Clin. Lab. 2018;64:XXX-XXX

SHORT COMMUNICATION

Verification of Bioanalytical Method for Quantification of Exogenous Insulin (Insulin Aspart) by the Analyser Advia Centaur® XP

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SUMMARY

Background: **In a number of cases the monitoring of patients with type I diabetes mellitus requires measurement of the exogenous insulin levels. For the purpose of a clinical investigation of the efficacy of a medical device for application of exogenous insulin aspart, a verification of the method for measurement of this synthetic analogue of the hormone was needed. The information in the available medical literature for the measurement of the different exogenous insulin analogs is insufficient. Thus, verification was required to be in compliance with the active standards in Republic of Bulgaria. A manufactured method developed for ADVIA Centaur XP Immunoassay, Siemens Healthcare, was used which we verified using standard solutions and a patient serum pool by adding the appropriate quantity exogenous insulin aspart.**
Methods: **The method was verified in accordance with the bioanalytical method verification criteria and regulatory requirements for using a standard method: CLIA chemiluminescence immunoassay ADVIA Centaur® XP.**
Results: **The following parameters are determined and monitored: intra-day precision and accuracy, inter-day precision and accuracy, limit of detection and lower limit of quantification, linearity, analytical recovery.**
Conclusions: **The routine application of the method for measurement of immunoreactive insulin using the analyzer ADVIA Centaur® XP is directed to the measurement of endogenous insulin. The method is applicable for measuring different types of exogenous insulin, including insulin aspart.**
(Clin. Lab. 2018;64:xx-xx. DOI: 10.7754/Clin.Lab.2017.171003)

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KEY WORDS

insulin, insulin aspart, method verification

LIST OF ABBREVIATIONS

CLIA - chemiluminescence immunoassay
C_{nom} - nominal concentration
LOD - limit of detection
LQC - quality control sample with low concentration
Sol - Solution
St - Standard

Short Communication accepted October 16, 2017

INTRODUCTION

The verification of the method for measurement of exogenous insulin aims to confirm the information in the available medical literature, as well as the analyzer's instructions, that the method is applicable for quantification of exogenous insulins in human serum [1,2].
The method was verified in accordance with the bioanalytical method verification criteria and regulatory requirements for using a standard method: CLIA chemiluminescence immunoassay ADVIA Centaur® XP, Siemens Healthcare. The ADVIA Centaur® XP Insulin assay is a two-site sandwich immunoassay using direct chemiluminescent technology which uses constant amounts of two antibodies. The first antibody, in the Lite Reagent, is monoclonal mouse anti-insulin antibody labelled with acridinium ester. The second antibody, in the Solid Phase, is a monoclonal mouse anti-insulin antibody, which is covalently coupled to paramagnetic particles.

MATERIALS AND METHODS

Master Curve Calibration for ADVIA Centaur XP Insulin assay was performed for each new lot number of the reagents (Lite Reagent and Solid Phase). The Master Curve card contains the Master Curve values, which is lot specific in the range of 0.5 - 300 µU/mL (two levels).
A serum pool was used to create five standards with concentrations as follows: St 1 (10 µU/mL); St 2 (30 µU/mL); St 3 (100 µU/mL); St 4 (200 µU/mL); St 5 (300 µU/mL).
The routine intra-laboratory quality control was performed using three levels of control samples, as follows: low concentration quality control (LQC BIO-RAD 1 Lot: 40321; Target Mean: 20.700 µU/mL); medium concentration quality control (MQC BIO-RAD 2 Lot: 40292; Target Mean: 88.500 µU/mL); high concentration quality control (HQC BIO-RAD 3 Lot: 40293; Target Mean: 228.0 µU/mL).
For the entire period this method is used, it is supported by stable Quality Control.

RESULTS

The following parameters are determined and monitored: intra-day precision and accuracy, inter-day precision and accuracy, limit of detection and lower limit of quantification, linearity, analytical recovery. The verification of the method is performed in August 2016. To prove proper operation of chemiluminescent immunoanalysis, before the procedures of validation as well as before the analysis of the clinical samples, a Luminometer technical check was performed.
The linearity of the method is tested, determined and imposed by the manufacturer of the method. The software of the analyzer extracts information from the database of the manufacturer specific for each batch number.
The linearity for this method extends from 0.5 µU/mL to 300 µU/mL.
The verification of the LOD was conducted using the data obtained for Standard 1. The serum pool, containing only endogenous insulin and no insulin aspart, was used to make this standard. Thus, it was appropriate for evaluation in terms of a sample free of the investigated compound (insulin aspart).
As can be seen, all other standards show recovery over 100% indicating the availability of the investigated compound (insulin aspart) except for Standard 1 (Table 2, Table 3).
The spiking recovery % was calculated for each standard concentration and juxtaposed with the data in the available literature [1,2]. The results showed higher levels of recovery than those expected for endogenous insulin, as stated in the Insulin (IRI) Assay Summary. Similar results were observed in a study of the Cross-Reactivity of Three Recombinant Insulin Analogs with Five Commercial Insulin Immunoassays [1,2].
In order to confirm the feasibility of the method developed by SIEMENS for the analyser ADVIA Centaur® XP Immunoassay System for the measurement of exogenous insulin, a procedure for analytical recovery was carried out in the following sequence:
A serum sample pool was collected from the laboratory's patients.
The serum was processed according to the laboratory's procedures; the sample was analyzed 10 times in two analytical runs; the level of insulin in the pool (endogenous insulin only) was calculated, and the fallowing values were obtained - mean 13.91, SD 0.32, % CV 2.3.
The Serum Pool was used to create five standard concentrations as follows:

