Calibrator: How Long is it Stable?

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Abstract

Calibration is a process of verifying, by comparison with a calibrator of known quantity for its accuracy and imprecision within its assay range. The calibration so performed will have validity and has to be repeated at the end of the validity. The stated stability of the calibrator material after reconstitution is 24hrs. Due to this limited stability, calibration per se, becomes a costly process since few parameters might require frequent calibration. With every calibration, new set of calibrators have to be used that would add to the cost of calibration and cost per reportable test. There are no literatures available which have studied calibrator stability beyond the manufacture's stated stability. Since the stability of the analytes are much longer than 24 hours in serum, we wanted to see if the same is applicable in calibrators as the matrix of calibrators is comparable to serum. Calibration data from January 2015 to December 2016 was collected. All calibrators reconstituted for the analytes to be studied were included. We assayed calibrator at day 0 after reconstitution for Glucose, Total cholesterol, Total protein, Iron and Lactate dehydrogenase. The mean at 0 days was compared with the mean for 3 different time intervals beyond 24 hours after storing aliquots at -20^{0}C. The calibrator for glucose was stable for up to 8 weeks, total cholesterol for 7 weeks, total protein for 10 weeks, iron for 28 weeks and LDH for 17 weeks.

Keywords: Calibration, Calibrator, Stability

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Introduction

Analytical calibration is an operation that establish a relationship between an instrument signal with analyte concentration, for a measuring system under specified conditions. The result of calibration is a model that may have the form of a conversion factor, a mathematical equation, or a graph. By means of this model, then it is possible to estimate analyte concentration.(1) It may also include adjustment of the instrument to bring it into alignment with the calibrator.(2)

Instrument calibration is intended to eliminate or reduce bias in an instrument's readings covering a range of interest for all continuous values. A functional relationship is established between the assigned values of the calibrator and the values obtained during measurement. The calibration so performed will have validity and has to be repeated at the end of the validity.(3)

The goal of calibration is to minimise any measurement uncertainty by ensuring the accuracy of test equipment. Calibration quantifies and control errors or uncertainties within measurement process to an acceptable level.(4)

Calibration is done by using commercially available calibrators or standards or reference material. This commercially available calibrator would have been assigned value by the manufacturer. This assigned value is established by using a definitive or reference method or by using reference materials traceable to primary standards. The calibrator is used to set the value reported by the laboratory's method or instrument. Calibrators are available as either as ready-to-use liquid stable state or in lyophilized state that has to be reconstituted before its use. These calibrators can be single constituent (analyte) or multi constituent (multi-analyte).(5)

Stability is "the ability of an *in vitro* diagnostic reagent, when kept under specified conditions, to retain throughout the shelf life its characteristics and/or performance within limits specified by the manufacturer". Firstly, stability is related to specified storage conditions for example, refrigerated (2°C-8°C), room temperature (15°C-25°C) and indeed frozen (≤ -20°C). Secondly, stability is related to the characteristics and performance limits established by the manufacturer. Performance limits may include recovery/ accuracy, precision, sensitivity etc. The stability can be tested by accelerated stability testing, real time stability testing, reconstituted stability testing, on board/ open vial stability and routine stability testing.(6)

Most often the stability of the calibrator material is 24hrs at 2-8ºC after reconstitution as stated by manufacturer. Due to this limited stability, calibration per se, becomes a costly process since few parameters might require frequent calibration. With every calibration, new set of calibrators must be used that would add to the cost of calibration and eventually to cost per reportable test. This could be minimised if the stability of calibrators were much longer as stated by the manufacturer. There are very few published articles available which have studied calibrator stability that challenges the manufacture's stated stability. The

stability of the analytes are much longer than 24 hours in serum.[4]

To minimise cost borne due to calibration without affecting the quality of the test results, we embarked on this study to evaluate the stability of the calibrators. The range of parameters for the study was selected to ensure that it covered enzyme activity, kinetic and end point calibrations.

