REPORT OF A TRIAL WITH LYOPHILIZED QC SERA IN THE CMCH/ACBI EQAS PROGRAMMES IN INDIA

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ABSTRACT

2000 vials of lyophilized QC of two different levels (low and high) were donated by Roche Diagnostics GmbH, through the IFCC and received by CMCH in June 2001. A total of 240 laboratories were enrolled for this 6 month pilot study. In addition to the 12 analytes in the liquid QC programme, six additional analytes, LDH, triglyceride, urate, total bilirubin, phosphate and amylase were included. It was also possible to measure sodium and potassium by ion selective electrode (ISE) methods in the QC for the first time.

The performance of the laboratories for the existing 12 analytes using liquid stabilized QC was compared to the performance using lyophilized QC. Using a statistical comparison of the methodwise mean variance index score (MVIS) values, five assays viz glucose, albumin, cholesterol, and SGOT and SGPT performance was the same in liquid QC and lyophilized QC. Three assays viz urea, calcium and creatinine were significantly better, and 4 assays total protein, sodium, potassium and ALP were significantly worse. However the overall VIS (OMVIS) for the laboratories was the same and the ranking pattern of this 6 month OMVIS was also unaltered.

The lyophilized QC scheme highlighted a negative bias between flame and ISE methods for sodium and potassium, and a definite standardization problem in reporting LDH and amylase results, but triglyceride, urate and total bilirubin assays were performing well.

It was concluded that the introduction of lyophilized QCs will not cause any deterioration of performance to participating laboratories. Stability of the material seems to be good and the laboratories are generally using a good reconstitution technique.

KEYWORDS

Lyophilized QC, performance, EQAS, MVIS, OMVIS

INTRODUCTION

The Clinical Biochemistry department at Christian Medical College & Hospital (CMCH), Vellore (1) organizes an external quality control scheme (EQAS) under the auspices of the Association of Clinical Biochemistry of India (ACBI). Participants enroll in the scheme for a cycle of 6 months, now lengthened to 1 year, and receive monthly by post a liquid stabilized 5ml QC vial of bovine sera, along with a proforma results sheet. The participant returns the result sheet after analysis of up to 12 parameters, and receives a monthly report on their performance. The scheme is very successful with approximately 1200 participating laboratories and has shown steady improvements in

performance of the analytes included in the scheme. Many laboratories have been able to switch over to better methods after beginning to monitor their performance.

The QC material is stabilized with 25% (v/v) ethanediol, according to the WHO recommendation (2). This means it is unsuitable for some analyte measurement eg sodium and potassium by ion selective electrodes.

It is now time to expand the range of analytes offered by the scheme and this can only be done using an alternative method of preserving the specimen ie lyophilization. A total of 2000 vials of lyophilized QC were generously donated by Roche Diagnostics, GmbH for a trial of this material, in our EQAS for Indian laboratories. This paper presents the outcome of the study undertaken using this lyophilized QC sera.

METHODS

2000 vials of two different levels low (green top) and high (red top) were donated by Roche through the IFCC and received by CMCH in June 2001. A total of 240

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laboratories who were already participating in our ongoing liquid EQAS and most of whom were in the ELITE group of our classification (participated in all six months and analysed between 10-12 parameters) were enrolled for this pilot study. In addition to the 12 analytes in the liquid QC programme, six additional analytes, LDH, triglyceride, urate, total bilirubin, phosphate and amylase were included in the pilot study.

According to the schedule each laboratory received 4 vials of the green QC and 4 vials of the red QC between July and December 2001. Green and red were dispatched together in July and December, green alone in August and October, and red alone in September and November. As usual the liquid stable QC was also sent to these laboratories, on a monthly basis. Every time after specimen dispatch the reference lab also analysed 5 vials of the dispatched material, after exposing them to room temperature for a period of 9 days, the mean values of which represents the reference lab mean value. This simulates the transport of the vials all over India, which can take up to 9 days.