St.1 concentration 10 µU/mL, preparation 2.81 mL dH2O + 7.19 mL serum pool
St.2 concentration 30 µU/mL, preparation 0.0810 mL Sol. + 9.919 mL serum pool
St.3 concentration 100 µU/mL, preparation 0.433 mL Sol + 9.567 mL serum pool
St.4 concentration 200 µU/mL, preparation 0.937 mL Sol. + 9.063 mL serum pool
St.5 concentration 300 µU/mL, preparation 1.440 mL Sol. + 8.560 mL serum pool

The standards were prepared: a) by adding a previously calculated amount of insulin aspart (Novo Nordisk) for Standards 2 to 5. The insulin aspart solution created had a concentration of 2000 µU/mL. A different amount of this solution was added to the serum pool in order to create the different concentration levels; b) by dilution of the serum pool for Standard 1 with distilled water. The standard insulin concentrations were measured. The results are shown in Table 2.
For these standards, an evaluation of the precision was carried out. Since they were analyzed in two series, two within-run %CV were calculated as well as a total %CV

Table 1. Main characteristics of the method.

1	2	3	4	5	6
Parameter	**Determined variable**	**Units**	**Limits**	**Results**	**Conclusion**
Low limit of quantification (LLOQ)	**concentration**	**µU/mL**	**-**	**30**	**Complies**
Linearity					
Slope	**CV**	**%**	**< 10.0**	**0.590**	**Complies**
Correlation coefficient	**r**	**dimensionless**	**> 0.990**	**0.994**	**Complies**
	CV	**%**	**< 10.0**	**0.007**	**Complies**
Analytical recovery					
For Insulin Aspart	**Analytical recovery**	**%**	**> 50.0**	**140.89**	**Complies**
	CV	**%**	**< 25.0%**	**21.30**	**Complies**
Intra-day accuracy and precision					
Accuracy					
Lower limit of quantification (LLOQ)	**d%**	**%**	**< ± 20.0**	**16.43**	**Complies**
Upper limit of quantification (ULOQ)	**d%**	**%**	**< ± 1.05**	**0.58**	**Complies**
Precision					
Lower limit of quantification (LLOQ)	**CV**	**%**	**< 20.0**	**2.44**	**Complies**
Upper limit of quantification (ULOQ)	**CV**	**%**	**< 15.0**	**2.84**	**Complies**
Inter-day accuracy and precision					
Accuracy					
Lower limit of quantification (LLOQ)	**d%**	**%**	**< ± 20.0**	**15.42**	**Complies**
Upper limit of quantification (ULOQ)	**d%**	**%**	**< ± 15.0**	**0.99**	**Complies**
Precision					
Lower limit of quantification (LLOQ)	**CV**	**%**	**< 20.0**	**1.93**	**Complies**
Upper limit of quantification (ULOQ)	**CV**	**%**	**< 15.0**	**1.94**	**Complies**

(presented in Table 2).
The acceptable limits are as follows:
- For LLOQ – CV < 20, bias < 20
- For ULOQ – CV < 15, bias < 15

As it became clear during this verification process using the Standards the availability of the exogenous insulin aspart is higher than the availability of the endogenous insulin. This explains the higher value of insulin measured by the analyzer in comparison to the amount of insulin actually placed in the sample. In order to counteract this specific feature of measuring insulin aspart and to make the result comparable to the endogenous insulin a multiplication factor was calculated named Restatement factor (RF). An average restatement factor was calculated for the entire linearity range, as well as three additional factors for three different concentration ranges within the linearity range. All individual results obtained for the patients' samples (containing insulin aspart) were multiplied by this factor (RF) in order to avoid the deviation in measuring the exogenous insulin. Detailed information is shown in Table 3.
Back-calculated concentrations of LLOQ (St 2) - mean 54.96, calculated after multiplication by the restement factor is 34.36, SD 0.67, CV% 1.93, bias 15.42, Cnom 30 and ULOQ (St 5) - mean 360, calculated after multiplication by the restement factor is 302.98, SD 5.88, CV% 1.94, bias 0.99, Cnom 300.
The values of precision (expressed by the coefficient of variation, CV%) and accuracy (in the tables as d%) across the whole concentration range are within the ac-

