BRIEF REPORT

Comparison of two commercial quality control sera for adrenocorticotropin (ACTH) used in Elecsys[®] immunoassay system

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Objectives: The purpose of our study was to investigate whether the storage time and temperature of internal quality control (IQC) material influence the result of ACTH in IQC measurements.

Design and methods: Five levels of IQC materials from two manufacturers were tested through the precision of ACTH, the three freeze/thaw cycles, and the storage time and temperature to evaluate the stability of IQC material. All commercial control materials were simultaneously tested three times a day for five consecutive days.

Results: Total precision of three levels of Bio-Rad IQC sera was 13.93%, 16.45%, and 15.98%, respectively, but repeatability was <2%. The concentration of ACTH decreased by 30%-50% after 3 freeze/thaw cycles. At room temperature, the concentration of ACTH from 3 levels decreased by 16.60%, 17.98%, and 17.20%, respectively, after 0.5 hours, and 70.54%, 74.36%, and 72.03%, respectively, after 4 hours. However, after 2 hours of storage at 4°C, the decline in the measured ACTH IQC was 8.04%, 11.84%, and 10.11%, respectively. Total precision of Roche IQC was 1.17% and 1.08%, respectively. After 3 freeze/thaw cycles, the concentration of ACTH decreased <5%. After 4 hours, the change of ACTH still steadied within 5% both at the room temperature and at 4°C.

Conclusion: Roche is a better choice for ACTH of IQC material in Elecsys[®] immunoassay system in our study. If Bio-Rad control materials be used in Elecsys[®] immunoassay system for ACTH IQC testing material, it should be stored at 4°C and testing should be completed within 1 hours.

KEYWORDS

adrenocorticotropin, internal quality control

1 | INTRODUCTION

It is now undeniable that the laboratory testing is vital for the diagnosis, prognostication, and therapeutic monitoring of human disease. Meanwhile, laboratories face inaccurate detection of the results, which can be caused by the measurement shifts and drifts adversely affecting the quality of testing. Internal quality control (IQC) is very crucial for medical biology laboratories to ensure results reliability.¹ The primary objective of IQC is to ensure daily consistency of an

analytical process and, thus, help to determine whether patients' results are reliable enough to be delivered to the clinicians.²

Adrenocorticotropin (ACTH) is a commonly used test in the differential diagnosis of hypothalamic-pituitary-adrenal disorders. Circadian rhythmicity of ACTH measurement is of great clinical significance for adrenocorticotrophic hormone-independent Cushing syndrome.³ The average coefficient of variation (CV) of ACTH IQC from Bio-Rad (Bio-Rad Laboratories, Inc., USA) fluctuated from 4% to 14% in our laboratory, which is considered to be unstable.

Meanwhile, as we know, ACTH concentration is interfered by store time and temperature of plasma samples of the patients.⁴ These facts prompted us to investigate whether the storage time and temperature of IQC material influence the result of ACTH in IQC measurements.

2 | METHODS

2.1 | Experimental design

Five levels of IQC materials from two manufacturers were tested through the precision of ACTH, the three freeze/thaw cycles, and the storage time and temperature to evaluate the stability of IQC material. All commercial control materials were simultaneously

tested three times a day for five consecutive days and whose aliquots were frozen and thawed three times. The lyophilized IQC is dissolved in accordance with the requirements of instruction for use from the manufacturer. ACTH was then measured either immediately or after 0.5-hour, 1-hour, 2-hour and 4-hour storage at 4°C or room temperature.

2.2 | Analytical methods

Three levels of IQC materials from Bio-Rad (Lyphochek® Immunoassay Plus Control, LOT: 40311, 40312, 40313) and two levels of Roche IQC materials (PeciControl Multimarker, LOT: 23764701) were used to check the impact of store time and temperature of control materials on ACTH IQC results which were measured on a cobas e602 module (Roche Diagnostics, Mannheim,