Instructions for reconstitution, storage, analysis and proforma for reporting results were sent along with the samples. The list of instructions is reproduced below.

1. Reconstitution

Utmost care is to be taken when reconstituting and analyzing the sample as per the following procedure.

- Switch off fan in the room where the vial is to be reconstituted.
- Open carefully, remove the rubber stopper and leave it upside down on a clean bench.
- Add 5ml of good quality distilled water using a good quality volumetric pipette.
- Replace the rubber stopper and leave at 20-30 °C for 15-20 minutes.
- After 15 minutes invert and mix gently several times to ensure homogeneity of the material.

The sample is now ready to carry out the various analyses listed in the proforma sheet of the lyophilized QC serum.

2. Analysis

The following precautions are to be taken while analyzing the reconstituted QC serum.

- Do not leave the reconstituted serum at room temperature for a long time (>1hour) since this material does not contain any preservative.
- Treat the serum like any other patient serum while analyzing.

- The reconstituted material may be stored at 2-8 °C for one full working day.
- For some tests for which analysis is not done on the same day, aliquot a small volume of the reconstituted sera sufficient for the analysis and freeze it.
- Avoid freezing and thawing the sample more than once,
- After completing the analysis of all the tests enter the results in the appropriate test method and mail the proforma sheet back to us as per the instructions given in the proforma sheet.

Statistical analysis of the results submitted by you will be carried out using the WHO recommended method and a summary will be sent to you at the end of the cycle.

Statistical Analysis

The statistical analysis was carried out using the same WHO method presently used in our liquid QC EQAS. The participants own method monthly specific mean and the chosen coefficient of variation (CCV) shown below were used to calculate the VIS in the pilot QC scheme. The list of CCV values is shown in Table 1. The values for CCV for the newly introduced parameters were based on those of the UKNEQAS scheme for Clinical Biochemistry (3). Table 1 also shows the value of 3x CCV.

To assess the performance of laboratories the statistical parameter Variance Index score (VIS) is employed, making use of the chosen coefficient of variation (CCV) described in table 1, and the designated value (DV) for that parameter. This is usually the mean result of the participating laboratories for that method after excluding outliers more than 3 standard deviations from the mean.

$$\% \text{ variation } [\%V] = \frac{\text{difference between participants result and DV}}{\text{DV}} \times 100$$

$$\text{VI} = \frac{\%V}{\text{CCV}} \times 100$$

When VI is less than 400 it is designated as VIS and has no sign

The closer the participants result to the DV the lower the VIS will be. When VIS is less than 50 it is regarded as a zero score. VIS values > 400 indicating grossly erroneous results still score 400 only. The objective is to get a VIS value below 100 which is very good. If the VIS is in the range 150-200 then there is room for improvement. VIS > 200 on one or more occasions for the same analyte means the standardization procedures should be reviewed. VIS >250 means wrong results are probably being reported on patient

samples and urgent steps should be taken to correct the method or standards.

A monthly VIS result is calculated for each laboratory for each analyte, the mean of this value over the study period of 6 months is called MVIS. Taking the mean of all the VIS scores for all the analytes reported by a laboratory in a single return gives the overall mean VIS or OMVIS.

The mean of the MVIS from all the individual participants was calculated for each analyte, as was the mean from all laboratories of the 6 month OMVIS.

The MVIS between the liquid QC scheme and lyophilized QC scheme was compared using the t-test for two samples assuming equal variances. Mean and standard deviation were calculated in standard fashion, percentage coefficient of variation (%cv) was

calculated as the SD divided by the mean for that analyte, times 100.

The mean result for the green QC and red QC was plotted, one point per laboratory, summarizing all their data for that analyte, results from green QC as X axis and red QC as y axis. Data was plotted as a two-sample plot or twin chart according to the guidelines of Elion-Gerritzen (4). That is the axes of the graphs are graduated in units of measurement rather than standard deviation intervals. This meant that less manipulation of the data was required.