In our study we hypothesised that there would be no significant difference in calibrator values when it is used beyond the stated stability period. To test this hypothesis, we have evaluated calibrators on the day of reconstitution and subsequently. The goal of calibration is to minimise any measurement uncertainty by ensuring the accuracy of test equipment. Calibration quantifies and control errors or uncertainties within measurement process to an acceptable level.

Materials and Method

This analytical study was carried out in a hospital based NABL accredited lab in department of Biochemistry, St. John's Medical College and Hospital, Bangalore after obtaining IEC approval. Calibration data was obtained from calibration records for a period of two years: retrospective data from January 2015 to December 2015 and prospective data from January 2016 to December 2016. We collected retrospective data to get more number of data points and to cover changes in reagent and calibrator lots. Chemistry assays chosen for evaluation were glucose, total cholesterol, total protein, Iron and Lactate dehydrogenase. The parameters were chosen to ensure that both high- and low-volume sample loads were included. All the parameters were calibrated using dedicated reagents and calibrators in a closed system on a random access autoanalyser (Siemens Healthcare Diagnostics, Dimension ExL, USA).

The calibrations that have passed the acceptance criteria (Table 1) were included in the study.

Table 1: Acceptance criteria for calibrations

Criteria	Linear Method
Slope	0.97 to 1.03
Intercept	0.0 or clinically insignificant
Correlation Coefficient	0.990 to 1.000

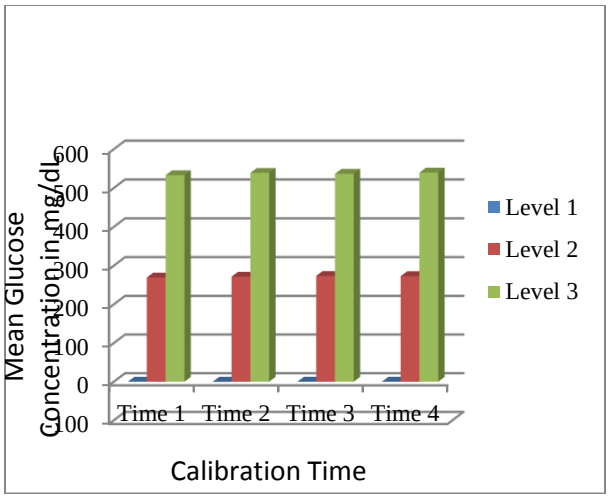

Fig. 1: Comparison of glucose calibration (3 levels) across four-time points

Calibrations with one or more value missing, value with error messages such as arithmetic or above assay range or high 'A' error or nan - nan designates "not a number" or assay range were not included for the analysis.

All calibrator packs have 2- 3 sets of calibrators with 3 levels in each set. The calibrators were analysed in triplicates during calibration. Calibrators for glucose, total cholesterol, total protein and iron are available as lyophilised powder. They were reconstituted with deionised water and mixed by gentle swirling. After reconstitution of calibrator, the aliquots prepared are labelled and stored in air tight vials at -20°C. Calibrator for LDI is available as a liquid stable, ready to use form which is stored at 2-8°C. Calibrators for all these parameters are a set of 3 levels with the first one being zero concentration/ blank. These vials are thawed before calibration. We have included atleast 5-6 calibrations at different time points and also calibrations done from 3 sets of calibrators. The first run was assigned as Time 1 i.e., on the day of reconstitution of calibrator. The subsequent runs (recalibrations done as per requirement) were assigned Time 2, Time 3 and Time 4. These recalibrations were done using the aliquots from the same bottle. We have considered 4 time –frames in our study (Table 2).