				Mean	SEM	CV%
Bio-Rad—level 1	20.21	20.08	20.08	20.12	0.08	0.37
	15.60	15.49	15.52	15.54	0.06	0.37
	14.32	14.53	14.74	14.53	0.21	1.45
	14.63	14.59	14.29	14.50	0.19	1.28
	15.06	14.79	14.77	14.87	0.16	1.09
Total				15.91	2.22	13.93
Bio-Rad—level 2	67.51	66.46	66.67	66.88	0.56	0.83
	49.32	49.20	49.30	49.27	0.06	0.13
	45.70	45.78	45.38	45.62	0.21	0.46
	45.41	45.03	44.99	45.14	0.23	0.51
	48.22	48.06	47.53	47.94	0.36	0.75
Total				50.97	8.39	16.45
Bio-Rad—level 3	103.70	103.90	102.89	103.50	0.53	0.52
	79.07	78.22	78.56	78.62	0.43	0.54
	71.46	71.26	70.29	71.00	0.63	0.88
	71.06	71.47	70.62	71.05	0.43	0.60
	74.14	73.08	73.80	73.67	0.54	0.73
Total				79.57	12.72	15.98
Roche—level 1	41.96	40.25	40.49	40.90	0.93	2.26
	40.03	40.33	40.51	40.29	0.24	0.60
	41.21	40.85	41.12	41.06	0.19	0.46
	40.25	40.82	40.85	40.64	0.34	0.83
	40.89	40.65	40.67	40.74	0.13	0.33
Total				40.73	0.48	1.17
Roche—level 2	864.9	878.5	869.3	870.90	6.94	0.80
	861.7	862.9	862.5	862.37	0.61	0.07
	857.0	864.2	854.3	858.50	5.12	0.60
	856.5	850.8	856.0	854.43	3.16	0.37
	841.2	846.6	852.2	846.67	5.50	0.65
Total				858.60	9.28	1.08

TABLE 1 The result of daily precision test of two internal quality control (IQC) sera

Mean, average value; SEM, standard error mean; CV%, coefficient of variation; the first five rows were within-run and the results of total are calculated based on 15 results of the same level

Germany) with electrochemiluminescence (ECL) technology in accordance with standard operating procedures (SOPs).

2.3 | Statistical analysis

Relative change was calculated according to the formula: $\% \text{change} = [(\text{ACTH IQC result} - \text{ACTH reference IQC result}) / \text{ACTH reference IQC result}] \times 100$, where ACTH reference IQC result was obtained without time delay after reconstitution of lyophilized control materials. A change of $>10\%$ from reference level was taken as evidence of significant interference.⁵ The precision of the method was determined using two different approaches, within-run and

total precision. The precision was calculated as the coefficient of variation (CV) of repeated measurements. Within-run precision was calculated by running three replicates of each level of IQC materials. Total precision was calculated according to CLSI protocol (EP15-A2), which suggests two levels, run 3 times per run for 5 days (15 replicates in all).⁶

3 | RESULT

Total precision of three levels of Bio-Rad IQC sera was 13.93%, 16.45%, and 15.98%, respectively, but repeatability was $<2\%$

FIGURE 1 Effect of freeze/thaw cycles on two internal quality control (IQC) sera A, Time of storage conditions and temperature effect on ACTH assay of BIO-RAD IQC sera B, and of Roche C, at $+4^{\circ}\text{C}$ and room temperature

(Table 1). The concentration of ACTH decreased by 30%-50% after 3 freeze/thaw cycles (Figure 1A). The concentration of ACTH from 3 levels of the one IQC decreased by 16.60%, 17.98%, and 17.20%, respectively, after 0.5 hours at room temperature. After 4 hours at room temperature, it had decreased by 70.54%, 74.36%, and 72.03%, respectively. However, after 2 hours of storage at 4°C, the decline in the measured ACTH IQC was 8.04%, 11.84%, and 10.11%, respectively (Figure 1B).

Total precision of Roche IQC was 1.17% and 1.08%, respectively (Table 1). After 3 freeze/thaw cycles, the concentration of ACTH decreased <5% (Figure 1A). After 4 hours, the change of ACTH still steadied within 5% both at the room temperature and at 4°C (Figure 1C).

4 | DISCUSSION

First, we tested the precision of ACTH test using three levels of Bio-Rad IQC. Performance for total precision was verified over five consecutive days using three levels of Bio-Rad IQC in triplicate, following the Clinical and Laboratory Standards Institute (CLSI) Evaluation Protocol 15 (EP15-A2) reference.⁶ The lyophilized IQC is dissolved in accordance with the requirements of instruction for use from the manufacturer and simultaneously tested three times a day for five consecutive days. Total precision of three levels of Bio-Rad IQC sera was 13.93%, 16.45%, and 15.98%, respectively, but repeatability was <2%. These results suggested that ACTH in IQC sera from Bio-Rad could probably be unstable. At the same time, two levels of IQC from Roche were tested. Total precision of Roche IQC was 1.17% and 1.08%, respectively. Samples of the all reconstituted commercial internal quality control materials were stored in a refrigerator and thus went through freeze/thaw cycles. To evaluate the effects of freeze/thaw cycles, we tested ACTH with all commercial control materials whose aliquots were frozen and thawed three times. The concentration of ACTH in Bio-Rad IQC decreased by 30%-50% after 3 freeze/thaw cycles.