Table 2. Analytical results for Standards, µU/mL before* and after multiplication by restatement factor.**

Method	St 1	d% **	St 2	d% **	St 3	d% **	St 4	d% **	St 5	d% **
CLIA	9.65 *	-	54.07 *	-	144.60 *	-	236.61 *	-	354.91 *	-
	9.65 **	-3.5	34.06 **	13.55	111.34 **	11.34	198.75 **	-0.62	298.12 **	-0.63
CLIA	9.42 *	-	55.50 *	-	138.07 *	-	238.32 *	-	351.89 *	-
	9.42 **	-5.8	34.97 **	16.55	106.31 **	6.31	200.19 **	0.09	295.59 **	-1.47
CLIA	9.65 *	-	56.78 *	-	140.33 *	-	238.59 *	-	370.86 *	-
	9.65 **	-3.5	35.77 **	19.24	108.05 **	8.05	200.42 **	0.21	311.52 **	3.84
Mean	9.57 *	-	55.45 *	-	141.00 *	-	237.84 *	-	359.22 *	-
	9.57 **	-	34.93 **	-	108.57 **	-	199.79 **	-	301.74 **	-
SD	0.13 *	-	1.36 *	-	3.32 *	-	1.07 *	-	10.19 *	
	0.13 **	-	0.85 **	-	2.55 **	-	0.90 **	-	8.56 **	-
% CV	1.39	-	2.44	-	2.35	-	0.45	-	2.84	-
CLIA	9.58 *	-	54.10 *	-	141.53 *	-	237.93 *	-	362.82 *	-
	9.58 **	-4.2	34.08 **	13.61	108.98 **	8.98	199.86 **	-0.07	304.77 **	1.59
CLIA	9.63	-	55.07 *	-	142.05 *	-	238.07 *	-	365.24 *	-
	9.63	-3.7	34.69 **	15.65	109.38 **	9.38	199.98 **	-0.01	306.80 **	2.27
CLIA	9.57	-	54.26 *	-	140.87 *	-	236.97 *	-	358.39 *	-
	9.57	-4.3	34.18 **	13.95	108.47 **	8.47	199.05 **	-0.47	301.05 **	0.35
Mean	9.59 *	-	54.48 *	-	141.48 *	-	237.66 *	-	362.15 *	-
	9.59 **	-	34.32 **	-	108.94 **	-	199.63 **	-	304.21 **	-
SD	0.03 *	-	0.52 *	-	0.59 *	-	0.60 *	-	3.47 *	-
	0.03 **	-	0.33 **	-	0.46 **	-	0.50 **	-	2.92 **	-
% CV	0.34	-	0.95	-	0.42	-	0.25	-	0.96	-
Mean	9.58 *	-	54.96 *	-	141.24 *	-	237.75 *	-	360.69 *	-
	9.58 **	-	34.63 **	-	108.76 **	-	199.71 **	-	302.98 **	-
SD	0.09 *	-	1.06 *	-	2.15 *	-	0.78 *	-	7.00 *	-
	0.09 **	-	0.67 **	-	1.65 **	-	0.66 **	-	5.88 **	-
CV, %	0.91	-	1.93	-	1.52	-	0.33	-	1.94	-
Bias **	-4.17	-	15.42	-	8.76	-	-0.15	-	0.99	-
Cnom	10.00	-	30.00	-	100.00	-	200.0	-	300.0	-

* - No calibration standards were excluded from calibration curve construction.

Table 3. Analytical recovery and restatement factor.

	Standard concentration µU/mL	Spiking Recovery %	RF	RF range
Standard 1	10	NA		NA
Standard 2	30	183.21	0.63	30 - 100 µU/mL
Standard 3	100	141.240		
Standard 4	200	118.87	0.77	100 - 200 µU/mL
Standard 5	300	120.23	0.84	200 - 300 µU/mL
-		average	0.73	

ceptable limits.
Back-calculated concentrations of individual patient samples using the currently in force calibration curve are presented in Table 3.

CONCLUSION

Verification and application of the method for quantification of insulin aspart in human serum were carried out in compliance with the bioanalytical method verification criteria and the regulations of Republic Bulgaria and European Medicines Agency.
The routine application of the method for measurement of immunoreactive insulin using the analyzer ADVIA Centaur® XP is directed to the measurement of endogenous insulin. The method is applicable for measuring different types of exogenous insulin, including insulin aspart.

Declaration of Interest:
The authors have nothing to declare.

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