International Journal of Biomedical and Advance Research
ISSN: 2229-3809 (Online); 2455-0558 (Print)
Journal DOI: https://doi.org/10.7439/ijbar
CODEN: IJBABN

Original Research Article

Effect of calibration frequency on coefficient of variation observed with internal quality control samples for Glucose, Total Protein, Iron and Unsaturated Iron Binding Capacity

Shilpi Shloka*, Manmeet Kochar, Niral Savaliya, Chapal Debnath and Shaileshkumar Manubhai Patel

Biochemistry Department, Government Medical College (GMC), Opp Income Tax Department Office, Majura Gate, Surat, Gujarat 395001 India

QR Code

***Correspondence Info:**
Dr. Shilpi Shloka,
Biochemistry Department,
Government Medical College (GMC),
Opp Income Tax Department Office,
Majura Gate, Surat, Gujarat 395001 India

***Article History:**
Received: 10/11/2018
Revised: 12/01/2018
Accepted: 12/01/2018
DOI: https://doi.org/10.7439/ijbar.v9i12.4955

Abstract

Objectives: The study aims at comparing precision of Glucose, Total Protein, Iron and Unsaturated Iron Binding Capacity assays between lot calibration and daily calibration.
Methods: For Glucose, Total Protein, Iron and Unsaturated Iron Binding Capacity calibration factors were derived during lot change as well as daily. Both types of calibration factors were used to derive results of internal quality control samples daily for 20 days. Coefficient of variation for IQC results for both lot-calibration and daily-calibration were measured.
Results and conclusions: CV% observed for daily calibration was higher than lot-calibration for total protein, glucose and Iron. While CV% observed for daily calibration were lower than lot-calibration for UIBC. It is concluded that optimum frequency of calibration differs for each examination and depends on robustness of testing process for each analytes.
Keywords: Calibration, Clinical Chemistry, Glucose, Total Protein, Iron, UIBC.

1. Introduction

Calibration is process of configuring an instrument to provide a result for a sample within an acceptable range of limits. Calibration [1] is checking the accuracy of a measurement instrument by comparing it to reference standards. The result of equipment calibration is higher accuracy.

As per ISO15189:2012 clause 5.3.1.4

The laboratory has a documented procedure for the calibration of equipment that directly or indirectly affects examination results [2]. This procedure includes:

- Taking into account conditions of use and the manufacturer's instructions;
- Recording the metrological traceability of the calibration standard and the traceable calibration of the item of equipment;
- Verifying the required measurement accuracy and the functioning of the measuring system at defined intervals;
- Recording the calibration status and date of recalibration;
- Ensuring that, where calibration gives rise to a set of correction factors, the previous calibration factors are correctly updated;
- Safeguards to prevent adjustments or tampering that might invalidate examination results.
- Metrological traceability is to a reference material or reference procedure of the higher metrological order available.

Note

Documentation of calibration traceability to a higher order reference material or reference procedure may be provided by an examination system manufacturer. Such documentation is acceptable as long as the manufacturer's examination system and calibration procedures are used without modification. Where this is not possible or relevant, other means for providing confidence in the results shall be applied, including but not limited to the following:

- Use of certified reference materials;
- Examination or calibration by another procedure;

– Mutual consent standards or methods which are clearly established, specified, characterized and mutually agreed upon by all parties concerned.

In addition to ISO 15189:2012 NABL 112 clause 5.3 states about factors for calibration frequencies [3].

a. Ruggedness of the equipment
b. Frequency of use
c. Life of the equipment
d. Quality and periodicity of maintenance, etc

The implication of ISO15189:2012 is that, a laboratory cannot solely on manufacturer's instructions for deciding calibration frequency. In addition to manufacturer's instructions, condition of use, frequency of use, life of the equipment, quality and periodicity of maintenance, which may differ from one installation to another?

One way to know about stability of calibration is coefficient of variation observed in internal quality control program of the laboratory. Observation of CV% for IQC sera obtained by different calibration strategy can give idea about optimum calibration frequencies. Present study aims to find appropriate calibration frequency for Glucose, total Protein, Iron, UIBC for Erba XL-640 at Biochemistry laboratory of New Civil Hospital and Government Medical College, Surat.

2. Materials and method

IQC sera of two levels were examined for Glucose, Total Protein, Iron [4] and UIBC [4] daily for 20 days. All four examinations were calibrated on putting new lot in use. While examining IQC sera, three level calibrators were also examined to find absorbance of calibrators and results of IQC were manually calculated using daily calibration factors obtained.