Second, we did experiments about the impact of store time and temperature of IQC material on ACTH results. The lyophilized IQC was dissolved in accordance with the requirements of instruction for use from manufacturer and was divided into two aliquots. One was put in room temperature, and the other was stored in a refrigerator at 4°C. They were tested after 0.5, 1, 2, and 4 hours of reconstitution, respectively.

Our results showed the concentration of ACTH from 3 levels of Bio-Rad IQC decreased by 16.60%, 17.98%, and 17.20%, respectively, after 0.5 hours at room temperature. With the extension of time, ACTH concentrations dropped even further. After 4 hours, it had decreased by 70.54%, 74.36%, and 72.03%, respectively. These results indicated that temperature played an important role in ACTH stability. Given a 10% change in ACTH concentration could be accepted in routine clinical practice,⁷ ACTH control material of Bio-Rad stored at room temperature could not be used in routine IQC practice.

Our results showed slower decrease in ACTH concentration, if Bio-Rad IQC was stored at 4°C instead of room temperature. It suggests that ACTH concentration remains stable (<5% change) up to 0.5 hours at 4°C and acceptable (<10% change) up to 1 hours at 4°C. However, after 2 hours of storage at 4°C, the decline in the measured ACTH IQC was 8.04%, 11.84%, and 10.11%, respectively. After 4 hours of storage at 4°C, the decline of ACTH reached 14.26%, 15.29%, and 12.01%, respectively. By contrast, storing at both room temperature and 4°C, there was a minimal change in Roche IQC. Both the samples steadied at <5% after 4 hours. These results indicated that ACTH concentration in Roche IQC can be kept stable for over 4 hours and does not depend on the surrounding temperature.

Therefore, we recommend Roche as a better choice for ACTH of IQC material in Elecsys[®] immunoassay system. However, Bio-Rad as a third-party quality control product, multiple markers can be detected at the same time. Once the lyophilized IQC dissolves in accordance with manufacturer's instructions cannot be used up at one time, how to save it is a problem. The results of our study suggest that if Bio-Rad control materials are used in Elecsys[®] immunoassay system, it should satisfy some conditions. ACTH IQC testing material should be stored at 4°C and testing should be completed within 1 hours. When the dissolved IQC was divided into a few aliquots, it cannot be freeze/thaw cycles.

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AUTHOR CONTRIBUTIONS

All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

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REFERENCES

1. Bugni E, Cohen R, Mazellier C. IQC Laboratory management strategy for medical biology. *Ann Biol Clin*. 2017;75:637-645.
2. Kinns H, Pitkin S, Housley D, Freedman DB. Internal quality control: best practice. *J Clin Pathol*. 2013;66(12):1027-1032.
3. Chen S, Li R, Lu L, et al. Efficacy of dexamethasone suppression test during the diagnosis of primary pigmented nodular adrenocortical disease in Chinese adrenocorticotrophic hormone-independent Cushing syndrome. *Endocrine*. 2018;59:183-190.
4. Wu ZQ, Xu HG. Preanalytical stability of adrenocorticotrophic hormone depends on both time to centrifugation and temperature. *J Clin Lab Anal*. 2017;31(5):<https://doi.org/10.1002/jcla.22081>.
5. Garinet S, Fellahi S, Marlin G, Capeau J, Lefevre G, Bastard JP. Differential interferences of hemoglobin and hemolysis on insulin assay with the Abbott Architect-Ci8200 immunoassay. *Clin Biochem*. 2014;47(6):445-447.

6. Clinical and Laboratory Standards Institute. *User Verification of Performance for Precision and Trueness; Approved Guideline - Second Edn.* Wayne, PA: Clinical and Laboratory Standards Institute; 2006.
7. Reisch N, Reincke M, Bidlingmaier M. Preanalytical stability of adrenocorticotrophic hormone depends on time to centrifugation rather than temperature. *Clin Chem.* 2007;53(2):358-359.

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