Journal of Advances in Medicine and Medical Research

32(5): 145-152, 2020; Article no.JAMMR.56169
ISSN: 2456-8899
(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)

PRE-EQC: A Proficiency Testing Program for Pre-Analytical and Analytical Monitoring Using Pooled Samples without Preservatives

Shyamali Pal[1*]

[1]JMD Diagnostics Private Limited, P-336, CIT Road Scheme VIM, Kolkata- 700054, India.

Author's contribution

The sole author designed, analyzed, interpreted, and prepared the manuscript.

Article Information

DOI: 10.9734/JAMMR/2020/v32i530423
Editor(s):
(1) Dr. Sinan Ince, University of Afyon Kocatepe, Turkey.
Reviewers:
(1) Tolga Dinc, Ankara Numune Training and Research Hospital, Turkey.
(2) Ibrahim Maman Laminou, Niger.
(3) Shamala Moodley, Mangosuthu University of Technology, South Africa.
Complete Peer review History: http://www.sdiarticle4.com/review-history/56169

Original Research Article

Received 15 February 2020
Accepted 21 April 2020
Published 27 April 2020

ABSTRACT

Aim: External Quality Assurance (EQA) is basic requirement of a medical laboratory to assess the quality assurance and achieve the accreditation. The available EQA schemes evaluate the analytical performances of the laboratory but neither evaluate pre analytical factors nor mimic actual laboratory process. PRE-EQC has combined both performances in single scheme and assisting the participant laboratories to take appropriate corrective action and interpretations.
Design: Pre-Analytical monitoring evaluates transport condition, correlation of the stability of samples and temperature, storage condition and environment of the laboratory of the participants and its effect on the results. A sample is specially prepared to estimate haemolysis, lipemic and icteric index.
Clinical Biochemistry: Serum, fluoride and biological fluid (CSF exempted) samples are pooled from the routine collection of specimens. Pooled fractions are homogenized in a rotary shaker for 10 minutes. The clear samples are poured in individual double pack primary containers, which are placed between two gel packs in a "biohazard" labelled plastic bag. Temperature is recorded, kept in insulated thermocol box and sent to the destination.
Urine Routine & Microalbumin, Creatinine Examination: Urine sample is stabilized using preservative.

**Corresponding author: E-mail: shy23_pal@yahoo.co.in;*

Methods: The process of sample pool to result submission has been completed within 6 days as samples are without preservative. Stability mimicking the transport, homogeneity and validation of assigned values were done.
Statistical Calculations: As per ISO 13528:2015.
Results and Discussion: The SD of the assigned values showed better performance than existing schemes and within the range of CLIA recommended SD.
Conclusion: The PRE-EQC sample from direct and microalbumin from direct specimen has achieved good reproducibility than lyophilized material.

Keywords: EQA; PTP; PRE-EQC.

1. INTRODUCTION

External Quality Assessment (EQA) is a requirement of ISO15189:2012 as per clause 5.6. Commercially available EQA samples are lyophilized samples with added preservative to enhance stability of samples for up to 2 years. Also, lyophilization facilitates supply in large quantity also. The awareness of proficiency testing (PT) should include both analytical performance and pre analytical assessments. In the year 2017, a study was done involving 769 laboratories for non-waived testing as per CLIA regulation [1]. Varying proportion of respondents used PT to identify problems in the pre analytic (48%), analytic (86%) and post analytic (76%) phases. It may be concluded that as pre analytical factors are one of the prime factors there should be regular evaluation of the same.

The lyophilized commercial controls do not exactly mimic the regular procedure of processing. The basic difference is that it is lyophilized, and regular samples are directly collected from human resources. In the 90s, it was observed that frozen stored pooled specimen of serum essentially produces identical apolipoprotein A-1&B when compared to the previous values before storage but after lyophilization approximately 30% deviation was observed [2]. The reason for such deviation has been clearly demonstrated by R. Rej [3]. The reasons include species origin, integrity of molecular species, matrix effect due to process such as lyophilization and addition of preservatives. The comparison of freeze dried, lyophilized and normal human samples showed differences in physical properties like Osmolality, density, surface tension, viscosity, pH at a particular temperature, particle size and absorbances at 600 nm in 1.0 cm cell [3].