Table 2: Time frame for all analytes

Analyte	Time 1	Time 2 (week)	Time 3 (week)	Time 4 (week)
Glucose	0	1	4	8
Total Cholesterol	0	2	4	7
Total Protein	0	2	5	10
Iron	0	4	12	28
LDH	0	1	6	17

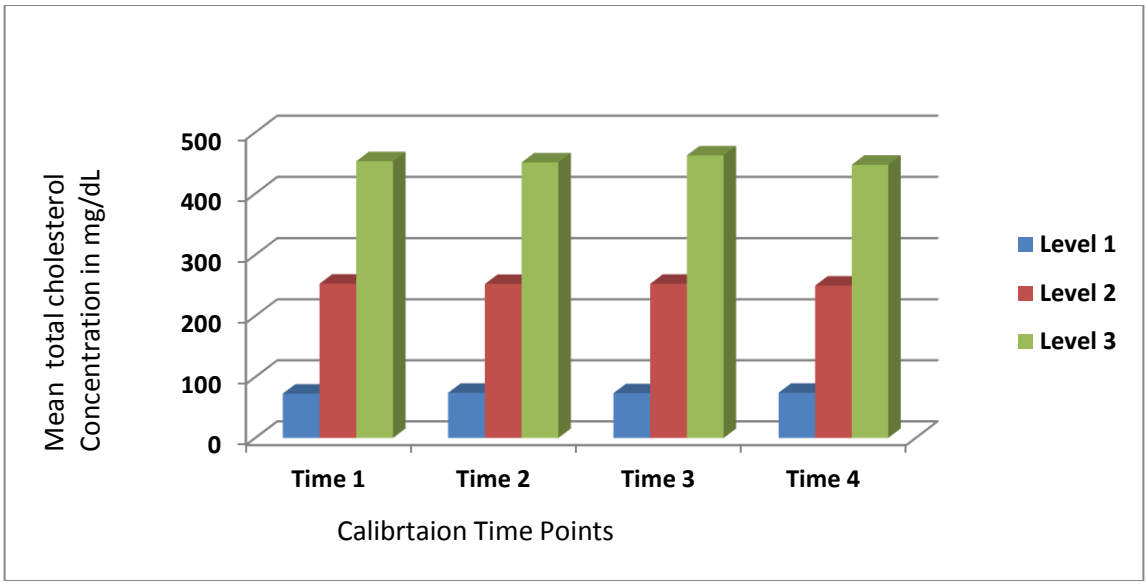

Fig. 2: Comparison of total cholesterol Calibration (m levels) across four-time points

Table 3: Descriptive statistics for glucose at varying time points

			Time 1	Time 2	Time 3	Time 4
Glucose (n=24)	Level 1	Mean	0.13	-0.33	0	0.04
		SD	0.85	1.63	0.66	0.91
		p value		0.41	0.82	0.88
	Level 2	Mean	269.5	271.17	273.58	273.04
		SD	5.02	4.24	4.52	3.52
		p value		0.40	0.04	0.08
	Level 3	Mean	533.08	538.92	536.67	539.79
		SD	6.20	8.26	7.73	11.15
		p value		0.14	0.36	0.09

