Physicochemical Interactions Between Analyte and Laboratory Material: Impact on Accuracy

M. Ruiz-Martínez, M. Pfenniger, P. Brennecke, M. Gröschl, C. Schiebl, P. Struwe
Celerion Switzerland AG, 8320 Fehraltorf, Switzerland

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Introduction

Celerion Switzerland AG developed an ELISA method for the quantification of C-peptide, which is a short endogenous serum peptide formed by proinsulin cleavage during insulin biosynthesis. Analysis of endogenous levels of C-peptide in human serum is of interest in diabetes related studies and to assess the functioning of beta-cells.

The developed biomarker assay consists of a sandwich ELISA method and is based on a commercially available analytical kit. 50 µL of sample are added to a pre-coated Streptavidin plate, together with Biotin- and HRP- conjugated capture and detection antibodies. The immobilized antigen-antibody complexes are detected by addition of TMB, a substrate for horse radish peroxidase (HRP) (Figure 1).

The method was developed with the optional use of a robotic liquid handling system Tecan genesis RSP 200 to allow for high-throughput analysis. Precision and accuracy were assessed during method validation for both manual and robotic performance.

Precision and Accuracy (P&A) assessments

Experimental design:

- 3 analytical runs performed manually
- 3 analytical runs performed by the Tecan Genesis RSP 200

Each P&A analytical run contained:

- One set of 9 calibration standards prepared in surrogate matrix
- Six sets of QC samples prepared in human serum (LLOQ as endogenous C-peptide, with spiked recombinant C-peptide to reach higher concentrations)

Acceptance criteria:

- Precision: %CV must be within 20% (25% for LLOQ and ULOQ)
- Accuracy: %Bias must be within 20% (25% for LLOQ and ULOQ)

Precision and Accuracy (P&A) results

Precision: All precision values were within acceptance criteria

Accuracy:

- Accuracy values were well within acceptance criteria in all runs performed manually (Table 1)
- In all robotic runs, several duplicates showed %Bias values outside the established limits (Table 2). Especially affected were LQC samples (Table 3)

		EXAMPLE MANUAL P&A RUN					
		Bias (%)					
QC sample	C-peptide (ng/mL)	Set Nr.1	Set Nr.2	Set Nr.3	Set Nr.4	Set Nr.5	Set Nr.6
LLOQ	0.1675	-1.0	5.7	5.1	3.7	7.3	7.7
LQC	0.4500	2.3	2.4	-1.2	0.2	2.8	1.4
MQC	2.000	0.7	0.2	0.9	0.4	-1.3	6.6
HQC	3.500	0.4	-0.7	2.9	0.0	1.9	0.3
ULOQ	5.000	-13.1	-4.2	-6.7	-6.8	-10.7	-10.9
DQC	10.00	0.5	2.9	3.3	5.0	3.3	3.9

		EXAMPLE ROBOTIC P&A RUN					
		Bias (%)					
QC sample	C-peptide (ng/mL)	Set Nr.1	Set Nr.2	Set Nr.3	Set Nr.4	Set Nr.5	Set Nr.6
LLOQ	0.1675	4.1	0.0	4.7	-7.7	-2.4	-3.0
LQC	0.4500	-2.3	-18.3	**-25.0**	**43.8**	7.7	-5.6
MQC	2.000	-1.9	**21.2**	2.3	-15.9	**-27.3**	-11.6
HQC	3.500	-6.2	-7.4	-6.9	-3.9	-3.7	-8.1
ULOQ	5.000	-8.9	-16.1	-10.3	-13.1	-13.5	-9.5
DQC	10.00	-0.6	-2.6	-0.3	-1.1	-2.4	3.9