Attempts were made to directly pool the sample and prepare aliquot and these samples were transported to participant laboratories and considered valid till 2019 by CLSI. The study was conducted to prepare secondary reference material for lipid and apolipoprotein measurements. The study showed potential reduction of inter method deviations [4,5]. The process is not only a validated method it is also inexpensive. The pool of samples for PT material may be from single/multiple donor. Pool from single donor is not ethically acceptable and if there is any interfering substance in the donor's sample the commutability would be affected [6,7,8]. Multidonor samples may be pooled from residual samples of a diagnostic laboratory which would satisfy the criteria:

1. Processing of sample follows the same manner of clinical sample.
2. Excess collection of samples from a single donor is not recommended.
3. If there's interfering substance from a single donor it gets diluted.
4. It is possible to get a higher range of values for all/a few analytes.

Disadvantage is interaction of serum/urine proteins from multiple donors may cause aggregation/precipitation of proteins [6,7,8]. So, PTP decided to discard the total pool if turbidity is observed as reports are available that filter paper may adsorb albumin resulting in lowering of albumin values [9]. The PTP decided to run the program using the pooled sample to exactly mimic the analytical process and to standardize optimum time and temperature for such samples without stabilizer. To start with, the outsourcing module is currently followed and maximum time limit for releasing the results is 7 days. Accordingly, it was decided to limit the time of submission of results within 6 days, as the PTP needs one day for preparation of samples and collection of infective marker results. The monitoring of pre analytical factor would automatically be available if a chart for recording of time and temperature can be prepared. The module of Proposal of European Federation for

Clinical Chemistry and Laboratory Medicine has been taken up and it was found out to be successful after completion of 3 rounds [10]. The process made participants aware of pre analytical factors and accumulation of data about stability of samples during transport and extent of tolerance of temperature.

2. MATERIALS AND METHODS

2.1 Materials

1. Pooled residual serum, fluoride plasma and biological fluid available in the diagnostic laboratory for routine testing.
2. Rotary shaker for homogenization.
3. Automated system and reagents for estimation of homogeneity and stability.
4. Dipsticks for urine analytes measurement.

2.2 Methods

2.2.1 Sample preparation

Serum & Fluoride sample: The samples are prepared from excess sample of the laboratory obtained for routine diagnostic investigations. Pooled serum/fluoride sample fractions are collected in a sterile plastic container of 50 ml capacity. Total requirement of sample is 30 ml for serum and 15 ml for fluoride plasma. The sample containers are placed in rotary shaker at 300 rpm for 20 minutes. If the sample turns turbid it is rejected. Only clear sample is considered as an appropriate EQA sample. The sample is divided in two subsets for homogeneity and stability testing [1,6,7,11,12].

Biological Fluid: The preparation is same as serum sample. The matrix may be ascitic, pleural, pericardial, or may be a mixture of all available biological fluid [1,6,7,11,12].

Sample for haemolytic, lipemic, icteric index and clot detection: The sample is prepared by mixing such samples as per the availability in the laboratory. For haemolysis detection, sample to be placed in cell counter rotor mixer before processing.

These four types of samples are directly transported to participants without addition of any preservative/stabilizer.

Urine sample: The urine sample is prepared for microalbumin, creatinine, routine chemistry analytes and physical property measurements. The sample is prepared from excess urine sample of the patients within 2 hrs of collection. Pooled urine samples are homogenized in a 500 ml sterile plastic container on a rotary shaker for 10 minutes. Preservative is added to the sample to prevent growth. The sample is preserved in two subsets for homogeneity and stability testing [13].

2.2.2 Aliquot preparation

Homogenized samples are aliquoted in respective double capped primary container for enrolled participants. Each participant receives 5 samples in separate primary containers. The primary containers are Hemogard™ containers to prevent splutter of samples and deposition of serum at the opening of the container. The Hemogard tubes are CLSI approved containers. Quantity and sample number is as follows:

Sample 1: Serum; 1.5 ml

Sample 2: Fluoride plasma; 0.5 ml

Sample 3: Biological fluid; 1 ml

Sample 4: Urine sample for microalbumin, creatinine and clinical pathology analytes such as glucose, protein, bilirubin, urobilinogen, ketone bodies etc.; 15 ml.