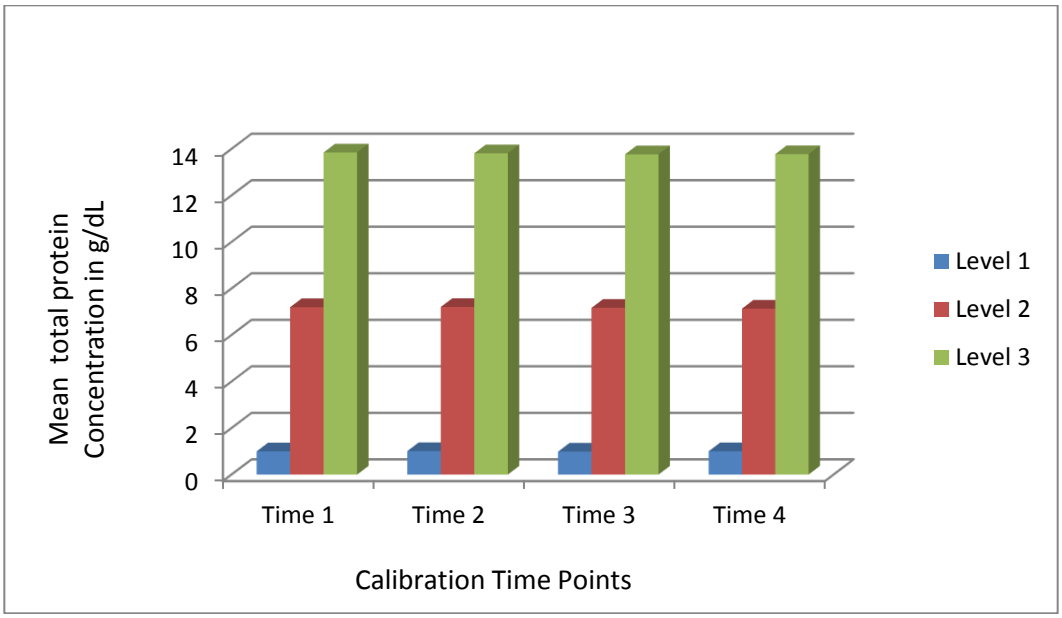

Fig. 3: Comparison of total protein calibration (3 levels) across four-time points

Table 4: Descriptive statistics for total cholesterol at varying time points

			Time 1	Time 2	Time 3	Time 4
Total Cholesterol (n=9)	Level 1	Mean	72.74	73.97	73.26	74.11
		SD	2.55	2.35	2.32	3.51
		p value		0.16	0.56	0.12
	Level 2	Mean	252.82	252.41	252.63	249.74
		SD	5.64	10.27	5.71	9.75
		p value		0.89	0.10	0.30
	Level 3	Mean	452.93	450.89	462.37	447.07
		SD	6.45	19.15	16.18	18.57
		p value		0.69	0.07	0.26

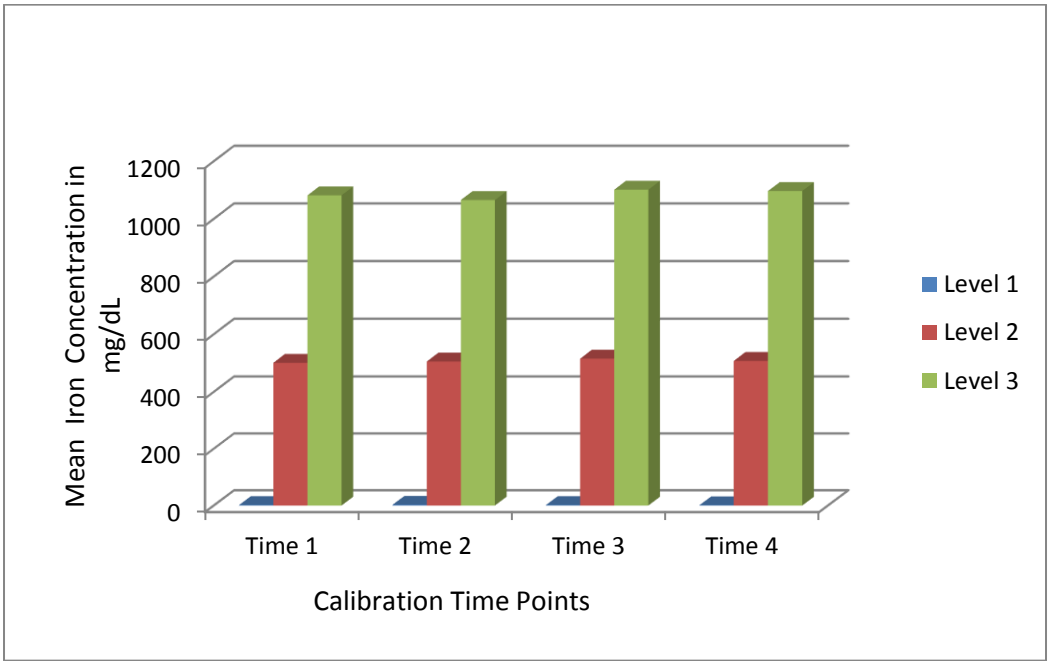

Fig. 4: Comparsion of iron calibration (3 levels) across four-time points

Table 5: Descriptive statistics for total protein at varying time points

			Time 1	Time 2	Time 3	Time 4
Total Protein (n=6)	Level 1	Mean	1.00	1.01	0.99	1.00
		SD	0.04	0.09	0.11	0.00
		p value		0.78	0.78	0.90
	Level 2	Mean	7.20	7.20	7.17	7.13
		SD	0.14	0.16	0.16	0.12
		p value		0.92	0.66	0.33
	Level 3	Mean	13.85	13.81	13.77	13.77
		SD	0.28	0.20	0.39	0.45
		p value		0.83	0.65	0.69