Three level calibrators are shown in Table 1 below

Table 1: Assigned value of calibrators

	Assigned Value			
Calibrator	**Glc(mg/dl)**	**TP(gm/dl)**	**Iron(µl/dl)**	**UIBC(µl/dl)**
Randox Normal Calibrator (Lot 1242N)[4]	112	5.94	107	153
Randox Abnormal Calibrator (Lot 961UE)[5]	274	4.60	201	99
Water Blank	0	0	0	0

Table 2: Shows test parameters used in Erba XL-640

	Glucose	**TP**	**Iron**	**UIBC**
Method	GOD-POD[6,7]	Biuret[8]	Ferrozine[9]	Ferrozine[9], Alkaline pH
Equipment	Erba XL-640	Erba XL-640	Erba XL-640	Erba XL-640
R1 vol	200µl	200µl	200µl	200µl
R2 vol	-	-	20µl	20µl
Sample Vol	2	4	34	50
Method	2 point	2 point	2 point	2 point
Wavelength	505	570	570	546
Table-2 Test parameters used in ERBA XL-640				

- 1 cup of normal calibrator, 1 cup of abnormal calibrator, 1 cup of human control assay 2, 1 cup of human control assay 3, 1 cup of water black were kept in same batch as IQC.
- Details of controls used are given in Table 3 below

Table 3: Assigned value of controls

Control	**Assigned Value**			
	Glc(mg/dl)	**TP (gm/dl)**	**Iron(µl/dl)**	**UIBC(µl/dl)**
Human control assay 2(Lot 1221UN)[10]	115	5.87	105	148
Human control assay 3(Lot 912UN)[10]	285	4.42	212	115

- OD of blank, calibrator and control, were obtained. All data was entered in a spreadsheet. Using OD of blank and calibrators daily factors was calculated. This factor was used to obtain value of control by multiplying OD of control with this factor. This process was continued for 20 days. Simultaneously values of control were also obtained using factor obtained during lot calibrations for all four parameters. All data were entered in a spreadsheet; average SD and CV% were calculated. CV% obtained for all parameters by two different method of calibration were calculated

3. Results

Table 4: Shows results for Total Protein

	Randox Human Assay Control 2		Randox Human Assay Control 3	
Day	**Result with Lot Calibration**	**Result with Daily Calibration**	**Result with Lot Calibration**	**Result with Daily Calibration**
1	5.80	5.76	4.50	4.41
2	6.00	5.50	4.60	4.19
3	6.20	5.64	4.60	4.36
4	5.80	5.63	4.70	4.38
5	6.10	5.76	4.80	4.41
6	5.90	7.70	4.70	4.34
7	5.80	5.58	4.70	4.36
8	5.80	5.54	4.70	4.39
9	5.90	5.36	4.60	4.31
10	5.90	5.68	4.50	4.38
11	6.10	5.77	4.70	4.41
12	6.00	5.76	4.60	4.47
13	5.90	5.28	4.60	4.05
14	6.00	5.58	4.60	4.33
15	5.90	5.89	4.70	4.53
16	5.90	5.69	4.80	4.57
17	5.90	5.67	4.40	4.65
18	5.70	5.67	4.50	4.41
19	5.80	5.94	4.60	4.12
20	5.90	5.56	4.60	4.33
Mean	5.92	5.65	4.63	4.37
SD	0.12	0.16	0.10	0.14
CV	**2.07**	**2.77**	**2.20**	**3.17**

Table 5: Shows results for Glucose

	Randox Human Assay Control 2		Randox Human Assay Control 3	
Day	**Result with Lot Calibration**	**Result with Daily Calibration**	**Result with Lot Calibration**	**Result with Daily Calibration**
1	114	115.9	290	285
2	119	111.7	283	276
3	117	110.9	292	278
4	114	115.9	282	285
5	117	111.7	297	276
6	118	117.2	288	276
7	114	115.4	295	280
8	114	114.2	282	291
9	117	109.7	285	273
10	114	115.2	289	297
11	114	119.0	289	288
12	115	119.3	289	299
13	115	109.7	284	275
14	114	114.4	285	289
15	116	124.1	280	298
16	115	122.3	293	293
17	114	113.4	280	304
18	116	115.1	282	292
19	118	116.2	276	268
20	114	114.5	280	286
Mean	115.45	115.30	286.05	285.34
SD	1.67	3.82	5.64	9.95
CV	**1.45**	**3.31**	**1.97**	**3.49**