Table 1 and Table 2. Example of accuracy values from manual and robotic runs

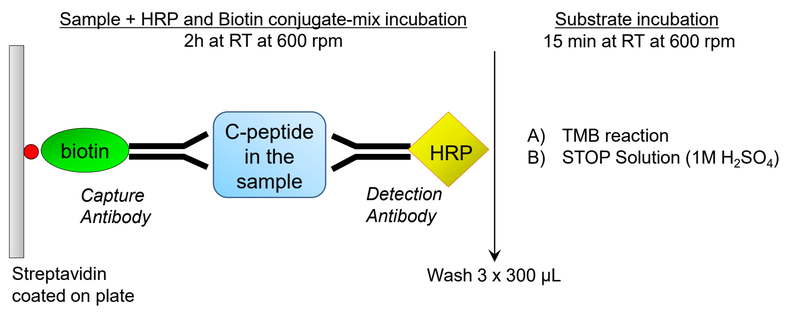

Figure 1. Overview of the C-peptide analytical method

Accuracy of LQC samples – closer look at robotic results

LQC samples pipetted in the centre of the plate were in all cases affected (Table 3). Preparation and handling processes were carefully reviewed:

Were these samples handled differently than the LQC samples pipetted in the outer columns of the plate, or than the samples pipetted manually?

	LQC Bias (%) in Robotic Runs					
	Set Nr.1	Set Nr.2	Set Nr.3	Set Nr.4	Set Nr.5	Set Nr.6
Run 1	11.8	-19.0	-19.3	**45.5**	11.5	1.4
Run 2	-2.3	-18.3	**-25.0**	**43.8**	7.7	-5.6
Run 3	-0.7	-18.4	-19.9	**-22.0**	4.6	-11.4

Table 3. Accuracy of LQC samples in the three robotic runs

Differences in handling

Set Nr. 1 and Nr. 6 were pipetted by the robot from a carrier dedicated to calibration standards and QC samples. The tubes placed on this carrier were the same polypropylene tubes where the QC samples had been prepared.

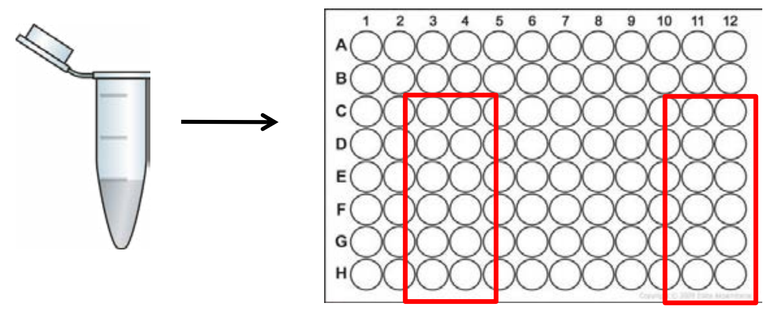

Figure 2. QC sample: tube directly to the robot carrier and sample pipetted into the assay plate

Set Nr. 2 to Nr. 5 were pipetted by the robot from a different carrier, usually dedicated to study samples. For this purpose, QC samples had to be re-aliquoted into new 3.6 mL polypropylene tubes before analysis.

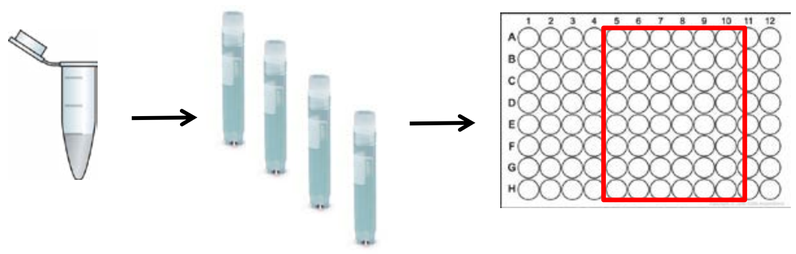

Figure 3. QC sample: from tube, lower volume re-aliquoted into new 3.6 mL tubes, which were then placed on the robot carrier and then pipetted into the assay plate

Conclusions and impact

- Additional aliquoting steps where lower volumes are transferred into new polypropylene tubes have an impact on accuracy.
- This effect is seen mostly in LQC and MQC samples, containing endogenous and recombinant C-peptide (but not on LLOQ samples, which contain only endogenous analyte). This indicates that recombinant C-peptide may establish different physicochemical interactions with polypropylene than endogenous C-peptide.
- When no re-aliquoting steps are performed in robotic runs, the accuracy of all samples is within acceptance criteria (data not shown).
- Since study samples are never re-aliquoted before analysis, these issues have no impact on the obtained study data.