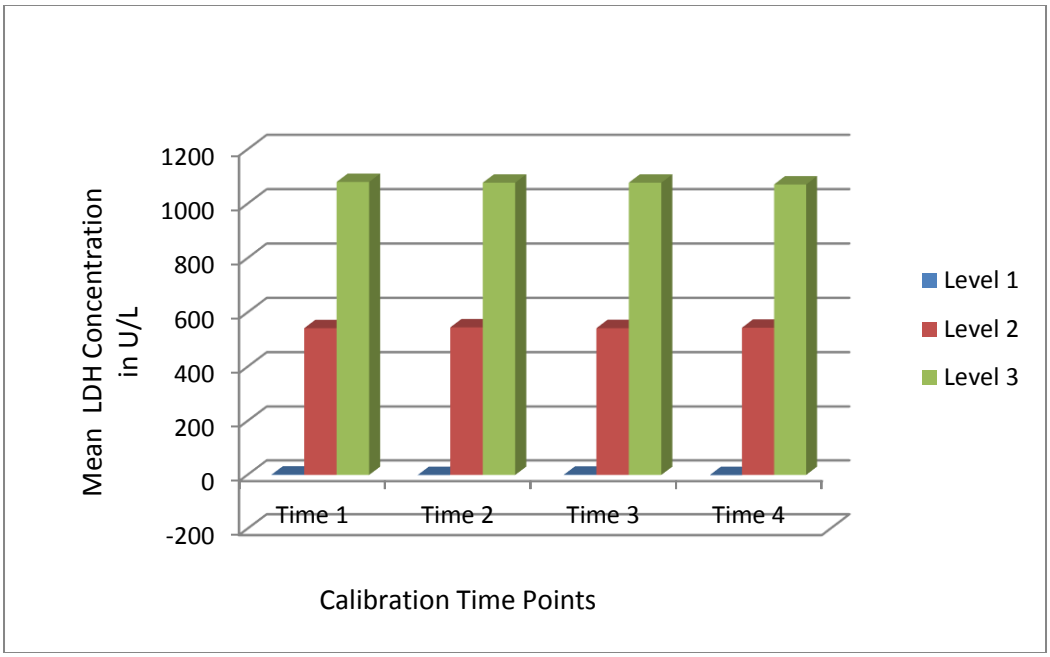

Fig. 5: Comparison of LDH calibration (3 levels) across four-time points

Table 6: Descriptive statistics for iron at varying time points

			Time 1	Time 2	Time 3	Time 4
Iron (n=5)	Level 1	Mean	2.00	2.80	2.00	1.40
		SD	1.87	2.28	1.58	1.34
		p value		0.18	0.69	0.69
	Level 2	Mean	501.00	505.40	515.40	507.60
		SD	4.74	6.47	18.49	12.46
		p value		0.58	0.07	0.45
	Level 3	Mean	1081.80	1065.60	1101.40	1096.80
		SD	30.79	38.93	28.18	26.61
		p value		0.38	0.35	0.52

Table 7: Descriptive statistics for LDH at varying time points

			Time 1	Time 2	Time 3	Time 4
LDH (n = 5)	Level 1	Mean	1.60	-0.20	1.00	-0.80
		SD	1.52	3.96	2.92	3.63
		p value		0.42	0.74	0.08
	Level 2	Mean	542.40	545.40	542.60	544.20
		SD	7.13	6.73	2.70	6.94
		p value		0.94	0.14	0.97
	Level 3	Mean	1080.60	1077.80	1078.00	1071.40
		SD	7.89	8.59	8.60	12.24
		p value		0.97	0.15	0.91

Subsequent calibrations runs were accepted based on the laid down criteria for calibration. As a part of lab protocol, third party Internal Quality Control (Bio Rad) were run and checked for its satisfactory performance. IQC value will be rejected if 1_{3s}, 2_{2s}, R_{4s}, 4_{1s}, 10_{x} rules are violated. For new lot of reagent, lot- to- lot verification was done for acceptability.