Table 6: shows results for Iron

	Randox Human Assay Control 2		Randox Human Assay Control 3	
Day	**Result with Lot Calibration**	**Result with Daily Calibration**	**Result with Lot Calibration**	**Result with Daily Calibration**
1	101	104	214	203
2	105	141	210	220
3	112	140	207	235
4	108	130	211	218
5	103	121	188	223
6	109	128	211	232
7	114	129	203	205
8	107	120	201	207
9	104	111	212	203
10	104	115	199	205
11	108	117	213	200
12	100	112	208	195
13	110	114	193	206
14	98	107	199	199
15	103	111	186	211
16	103	116	202	207
17	102	114	201	210
18	105	116	203	180
19	102	113	205	188
20	114	105	204	197
Mean	105.60	118.20	203.50	207.20
SD	4.52	10.48	7.86	13.51
CV	**4.28**	**8.86**	**3.86**	**6.52**

Table 7: Shows results for UIBC

	Randox Human Assay Control 2		Randox Human Assay Control 3	
Day	**Result with Lot Calibration**	**Result with Daily Calibration**	**Result with Lot Calibration**	**Result with Daily Calibration**
1	153	146	132	122
2	151	148	135	113
3	171	146	137	114
4	166	150	138	115
5	187	146	144	112
6	181	154	147	114
7	199	154	164	114
8	196	148	156	115
9	191	154	162	115
10	179	139	122	119
11	176	155	136	122
12	184	143	144	121
13	178	162	144	102
14	161	147	141	103
15	179	162	122	108
16	176	147	136	102
17	184	170	144	103
18	178	156	141	110
19	161	163	128	107
20	161	157	120	110
Mean	175.61	152.37	139.63	112.09
SD	13.39	7.74	12.08	6.38
CV	**7.62**	**5.08**	**8.65**	**5.69**

Table 8: Shows summary of CV% obtained for various examinations for both calibration strategies

	Comparison of CV % between different calibration strategy			
Test	**Result with Lot Calibration**	**Result with Daily Calibration**	**Result with Lot Calibration**	**Result with Daily Calibration**
Total protein	2.07	2.77	2.20	3.17
Glucose	1.45	3.31	1.97	3.49
Iron	4.28	8.86	3.86	6.52
UIBC	7.54	5.18	8.15	5.83

4. Discussion

Table-8 shows that for Total Protein, glucose and Iron, CV obtained is lower with Lot calibration as compared to daily calibration. While, for UIBC CV obtained is higher with Lot calibration as compared to daily calibration.

Certain variation in measurement system are momentary, lasting few minutes, certain variation lasting few hours and certain lasting for days to weeks.

For example, electrical fluctuation, resulting in variation in result last for few moments only. A blockage in washing station develops over few days. Change in reagent quality occur only once in a month when lot changes, if reagent is stable. While an unstable reagent develops increasing reagent blank over days.

Depending of nature of laboratory certain factors may be more or less important. If variation in such factors is to be considered, laboratory may need to define their own criteria and conditions for re-calibrating assay.

In the study, it was found that, some examinations are stable over a period of weeks and months (like Total Protein, Glucose and Iron). While stability of certain assay like UIBC is for a day. Thereby, laboratory needs to perform daily calibration of such unstable assay in order to improve precision.

Moreover, the example of Glucose, Total Protein and Iron shows that, unnecessary calibration not only add cost to the laboratory, but also are counter-productive and increase assay variability.

5. Conclusion

The study concludes that optimum calibration frequency of an assay depend on laboratory environment and robustness of assay. Finding optimum calibration frequency can help laboratory improve assay impression.

References

[1]. What is Calibration? Advanced Instruments [Internet]. Aicompanies.com. 2018 [cited 9 November 2018]. Available from: https://www.aicompanies.com/

[2]. Bureau of Indian Standards. IS/ISO 15189:2012. Medical laboratories - Requirements for quality and competence. New Delhi: BIS; 2012.

[3]. National Laboratories. Accreditation Board for Testing and Calibration NABL-112. Specific Criteria for Accreditation of Medical Laboratories. New Delhi: NABL;2018

[4]. Randox. *Calibration Serum–Level 2.*United Kingdom: Randox;2015

[5]. Randox. *Calibration Serum –Level 3.*United Kingdom: Randox;2015

[6]. Erba Manheim. *Glucose Reagent GOD-POD.* Germany: Erba Diagnostics Manheim Gmbh;2018

[7]. Trinder, Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. P. *Ann. Clin. Biochem*.1969; 22(2):158-61.

[8]. Basil T. Doumas, David D. Bryce, Richard J. Carter, Theodore Peters Jr., Robert Schaffer. A Candidate Reference Method for Determination of Total Protein in Serum: *Clinicalchemistry.*1981;27(10):1642-1650

[9]. Jesse F. Goodwin, Bramlett Murphy, Marcel Guillemette. Direct Measurement of Serum Iron and Binding Capacity: *Clinical Chemistry* 1966; 12(2): 47-57.

[10]. Randox. *Human Assay Multi-sera–Level 2.* United Kingdom: Randox;2017