Sample 5: Serum sample for detection of Haemolysis, Icterus, lipemia and clot detection; 1.5 ml [14].

2.2.3 Packaging and transport [15]

Each sample is wrapped with cotton. Then wrapped in small plastic wrapper. Total 5 number of samples are wrapped individually. The plastic “Biohazard’’ bag is taken, and two appropriately sized cool pack inserted in the bag. The wrapped primary sample containers are placed between two cool packs. The position of primary containers is such that their position is straight and no chance of getting tilted. Temperature is recorded. The Biohazard bag is tightly secured with a rubber band. Urine sample is poured in 30 ml sterile urine container and packed separately in plastic wrapper and “Biohazard” bag. Both blood and urine sample packs are placed in an insulated thermocol box which is tightly sealed to assure insulation. The thermocol box is placed in an appropriately sized hard brown paper box. Label contains the information of sender (PT Provider), participant’s details, unique ID of the

participant of every round. Unique ID generation is necessary on every round to make the result available in the website from virtual clouds. Declaration for transport of biohazard material is prepared and submitted to courier service. Time of sending of samples and receipt is recorded.

Time limit for submission of results: During enrolment it is taken into consideration that sample must reach the participant within day 4. By day 6 results are to be submitted.

2.2.4 Homogeneity and stability testing

The sample is divided in two subsets. Homogeneity is tested on day one i.e. the day of transport 10 tests from each subset (10*2). The list of tests consists of 35 analytes and number of rounds are 4 in a cycle. 9 analytes tested for homogeneity in every round covering all analytes in a cycle. Stability is tested from two subsets on day 1, each subset being tested twice. Arrangement is made with one of the participants who would receive three sets of samples, test and submit results from one set and return two sets on day 6 to mimic the transport process. On day 7, the stability of the received samples from participant is tested. If homogeneity and stability fail, the round is called off [16].

2.2.5 Tests performance

In EM 200 System, Easylyte Electrolyte analyzer. Reagents and calibrators are system specific.

2.2.6 Release of results

In the website: www.pre-eqc.com

2.2.7 User's instruction

Provided in the website.

2.2.8 Disposal of samples

As per local guideline.

2.2.9 Test for infective markers

HIV (I+II) Antibody & P24 antigen, Anti HCV antibody, HbSAg are tested and samples are only transported if the results are negative.

2.2.10 Number of rounds

4 times in a cycle. July, October, January and April.

Calculations:

Time and temperature calculation for pre analytical measurements:

- Recording of time from completion of sample preparation to receipt by the participant.
- Recording of time of transport from PTP to participant.
- Time and temperature when received by the participant.
- Preservation temperature and duration of preservation.
- Time of keeping the sample at room temperature before performance of tests.
- Time lag between two repeat tests.

Statistical calculations:

Assigned value: The assigned value is obtained from consensus of the participants performing the test by same method. Robust mean and Robust SD are calculated. Z-Score is reported for the analyte having ≥18 participants and prime z-score for <18 number of results. The PT Provider (PTP) calculates Standard Error Mean (SEM) also and provides the data of 95% and 99% Confidence Interval (CI). The CI data is more stringent and being provided for continual improvement [17,18].

Reproducibility check: Every analyte is tested twice to check the reproducibility. Reproducibility is measured by % deviation. Deviation from 1st estimated value.

Validation of assigned value: This is the record of PTP. The assigned value obtained from the participant's consensus is compared with laboratory assigned value. The laboratory obtained the results by using calibrators traceable to international standard [19].

3. RESULTS

The PTP completed 3 rounds of a cycle consisting of 4 rounds. The Robust SD (RSD) is compared with one internationally distributed (BIORAD) and one nationally distributed accredited lyophilized EQA (CMC Vellore). The SD of PRE-EQC was less compared to the two already running programs. Stability and homogeneity were passed in all rounds.

The urine chemistry analysis is qualitative as quantitative analysis is still not popular. The reporting pattern is presented in Table 2.