RESULTS

The results of all the analyses for the lyophilized QC green top and red top are shown in Table 2 for analytes and methods with more than 7 participants, the remainder of results with less than 7 participants are shown in Appendix 1. All values in table 2 and

Appendix - I

Lyophilised QC scheme: summary of all data from methods with less than seven participants

ANALYTE	METHOD	GREEN QC			RED QC			LABS % cv	
		REF OVERALL MEAN	NO. OF LABS	LABS OVERALL MEAN	LABS % cv	REF OVERALL MEAN	NO. OF LABS		LABS OVERALL MEAN
GLUCOSE	O-TOLUIDINE		3	107.0	10.3		4	250.0	13.5
	FOLIN-WU		1	94			1	221.0	
UREA	UREASE		6	40.0	25.3		7	128.0	25.2
	NESSLER								
ALBUMIN	BCP		2	3.5	8.6		2	3.0	13.2
CALCIUM	EDTA		5	8.3	18.3		5	11.4	25.8
	TITRATION								
CHOLESTEROL	CARR- DREKTER		1	132.0			2	212.0	12.0
	CARR- DREKTER		2	133.0	7.8		2	214.0	6.8
	DIRECT								
	WYBENGA		3	125.0	13.3		3	219.0	13.7
	DIRECT								
	ZAK		3	144.0	32.0		3	198.0	23.6
SODIUM	COLORIME		5	138.0	8.0		6	139.0	10.6
	TRIC								
POTASSIUM	COLORIMETRIC		5	3.0	35.8		6	5.3	17.4
CREATININE	JAFFE- DIRECT		5	1.5	17.6		6	3.6	18.7
ALP	KING- ARMSTRONG		6	7.1	29.4		5	17.1	36.1
	KIND-KING		5	8.6	24.8		6	21.8	24.8
	PNP DEA		6	156.0	28.2		6	323.0	41.0
	END POINT								
	PNP AMP		4	117.0	19.1		5	303.0	18.8
	END POINT								

KEY : Reference overall mean, Laboratories overall mean, Number of laboratories participating and Laboratories % cv are all the mean of each of 4 months analysis

appendix are the mean value for each reference value, analyte value, %cv, and number of laboratories, over 4 cycles of analysis corresponding with the 4 cycles of dispatch of each vial to the participants, with no exclusions

In Table 2 the values for the reference lab mean differs by more than 10% from the participants mean, using the same method analysis, for the following assays: glucose high QC ((+13.1%), SGPT high QC (+19.5%), ALP high and low QC's (-15.6%) LDH high and low QC's (+25.9%), bilirubin high QC (+19.5%), phosphate high QC (+10.2%) and amylase both QC's which differ by a factor of +2.2 x higher.

The degree of variation of all participants results from the designated mean can be assessed by either the mean %cv of that mean (Table 2), or the MVIS for that method (Table 3) or by examination of the two- sample plots (Figure 1A-F).

The data in Table 2 was examined using mean %cv data and looking for values greater than 3 times the %CCV for that analyte (see Table 1). Data exceeded these values for many methods of analysis : total

Table 1: List of chosen coefficients of variation used for calculation of VIS

Analyte	%CCV	3x %CCV
GLUCOSE	7.7	23.1
UREA	10.0	30.0
TOTAL PROTEIN	7.5	22.5
ALBUMIN	7.5	22.5
CALCIUM	6.0	18.0
CHOLESTEROL	7.5	22.5
SODIUM	2.3	6.9
POTASSIUM	5.0	15.0
CREATININE	10.0	30.0
SGOT/AST	12.5	27.5
SGPT/ALT	17.3	51.9
ALP	15.5	46.5
LDH	15.5	46.5
TRIGLYCERIDES	14.0	42.0
URATE	7.7	23.1
BILIRUBIN	19.2	47.6
PHOSPHATE	7.8	23.4
AMYLASE	15.5	46.5

protein, low QC by Biuret method, calcium high QC by CPC AMP method, calcium, both QCs, by Trinders method, high QC by CPC AMP method, and low QC CPC DEA method, sodium, both QCs, flame and ISE methods, potassium both QCs by the flame method, creatinine low QC, end point method, SGOT both QC's and SGPT high QC by Reitman Frankel method,

LDH both QC's King method, phosphate, both QC's reduction and kinetic methods, and amylase, both QC's, Somogyi and kinetic methods.