Statistical analysis was performed using SPSS (17.0 version). Triplicate's average data was taken and comparison was done within bottle levels for glucose,

total cholesterol, total protein, Iron and LDH. The mean for each time point -Time 1, Time 2, Time 3 and Time 4 was compared with Time 1 using linear mixed models. Statistical significance was considered at $p < 0.05$.

Results

The mean values for each set of calibrator were compared across respective levels for analyte. There was no significant difference in the calibrator recovery between time 1 and all the other 3 time points for total cholesterol, total protein, iron and LDH (Table 4-7 and Fig. 2-5). There was no significant difference between time 1 and all the other 3 time points for level 1 and 3 of glucose. However, significant difference was noted for level 2 between time1 and time 3 (Table 3 and Fig. 1). The highest coefficient of variation obtained for the analytes during the study period was glucose- 3.9%, total cholesterol- 3.3%, total protein- 1.7%, iron- 2.7% and LDH- 4.61%.

From the results, it can be inferred that calibrator for glucose was stable for up to 8 weeks, total cholesterol for 7 weeks, total protein for 10 weeks, iron for 28 weeks and LDH for 17 weeks.

Discussion

Stability of serum glucose, total cholesterol, total protein and iron at -20°C is 28 days, one year, 6 months, 2 months respectively and LDH at room temperature for 3 days.[7] Manufacturer states that the stability of lyophilised calibrators after reconstitution is 24 hours at 2-8^{0}C. Stability of these calibrators at -20°C is not mentioned in the calibrator pack insert. The stability of liquid stable, ready to use LDH calibrator is 28 days at 2-8°C.[8] There is no literature regarding the stability of frozen calibrators beyond 24 hours. We initiated this study, keeping the stability of serum in mind. We also wanted to bring down the cost due to frequent purchase of calibrators. In our study, we did not find any significant difference between the first run and the last run in total cholesterol, total protein, lactate dehydrogenase and iron. Only with glucose, the penultimate run had a significant difference from the first run whereas the last run was not statistically significant. But the same difference was neither clinically significant nor significantly different from the assigned value to reject the calibration. This could have been just a random error as run 1 was slightly lesser than the assigned value for the calibrator and we obtained the assigned value during run 3.

From our study, we have seen that the calibrator for glucose was stable for up to 8 weeks, total cholesterol for 7 weeks, total protein for 10 weeks, iron for 28 weeks and LDH for 17 weeks. This stability is more than the stability quoted by manufacturers. From our study, we can infer that the calibrators are stable for longer period than stated by the manufacturer when capped tightly and stored at -20^{0}Cfor lyophilised and at 2-8^{0}C for liquid stable calibrators. We have not studied the stability beyond the time points mentioned since the calibrators were exhausted.

According to literature, serum LDH should be stored at room temperature and is not stable if frozen. In our study, we have frozen the LDH calibrator aliquots at -20^{0}C. On subsequent runs, we found that there was no significant difference between the values obtained with subsequent runs. We have seen that LDH is stable at -20^{0}C for 5 months. Therefore, we have seen that calibrators can be aliquoted and frozen for longer storage.

Conclusion

Manufacturer stated stability of calibrators is less than 24 hours. But we wanted to study if it will be stable for longer periods on freezing. We compared the recovery at day 0 with the mean of recovery on 3 different time points on freezing aliquots at -20°C. From the results, it can be inferred that calibrator for glucose was stable for up to 8 weeks, total cholesterol for 7 weeks, total protein for 10 weeks, iron for 28 weeks and LDH for 17 weeks.

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