Table 1. Clinical biochemistry

Analyte	SD, July 2019 PRE-EQC	SD, Oct. 2019, PRE-EQC	SD, Jan 2020 PRE-EQC	SD Max BIORAD	SD Max CMC Vellore
Glucose	4	7.4	7.55	11.8	21.5
Urea	1.07	0.98	2.35	11.4	14.9
Creatinine	0.04	0.1	0.05	0.25	0.44
TBil	0.06	0.09	0.11	0.61	0.67
DBil	0.04	0.04	0.15	0.52	Not in the program
T Protein	0.15	0.25	0.18	0.36	0.59
Albumin	0.11	0.16	0.15	0.25	0.31
ALP	12.5	7.55	12.5	60	41.8
ALT	2.35	5.5	6	26.6	44.2
AST	2.5	1.75	8.35	34.1	48.4
T Cholesterol	4.25	4.75	4.83	21.5	13.8
Triglycerides	3.2	8.5	6.15	11.3	34.4
HDLC	2.3	4	2.95	4.63	5.62
LDLC	3.2	4.75	6.3	21.4	Not in the program
UA	0.1	0.18	0.25	0.63	1.09
Na	1.31	2.5	2.45	4.95	8.13
K	0.24	0.15	0.32	0.28	0.48
Cl	3	7.5	2.95	5.94	7.43
Amylase	8	7.5	8.05	120	45.5
Lipase	4.65	12.31	5.5	14.5	Not in the program
LDH	22.4	12.5	19.5	29.1	Not in the program
CK	3	3.8	4.92	42.6	48.3
GGT	1.75	1.65	0.6	31.2	Not in the program
T. Calcium	0.3	0.35	0.18	0.5	1.07
Phosphorus	0.07	0.16	0.52	0.33	Not in the program
CO2	1.08	1.66	1.2	3.42	-do-
Fe	4.1	4.88	3.9	31.4	-do-
UIBC	20.5	10.05	41	15.3	-do-
Glucose (Biological Fluid)	2.75	6.74	5.5	Not in the program	-do-
T Protein (Biological Fluid)	0.14	0.14	0.12	-do-	-do-
Albumin (Biological Fluid)	0.06	0.15	0.06	-do-	-do-
LDH (Biological Fluid)	6.2	8.95	44.46	-do-	-do-
Microalbumin (Urine)	9	7.23	4.7		52.3
Creatinine (Urine)	1.1	3.72	1.96		11
CKMB	0.05	4.3	1.4		

4. DISCUSSION

The $V_{Max\ in}$ Table 3 shows that PTP of PRE-EQC completed sample preparation to submission of results within 7 days (10080 minutes). Hence, as stated the transport to release of results took place within 6 days. Maximum time taken to reach the participant at a distant place is 7200 minutes i.e. 5 days keeping the option for the participant to perform the tests within 1440 minutes (1 day). So, geographical position and courier services availability is a concern for such a proficiency testing scheme. The PTP assures receipt of PT material within 4 days considering the necessary time lag for any unforeseen situation which may delay the process.

Table 2. Urine chemistry

Analyte	Remarks
Colour	Yellow/pale yellow/straw colour/colourless
Appearance	Clear/Hazy/Slightly hazy
Deposit	Present/Not present
Protein	Negative/trace/+/++/+++/++++
Glucose	Negative/trace/+/++/+++/++++
pH	5.0-9.0
Blood	Negative/trace/+/++/+++/++++
Specific gravity	1.000-1.060
Ketone bodies	Negative/trace/+/++/+++/++++
Bilirubin	Negative/trace/+/++/+++/++++
Urobilinogen	Negative/trace/+/++/+++/++++
Nitrate	Present/Not present
Phosphate	Present/Not present

