

Measurement of Albumin in Human Urine by Liquid Chromatography-Isotope Dilution Tandem Mass Spectrometry

Protein Analysis Working Group Meeting

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- Urine albumin is an important biomarker for assessing the health status of the kidneys.
- Urine albumin-to-creatinine ratio (ACR) helps to identify early-stage kidney disease.
- Diabetes and hypertension are the leading causes for kidney disease, it is imperative to routinely measure the urine albumin of patients with these chronic diseases in order to provide timely treatment and prevent the onset of kidney failure.
- The IFCC has formed a Working Group for standardisation of albumin in urine with the objectives of developing reference measurement procedures and commutable CRMs, as well as harmonising routine measurement procedures with reference measurement procedures.

Objectives of Our Research

- To develop an IDMS method for the measurement of albumin in urine using peptide and/or protein calibration standards.
- To provide metrologically traceable assigned values in the HSA External Quality Assessment (EQA) Programmes, and use the assigned values to evaluate the results from the participating clinical laboratories.
- To produce albumin in urine certified reference materials (CRMs) with certified values determined by the IDMS method.
- To contribute to standardisation of albumin in urine by collaborating with other NMIs/reference laboratories.

Choice of Calibrators and Internal Standards for the IDMS Method

Peptide Calibrator with Isotope-labelled Peptide Internal Standard

Pros:

- Purity can be determined by peptide impurity corrected amino acid (PICAA) methods.
- Materials and internal standards are readily available from custom synthesis.

Cons:

- Accuracy may be affected by incomplete proteolysis, matrix effect and/or poor stability of the peptides during proteolysis.

Protein Calibrator with Isotope-labelled Albumin Internal Standard

Pros:

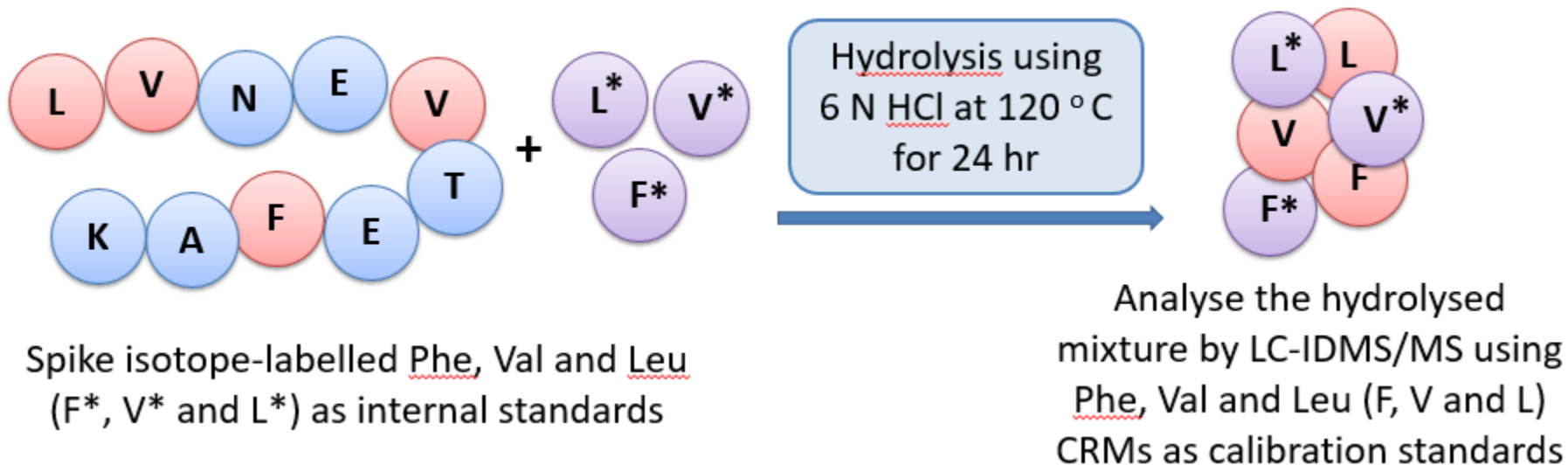
- Less influence from incomplete proteolysis, matrix effect and/or poor stability of the peptides.
- Pure albumin CRMs from NMIs are readily available.

Cons:

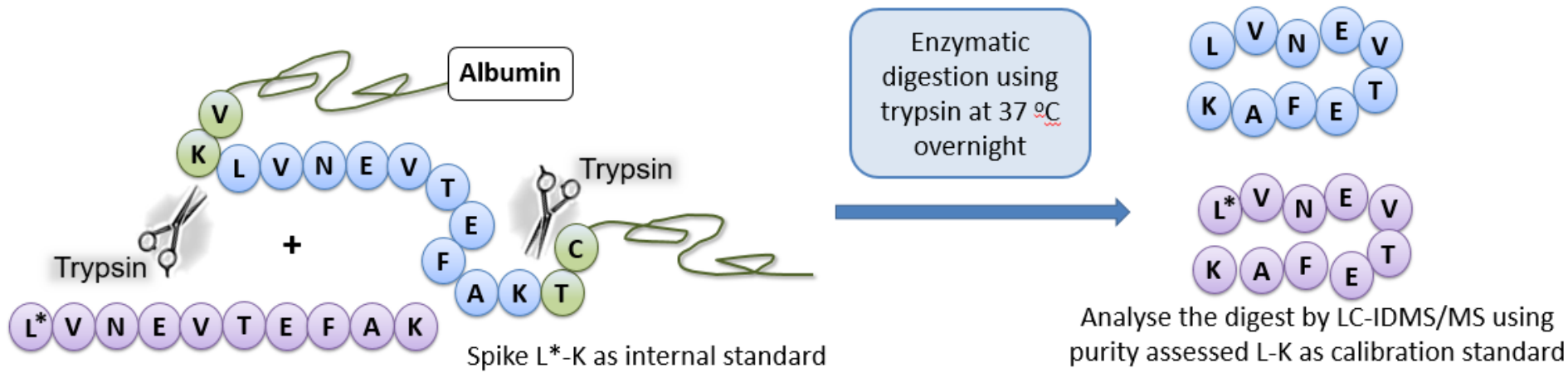
- Isotope-labelled albumin is costly and relatively hard to obtain.

