.96	9.81	5.17	9.94	10.44	11.51	11.05	
Creatinine	E	11.21	4.69	12.63	9.30	15.68	11.05	9.07	4.67	3.03	9.01	5.03	11.13	<0.001
	G	12.34	13.2	17.23	12.79	21.74	18.73	18.83	14.34	8.54	13.78	11.80	14.31	
Cholesterol	E	8.15	10.55	4.76	5.26	4.16	4.63	4.28	4.20	3.23	4.09	2.71	2.75	0.084
	G	6.74	7.75	5.12	5.75	4.99	5.93	7.32	7.20	6.55	7.75	5.52	4.38	
Triglyceride	E	1.69	5.73	4.95	3.61	4.70	1.75	2.51	6.15	3.21	3.66	4.66	3.66	0.001
	G	5.26	6.11	5.81	4.98	6.56	4.41	7.42	11.98	5.71	5.34	6.55	9.5	
Uric acid	E	4.42	7.98	2.23	2.31	3.27	2.64	2.32	2.64	3.04	1.86	3.24	4.68	<0.001
	G	6.94	8.08	6.52	5.78	8.73	5.98	11.17	9.5	8.25	9.65	10.07	10.15	
AST	E	15.69	19.48	17.47	9.19	11.82	8.64	16.78	10.39	8.52	9.01	11.46	8.53	0.054
	G	13.69	17.07	19.07	16.21	17.35	9.72	22.20	13.29	15.83	46.98	13.72	15.04	
ALT	E	24.96	25.54	33.2	19.48	13.96	10.79	20.69	22.9	11.72	10.99	12.79	7.56	0.389
	G	20.74	19.96	39.51	19.66	19.17	15.33	16.43	23.45	18.39	16.04	21.04	15.65	
GGT	E	3.86	7.32	9.54	3.98	7.05	3.76	7.26	4.97	8.29	5.92	7.54	5.97	<0.001
	G	18.76	23.60	12.26	11.25	11.23	9.32	11.3	7.24	15.59	12.03	9.11	9.89	

E: expert group; G: all participants.

The coefficient of variation (CV) of the expert group and all participants is depicted in Table 1. The expert group's CVs of glucose, urea, creatinine, triglyceride, uric acid, and GGT were significantly lower than those of general participants. The CVs of cholesterol, AST, and ALT in both groups were similar. However, the CVs for the enzymes AST and ALT were more significant than those for the other parameters.

In Fig. 4, the bias of glucose, urea, cholesterol, and triglyceride in the general group oscillated in a narrow range, almost near 5% on two sides. On the other hand, the biases of creatinine, uric acid, AST, ALT, and GGT are more visible than the others in which GGT has a negative value in all twelve samples. At high concentrations, negative bias was met for glucose and creatinine, while the other parameters had two sides. For glucose, the level of negative bias is near the upper linearity range (28.73 mmol/L). For creatinine parameter, four high-concentration samples range from 154,16 to 443,2 μmol/L.

4. Discussion

Developing countries such as Vietnam have diverse rates from small laboratories with a lack of high-quality technicians on the Vietnam Central Coast and Central Highlands to modern laboratories in large cities. Many analytical variations affect the assay system, including staffing, environment, and characteristics of the methods. Human factors need to be removed to limit the variables. Therefore, many modern laboratories invest in fully automatic biochemistry analyzers to keep variables at a minimum; all laboratories in expert group sites in one of the largest cities in Vietnam are Ha Noi, Ho Chi Minh City, and Da Nang [10]. In contrast, in developing countries such as Vietnam, especially in the Central Coast and Central Highlands, with poor infrastructure, some laboratories still use the semi-auto biochemistry analyzer that covers human variable factors (Fig. 1).

On the other hand, the lyophilized samples were delivered to ensure quality and stability under normal conditions because of the complex geographical topography of the management area and transportation difficulties. In Brazil, the National Program of Quality Control sent the lyophilized EQA samples to participants to maintain superior quality conditions [11].

Depending on the area's characteristics, the EQA providers make suitable decisions to determine the assigned value [3]. The consensus from the expert group was used as an assigned value, especially in developing countries where the quality management system of the laboratories was not accredited against ISO 15189. In the study of S.K. Wong, the deviation of the assigned value from the true value could be as large as 40%, depending on sample homogeneity, the number of participant laboratories, concentration levels of the sample, method characteristics, and laboratory bias [12]. For example, in the creatinine assay (Fig. 2), there were 7 per 12 different consensus values between the two groups. Moreover, the consensus value of the general group shows a negative bias toward the expert group in the reference range (Fig. 4). Nevertheless, vice versa, the abnormal range may cause a biased calculation of the estimated glomerular filtration rate [13]. The difference can be explained by the poor quality control plan and the unimplemented method verification of some participants in the non-expert group. In this case, the expert group makes the best choice for the assigned value. In some cases, spiking pure material to get pathology levels in EQA samples may cause matrix effects, and bias due to matrix effects in EQA samples has the potential to affect the analytical performance specification of participants. In this study, the commutability of EQAS samples (5 samples including high-level samples) was checked following CLSI EP 14 A3 between two procedures: the homogeneous system is Beckman Coulter AU480, and the semi-automatic system is Teco Diagnostic TC 3000 with Cormay reagents. The result of the study showed that there was no matrix effect with patient samples. Because the EQA samples are not assessed by all