Table 3. Pre-analytical measurement outcome

Analyte	V_{Max} July 2019	V_{Max} Oct 2019	V_{Max} Jan 2019	Max value from 3 rounds
Time interval of sample preparation and receipt (minutes)	7200	10080	8490	10080
Time interval from core lab to participant (minutes)	5760	7200	4320	7200
Time interval from receipt to analysis (minutes)	1440	1440	1440	1440
Temperature when received (°C)	19.05	34.5	28.8	34.5
Preservation temperature (°C)	8	8	8	8
Temperature during analysis (°C)	26	28	27	28
Time lag between two performances (minutes)	300	300	240	300
Haemolysis(g/dL)	0.2-0.22	1.6	2.42-2.52	2.52
Icterus (mg/dL)	0.11-0.12	2.41-2.45	0.89-0.91	2.45
Lipemia(mg/dL)	100-109	75-81	155-161	161

V_{Max} = Maximum value

The insulated packing and gel pack cover cannot in practice maintain the temperature within 8°C. The need of outsourcing is growing day by day and the temperature of transported sample cannot remain constant throughout transport process. The PT preparation is without any added preservative, maximum temperature noted by the participant /participants is 34°C and time 5 days. The maximum SD after the process of transport seemed to be less than established EQAS. It may be concluded that transport system and selection of location before enrolment are ideal.

The number of participants is 20, for some of the analytes less than 18. In such situation the scheme cannot be both method and system specific. So, the participation is method specific. The SD was thought to be high as instrument bias is an important factor [19]. PRE-EQC has started with the idea of method specificity as specific analytical performances like method, instrument, commutability of calibrators varies widely resulting wide variation from one group of participants to other and basic need is uniformity of results irrespective of specific analytical performance. It was felt that EQA should have no bias attributable to the instrument as before launching it has passed through CLSI evaluation processes. A linear model was proposed. In ISO 13528: 2015 the bias attributes are expressed by uncertainty [20].

If uncertainty criteria are satisfactory that proves the PT sample, process and method are appropriate. The uncertainty of three rounds showed satisfactory uncertainty proving commutability of PT specimen.

Urine sample: Microalbumin and creatinine: It has been observed that result of microalbumin from lyophilized urine sample received by the participant as PT sample often varies widely (Table 1). The pooled urine sample of PRE-EQC has nicely sorted out the problem (SD, Table 1).

Clinical Pathology (CP): The recommendation of Indian Accreditation Body is inter laboratory comparison. PT is not essential for accreditation. During evaluation of CP it was observed a PT program would have been best option. The difference between qualitative and quantitative measurement is the quantitative estimation presents a data whereas qualitative is impression and observation-based result. Hence, one PT Scheme would have standardized the process [21].

HIL samples: The results of HIL in 3 rounds show that samples may not need to be rejected up to the index of 2.52,2.45 and 161. The range of results is showing consistency. The best method of judgement is the consistency of triglycerides result(L) as in homogeneity study triglycerides is considered as one of the prime markers [22].

5. CONCLUSION

Any scientific endeavour has its merits and limitations.

Advantages of PRE-EQC:

- An inexpensive and simple process.
- The scope of the program includes some uncommon tests along with the commonly available tests which are not available commercially.
- The tests from other fluid matrix are evaluated.
- Proved the necessity of EQA in CP instead of ILC. ILC is having limitation of becoming concentrated during transport from one lab to other and “on the table” time lag.
- The provision of direct interaction with the PTP regarding corrective actions.

Disadvantage:

The project is difficult to commercialize. The organizer needs to consider geographical locations and transport availability within time frame. The disadvantage may be sorted out by preparing a team of four/five members who would independently run the program. Such members would perform one master proficiency program among themselves. The scheme is in budding state but to create an organogram and propagate such program is not impossible.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

PRE-EQC uses residual sample of patients who are giving consent to let the sample utilize after test performance. Consent form is signed and the patient is informed about the procedure.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

ACKNOWLEDGEMENTS

1. JMD Diagnostics Private Limited.
2. Saptarshi Pal, Web designer.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Earley MC, Astles JR, Breckenridge K. Practices and perceived value of proficiency testing in clinical laboratories. Journal of American Laboratory Medicine. 2017;1:415-420.
2. Marcovina SM, Adolphson JL, Parlavecchla M, Albers JA. Effect of lyophilization of serum on the measure-ment of apolipoprotein-1 & B. Clin Chem. 1990;36(2):366-369.
3. Rej R. Accurate enzyme activity measurements: Two decades of development in the commutability of enzyme control materials. Archives of Pathology and Laboratory Medicine. 1993;117(4):352-364,.
4. Cobbaert C, Weycamp C, Baadenhuigsen H, Kuypers A, Lindmans J, Jansen R. Selection, preparation and characterization of commutable frozen human serum pools as potential secondary reference materials for lipid and apolipoprotein measurements. Study within the Framework of the Dutch Project “Calibration 2000”. Clin Chem. 2002;48(9):1526-1538.