Table 3 shows the liquid QC scheme mean value for MVIS and its standard deviation for all methods of analysis for a particular analyte in comparison with the green and red lyophilized QC performance. The result of the comparison of these MVIS values is described as significantly better or worse only if both probability values are significant and the change is in the same direction ie both better or worse. Better means statistically significant lower MVIS and worse means higher MVIS.

This comparison data is only available for the established analytes, but the MVIS for the additional analytes is also shown. Looking at Table 3, all MVIS values are <200 for both liquid and lyophilized QC, except for LDH lyophilized QCs and calcium liquid QC. Values between 150-200 mean the assay requires some attention. This is true for liquid QC urea and creatinine, and for lyophilized QC calcium, sodium, potassium, ALP and amylase. Table 4 shows how the ranking of the 240 laboratories is affected by using lyophilized QC material.

Table 4: Rank of the participating laboratories using 6 month omvis for each laboratory

VIS RANK 6 MONTH OMVIS	LIQUID QC	LYOPHILISED QC
<50	-	1
50 - 100	41	44
100 - 150	112	98
150 - 200	63	61
200 - 250	16	22
250 - 300	1	5
300 - 350	-	2
TOTAL NO. OF LABS	233	233

The lyophilized QC data for each method for which more than 7 laboratories returned values were plotted as two-sample plots. Each laboratory value is the mean result for analysis of both the green and red QC on all occasions on which it was analyzed. Participants were not included unless they reported at least one value for the green and red QC. Data was not plotted as Youden-Tonks graphs, which use standard deviation intervals, but rather as two-sample plots, using units of measurement. The box drawn around the data was at the $\pm 2SD$ limits of the stated mean values in Table 2.

Figure 1. Two-sample plots for lyophilized QC data for LDH, triglyceride, urate, total bilirubin, phosphate and amylase.

A- LDH, UV Kinetic method, n= 106, 2 SD box limits 190-362, and 282 – 534 U/L,

B- Triglyceride, Enzymatic method, n=190, 2SD box limits, 90-160, and 138-245 mg/dl,

C- Urate, Enzymatic method, n= 115, 2 SD box limits 4.1 – 5.5, and 8.9 – 12.1 mg/dL,

D- Total Bilirubin, Gendrassik Grof method, n=130, 2 SD box limits 0.7 – 1.5 and 2.5 – 5.7,

E- Phosphate, Kinetic method, n=99, 2 SD box limits 3.3 – 4.5 and 5.0 – 6.0 mg/dL,

F- Amylase, Kinetic method, n=128 (11 labs excluded), 2 SD box limits 61 –113 and 115 – 213 U/L

Table 2:
Lyophilised QC scheme: summary of all data from methods with less than seven participants