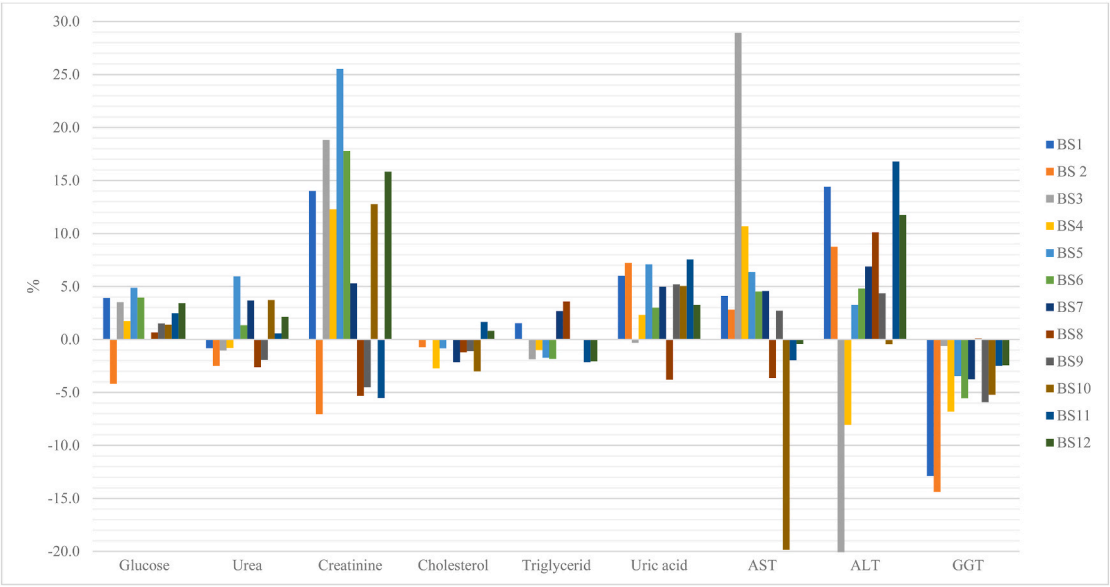

Fig. 4. The bias of the consensus value from all participants to the expert group of nine parameters. (BS: bias sample).

measurement procedures, spiking is a possible cause of the dispersion.

On the other hand, Fig. 2 showed that glucose, uric acid, and ALT have two different consensus values, and cholesterol and AST have one different consensus value between the two groups without any trend. There was no difference in consensus value between the two groups in urea, triglyceride, and GGT parameters. In general, the expert group's consensus values are the suitable choice for assigned values in Vietnam. Many examples use the assigned value from the reference procedures and consensus values from expert groups in the EQA scheme because participants' results might be biased on average [7,14–19].

The CV% shows the extent of variability in the population's mean. Therefore, higher CV% of the expert group on cholesterol, AST, and ALT in samples 1, 2, and ALT in sample 7 were observed. The reason could be explained firstly by the low activity of AST, ALT in samples 1, 2, 7 (15.1-11.6 U/L; 17.1-13.1 U/L; 8 U/L, respectively) and secondly due to the lowest number of participants in the expert group in samples 1 and 2 in this research. So the number of participants in the expert group should be more than 5.

Although the number of participants in the expert group was less than five times the number in the general group, the CV% in the expert group was lower than that in the general group, except for cholesterol, AST, and ALT (Table 1). The CV% in the expert group may achieve the performance standard in the developed countries in glucose, urea, cholesterol, triglyceride, uric acid, and GGT due to the quality of this group achieving the international standard [20]. With CV% of creatinine, ALT, and AST, there was no difference between the two groups, but these CV% were less than the CV% of the same analytes in the study in Bhutan [17]. Although there was a change in the number of participating laboratories in both groups, the CV% of analytes in the programs did not change significantly, especially for glucose testing, so the change in the number of participating laboratories did not affect the research results.

The limitation of this study is comparing the two consensus values of different parameters between the expert group and all participants group. In some cases the experts represented one-quarter to one-third of all participants so the difference between the two groups is relatively small, the bias may be appreciable. Another limitation of this study is that clinically highly important electrolytes (Na, K, Cl, and bicarbonate) were not included in this study. The study of these electrolytes will be carried out in our next research.

Similar to developing countries, out of 59 participants, the percentage of SAA in this EQA program ranges from 14.46% to 23.37%, depending on the parameter in Fig. 1. Although with the development of medical technology, automatic analyzers account for a large proportion in developed countries, in developing countries such as Bhutan, SAA still maintains a significant position in biochemistry laboratories with 17/19 analyzers [17,21]. Another reason is the size of the laboratory and the capacity of SAA to meet the demand of district hospitals, clinics, and health centers with limited tests with 10–30 patients per day. Generally, the participants with SAA obtained unacceptable results more than those with HAA, and OAA, especially for cholesterol, glucose, urea, ALT, and AST (Fig. 3). The reason may be caused by human variation, such as an inconstant incubation time for reactions, reagents, and sample pipetting. Furthermore, the participants with heterogeneous systems need to validate the measuring procedure before implementing the service, this step is still lacking in laboratories with poor-quality management systems potentially causing errors.

In conclusion, with the limited conditions of EQA for clinical chemistry programs in developing countries such as Vietnam, using the consensus values of expert groups as the assigned values of the EQA program is a suitable strategy to increase the quality of testing.

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CRediT authorship contribution statement

Hy Triet Van: Conceptualization, Methodology, Validation, Writing – original draft. **Van Thanh Tran:** Writing – review & editing. **Manh Tuan Ha:** Data curation. **Quang Huy Vu:** Visualization, Investigation, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plabm.2023.e00339.

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