5. CLSI Document-C-37A. Preparation and validation of commutable frozen human serum pools as secondary reference materials for cholesterol measurement procedures, 1st Ed.; 1999.
6. Greg Miller W, Graham RDJ, Horowitz GL, Weycamp C. Proficiency testing/EQA: Current challenges and future directions. Clin Chem. 2011;57(12):1670-1680.
7. Ferrero CA, Carobene A, Ceriotti F, Modenese A, Arcelloni C. Behaviour of frozen serum pools and lyophilized sera in an external quality assessment scheme. Clin Chem. 1995;41(4):575-580.
8. Naito HK, Kwak YS, Park JK, Mayers GL, Hartmann AE, et al. Matrix effects on proficiency testing materials. Impact on accuracy of cholesterol measurement in laboratories in the nation's longest hospital system. Arch Pathol Lab Med. 1993;117(4):345-351.
9. Harris R. The adsorption of protein by filter paper in the estimation of albumin in blood serum. J. Biol. Chem. 1939;127:751-756.
10. Lippi G, Banfi G, Church S, Cornes M, Plebani M, et al. Pre analytical quality improvement. In pursuit of harmony on behalf of European Federation for Clinical Chemistry & Laboratory Medicine working group for pre analytical phase. Clin Chem Lab Med. 2015;53(3):357-370.
11. Premachandra P, Wood PL, Hill PG, Browning DM, Olazabal DA. Preparation and stability of low-cost liquid quality - control serum stabilized with ethanediol. Clin Chem. 1987;33(6):851-852.
12. Lalani R, Molla A, Khurshid M. Efficacy of a homemade quality control serum. J Pak Med Assoc. 1989;39(12):317-320.
13. Ercan M, Akbulut ED, Abusoqlu S, Yilmaz FM, Oztekin V, et al. Stability of urine specimens stored with and without preservatives at room temperature and on ice prior to urinalysis. Clin Biochem. 2015;48:919-922.
14. Lippi G, Favalaro EJ, Franchini M. Haemolysis index for the screening of intravascular haemolysis: A novel diagnostic opportunity. Blood Transfusion. 2018;16(5):433-437.
15. Guidelines for the safe transport of infectious substances and diagnostic specimens. World Health Organization; 1997.
16. Guidelines for homogeneity and stability of proficiency testing items. Statistical method for use in proficiency testing by interlaboratory comparison. International Standard. ISO 13528:2015, 2nd Ed. 2018;44-51.
17. Determination of assigned value and its standard uncertainty. Statistical method for use in proficiency testing by interlaboratory comparison. International Standard. ISO 13528:2015, 2nd Ed. 2018;13-19.
18. Everitt BS. The Cambridge dictionary of statistics. CUP; 2003.
    [ISBN: 978-0-521-81099-9]
19. Ceriotti F. The role of external quality assessment schemes in monitoring and improving the standardization process. Clinica Chimica Acta; International Journal of Clinical Chemistry. 2014;432:77-81.
20. Lim J, Sungho W, Suyeon P, Kim J, Sun Hoe Koo, Kwon GC. External quality assessment of institutions and instruments using a linear mixed model. Journal of Laboratory Medicine and quality assurance. 2016;38:43-51.
21. Design and analysis of qualitative proficiency testing schemes. Statistical method for use in proficiency testing by interlaboratory comparison. International Standard. ISO 13528:2015, 2nd Ed. 2018;37-39.
22. Getahun T, Alemu A, Mulugeta F, Sileshi M, Habtu W, Wolde H, et al. Evaluation of visual serum indices measurements and potential false result risks in routine clinical chemistry tests in Addis Ababa, Ethiopia. EJIFCC. 2019;30(3):276-287.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/56169