ANALYTE	METHOD	GREEN QC			RED QC		
		REF OVERALL MEAN	No. of Labs	LABS OVERALL MEAN	LABS % cv	REF OVERALL MEAN	No. of Labs
GLUCOSE	* GOD- POD	111.0	197	99.0	16.3	259.0	196
UREA	DAM-TSC		25	40.3	23.5		24
	UREASE- BERTHELOT		73	40.3	24.4		74
UREA	UREA-S		10	41.3	18.1		11
	* CREASE- GLDH	43.0	85	40.1	27.5	119.0	84
TOTAL PROTEIN	* BIURET	5.3	190	5.3	25.2	5.2	190
ALBUMIN	* BCG	3.3	167	3.4	13.2	3.1	168
ALBUMIN	BIURET		21	3.4	15.8		20
	* CPC AMP	9.2	94	8.9	14.3	12.7	91
CALCIUM	CPC DEA		26	9.1	18.2		27
	TRINDERS		9	8.7	24.4		9
OTHER	DYE		31	8.8	15.9		35
	* CHOD- PAP	103.0	190	107.0	18.1	186.0	190
SODIUM	FLAME		99.0	138.0	8.4		99
	* ISE	139.0	35	145.0	8.7	157.0	33
POTASSIUM	FLAME		99	3.7	18.3		99
CREATININE	* ISE	3.5	34	3.8	12.5	6.6	34
	JAFFE - END POINT		36	1.3	40.1		36
SGOT	* JAFFE KINETIC	1.3	156	1.3	26.3	3.7	156
	REITMAN- FRANKEL		11	34.0	38.1		14
						92.0	44.6
						225.0	15.0
						115.0	21.9
						118.0	22.1
						122.0	20.9
						127.0	19.7
						5.3	15.2
						3.3	13.4
						3.2	15.5
						12.6	18.3
						12.4	14.6
						12.6	24.7
						12.6	15.2
						186.0	12.4
						149.0	8.9
						162.0	9.2
						5.8	18.5
						6.7	10.6
						3.4	21.5
						3.4	24.0

Table 2 (Cont.)

SGOT	* UV KINETIC	39.0	175	43.0	36.7	135.0	171	126.0	25.8
SGPT	REITMAN-FRANKEL		10	25.0	47.0		13	71.0	54.9
	* UV KINETIC	44.0	175	43.0	36.7	141.0	173	118.0	31.7
ALP	PNP DEA KINETIC		49	136.0	32.4		56	328.0	30.4
	* PNP AMP KINETIC	90.0	107	113.0	38.1	241.0	100	270.0	41.0
LDH	* UV - KINETIC	339.0	81	276.0	42.9	526.0	82	408.0	41.3
TRIGLYC-ERIDES	KING CHEMICAL * ENZY-MATIC		10	186.0	50.2		11	291.0	46.5
	REDUCTION	121.0	160	125.0	23.6	200.0	158	191.0	18.9
URATE	* ENZY-MATIC	4.4	8	4.1	24.2		8	8.9	20.9
	DIAZO		97	4.8	19.6	10.5	96	10.5	33.6
BILIRUBIN	* GENDR-ASSIK GROF	1.1	81	1.0	48.6		84	3.8	32.1
	REDUCTION		96	1.1	36.4	4.9	94	4.1	27.4
PHOSPHATE	* KIN-ETIC	4.1	31	4.1	28.8		33	6.1	25.4
	SOMOGYI		76	3.9	32.8	6.5	75	5.9	39.8
AMYLASE	* KIN-ETIC	181.0	22	98.0	87.3		23	164.0	83.7
			116.0	87.0	56.2	451.0	116	198.0	48.4

Reference overall mean, Laboratories overall mean, Number of laboratories participating and Laboratories %cv are all the mean of each of 4 months analysis

TABLE 2 (cont.)

Lyophilised QC scheme: summary of all data from methods with more than 7 participants

Table 3:
Lyophilized QC scheme: Six month MVIS for individual methods and comparison of OMVIS between liquid and lyophilized QC for all methods

ANALYTE	LIQ MVIS	LIQ SD	GREEN MVIS	GREEN SD	PROBABILITY (SD) SIG. (NS) NOT SIG.	RED MVIS	RED SD	PROBABILITY (S) SIG. (NS) NOT SIG.	COMPARISON
GLUCOSE	106.6	60.1	104.4	72.0	NS	106.5	73.4	NS	SAME
UREA	156.3	61.0	111.8	82.2	<0.001 SD	134.5	86.0	<0.001 SD	BETTER
TOTAL	87.1	55.6	115.3	78.2	<0.001 SD	117.3	86.9	<0.001 SD	WORSE
PROTEIN									
ALBUMIN	111.7	79.0	127.5	84.2	<0.02 SD	118.8	81.9	NS	SAME
CALCIUM	208.2	95.4	163.2	88.6	<0.001 SD	181.0	101.4	<0.003 SD	BETTER
CHOLESTEROL	112.9	70.5	134.7	81.6	<0.001 SD	100.0	75.9	<0.03 SD	SAME
SODIUM	107.7	95.2	175.6	95.6	<0.001 SD	188.8	102.2	<0.001 SD	WORSE
POTASSIUM	115.9	105.4	176.6	104.5	<0.001 SD	167.6	103.5	<0.001 SD	WORSE
CREATININE	174.9	65.8	160.5	94.0	<0.03 SD	144.9	91.5	<0.001 SD	BETTER
SGOT	109.0	84.4	135.8	94.4	<0.001 SD	112.1	102.8	NS	SAME
SGPT	119.2	82.0	106.7	86.4	NS	97.5	90.2	<0.004 SD	SAME
ALP	130.4	92.4	150.2	88.0	<0.01 SD	165.4	95.8	<0.001 SD	WORSE
LDH	-	-	219.5	105.3	-	224.9	103.6	-	-
TRIGLYCERIDE	-	-	94.8	74.3	-	80.9	77.9	-	-
URATE	-	-	142.9	91.0	-	137.9	94.0	-	-
BILIRUBIN	-	-	110.2	85.1	-	103.7	78.3	-	-
PHOSPHATE	-	-	190.4	101.9	-	188.4	107.9	-	-
AMYLASE	-	-	178.3	112.0	-	165.1	123.5	-	-
6 MONTH OMVIS ALL METHODS	137.1	41.8	142.8	49.7	NS	142.8	49.7	NS	SAME

Key:

Green and red lyophilized QC sent on 4 distributions. Only labs which ran each lyophilized QC at least once are included Probability calculated by t-test two sample assuming equal variances. Probability <0.05 refers to liquid QC OMVIS and Green or Red QC having a mean which is significantly different. For the liquid or lyophilized QC to have performed significantly better or worse than the other, then both the red and green QC OMVIS should be significant and consistent difference.

6 month OMVIS for lyophilized QC is a combined figure for both red and green QC

Examples of these graphs are shown in Figure 1 A-F for the predominant assay used for the new analytes. An estimate of the scatter of the participants reported values can be found by counting how many of the laboratory values fall outside the 2 SD box. In ascending order the values were: for triglyceride 10/190 or 5.3% of the values are outside the box, for total bilirubin it is 12/130 or 9.2%, for amylase 19/128 or 14.8%, for urate 26/115 or 22.6%, for phosphate 45/99 or 45.5%, and for LDH 66/106 or 62.2% of values.

CONCLUSIONS

This is the first time that we have been able to monitor the performance of methods for the analysis of , LDH, triglyceride, urate, total bilirubin, phosphate and amylase

It is also the first time that the performance of ISE methods for sodium and potassium has been analysed. The flame method for sodium has a negative bias of -4.8% compared to the ISE method for the low QC (green) and -8.0% for the high QC (red) . For potassium flame method the negative bias is -2.6% for the low QC and -13.4 % for the high QC. Theoretically the ISE method should give values 7% higher than the flame method , provided that the sodium and potassium ions are not bound or dissolved in the protein phase. This is because the ISE is measuring ion activity in the water phase of the serum (molality) while the flame is measuring ion concentration per volume of serum (molarity). Serum normally consists of 93% water phase and 7% protein and lipid ie mass concentration of water is 0.93 kg/L. In practice a somewhat smaller difference in values of 3-5% is found. Companies correct their ISE measurements electronically so that values obtained are identical with those obtained by flame photometry on normal specimens. This obviates the need for changing reference ranges when using ISE systems but it means that results for aqueous solutions measured by ISE will be approximately 3% lower than the nominal content of sodium and potassium, provided the ionic strength of the solution is similar to serum ie 160 mmol/L. (5).

Pathological specimens with severe hyperlipidaemia, or hyperproteinaemia may have mass concentration of water as low as 0.8 kg/L so that concentration of sodium may be 20% lower than molality ie pseudohyponatraemia (6). This difference will also be seen using indirect ISE methods ie in which a fixed volume of specimen is diluted before taking the ISE reading. In the case of our lyophilized QC specimens neither hyperlipidaemia nor hyper proteinaemia is present to account for the discrepancies. The reference laboratory is using an indirect ISE measurement, but the majority of ISE participants will be using a direct ISE method. Our laboratory gets a

value for the high sodium lyophilized QC intermediate between the flame and other ISE users.

These comparisons must be closely monitored in the future to see if this trend continues.

Compared to the other labs, the reference laboratory values for amylase are a mean of 2.2 x higher than most of the other participants. This is explained by the use of a factor of 2.2 in the department to relate results obtained by the current Roche amylase method with a serum reference range up to 98 U/L to their older assay with a range up to 220 U/L, as explained in the manufacturers kit insert. This newer Roche method uses a maltoheptose (G7) substrate with 4-nitrophenol (NP) bound to the reducing end and a blocking group 4,6 ethylidene at the non reducing end. This blocking group is necessary to prevent hydrolysis of the molecule by α -glucosidase. This enzyme is present to release 4NP from the 4NP-G2, 4NP-G3 and 4NP-G4 produced by amylase action on the G7 substrate(7) This may also be the case for the 10 other participant labs with very high values. This problem of standardization may also be affecting the LDH assays.

From examination of the %cv values in Table 2, Individual methods which performed badly with lyophilized QC were sodium and potassium by flame, SGOT and SGPT by the Reitman-Frankel method, and LDH by the King method. The phosphate reduction assay has a positive bias of 3.3 to 5.1 % compared to the kinetic assay, and both have high %cvs.

Looking at both liquid QC and lyophilized QC performance, a ssays performing badly in terms of MVIS >200 are calcium, using liquid QC, and LDH using lyophilized QC. Assays which could improve (MVIS 150-200) are urea and creatinine using liquid QC, and calcium, sodium, potassium, creatinine, ALP, LDH, phosphate and amylase using lyophilized QC.

The two-sample plots in Figure 1A-F give a very good visual presentation of the data. Ranking of the new analytes using MVIS shows the order of increasing MVIS as triglyceride , total bilirubin, urate, amylase, phosphate and finally LDH. This order is identical to that using the plots except amylase and urate have changed places. So LDH, amylase and phosphate assays need to show improvement.

Table 4 shows in the ranking of the laboratories that switching to lyophilized QC would not adversely affect laboratory performances. The stability of analytes like glucose and the enzymes is also good in the lyophilized material. The poor performance of the electrolyte assays using lyophilized QC would have to be closely monitored in the future.

Data shown in Appendix 1 for ALP are using different units and so are not easily comparable to other data.

The colorimetric assay for sodium is comparable to the flame assay for the low QC but only 93.2% of the expected value for high QC. This figure becomes 86% when compared to ISE. Values are also low for potassium compared to flame method. The cholesterol methods listed in the appendix have a strong positive bias, calcium by EDTA titration and glucose by Folin Wu a strong negative bias. These differences should be appreciated by the participants using these methods of analysis.

The cost of commercially produced lyophilized QC is prohibitively expensive for most Indian laboratories. So that any CMCH/ACBI EQA scheme would have to use indigenously produced lyophilized QC. Participants must have reconstituted the lyophilized material correctly since performance was comparable in both schemes. Since we have established that lyophilized QC is as stable as liquid QC, so we can now move on to investigate the best financial option for indigenous lyophilization.

The advantage of lyophilized QC is the additional analytes which can be included in the monthly survey including the six new analytes, which are being requested by over 250 of our laboratories and the assay of sodium and potassium using ion selective electrodes.

ACKNOWLEDGMENTS

We gratefully acknowledge the generosity of Roche Diagnostics, GmbH in providing the lyophilized QC specimens and the role of the IFCC in coordinating the project.

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