Procedure of Method 1 (Peptide Calibrator) – Peptide Purity

A few signature peptides of albumin were tested. Only one peptide, LVNEVTEFAK (L-K), was found to be suitable for quantification. The purity of L-K was determined by PICAA method.



Procedure of Method 1 (Peptide Calibrator) – Determination of Albumin Concentration



Calibrator: Custom synthesised peptide, LVNEVTEFAK

Internal Standard: Isotope-labelled peptide, L*-VNEVTEFAK

Recovery Test using Method 1 (Peptide Calibrator)

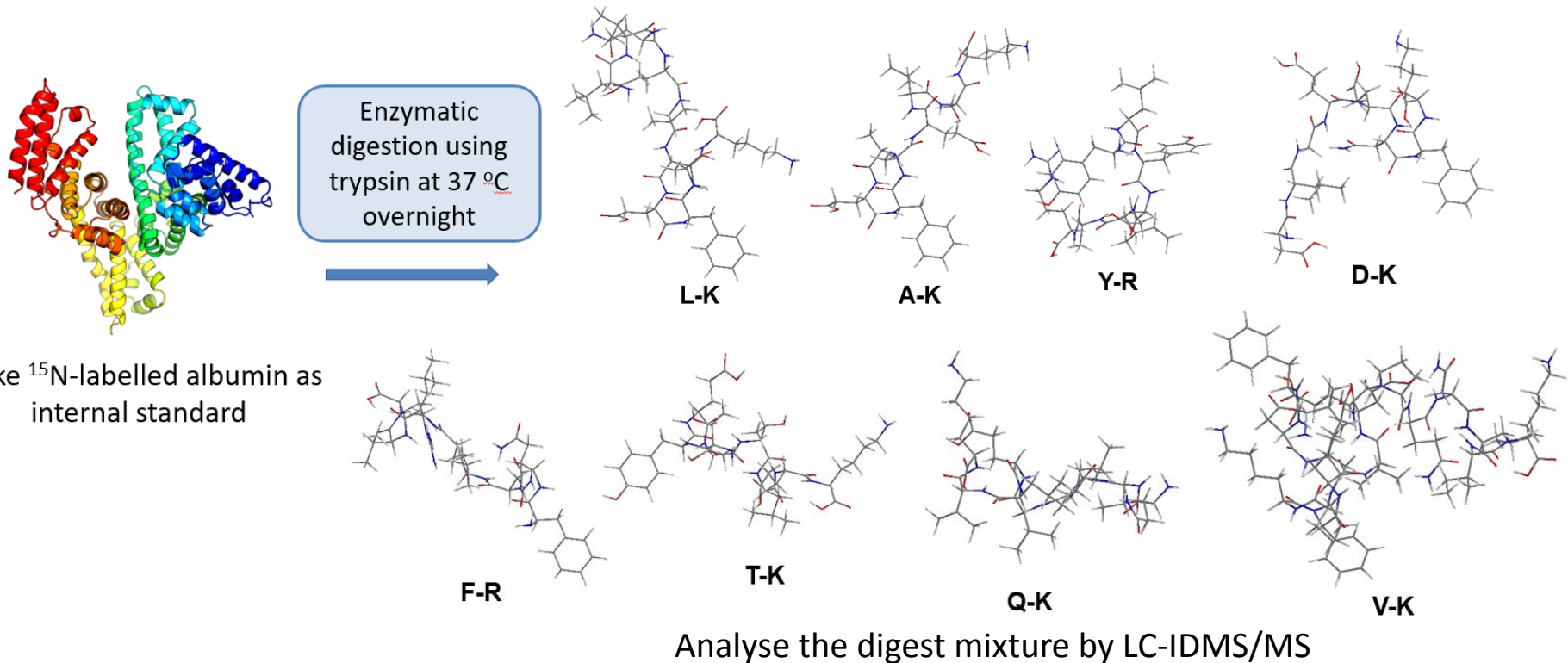
	<u>Mid Level (~ 40 mg/kg)</u>	<u>High Level (~ 220 mg/kg)</u>
Replicates, n	8	6
% Recovery	96.4	98.8
% CV	1.04	2.33

Recovery = (Obtained Conc. – Intrinsic Conc.)/Spiked Conc.

- Albumin CRM from NMIJ was spiked into human urine samples with two different concentrations.
- Good recoveries (> 95%) were obtained when urine albumin concentrations were not very low.
- Not suitable for low concentrations of albumin in urine.

Procedure of Method 2 (Protein Calibrator) – Peptide Purity

Urine samples were digested using trypsin and eight resulting peptides, L-K, AEFAEVSK (A-K), YLYEIAR (Y-R), DLGEENFK (D-K), FQNALLVR (F-R), TYETTLEK (T-K), QTALVELVK (Q-K), and VFDEFKPLVEEPQNLIK (V-K), were quantified by LC-IDMS/MS simultaneously.



Calibrator: Albumin solution CRM from NMIJ

Internal Standard: Recombinant isotope-labelled albumin

Recovery Test using Method 2 (Protein Calibrator) – Results from Different Peptides

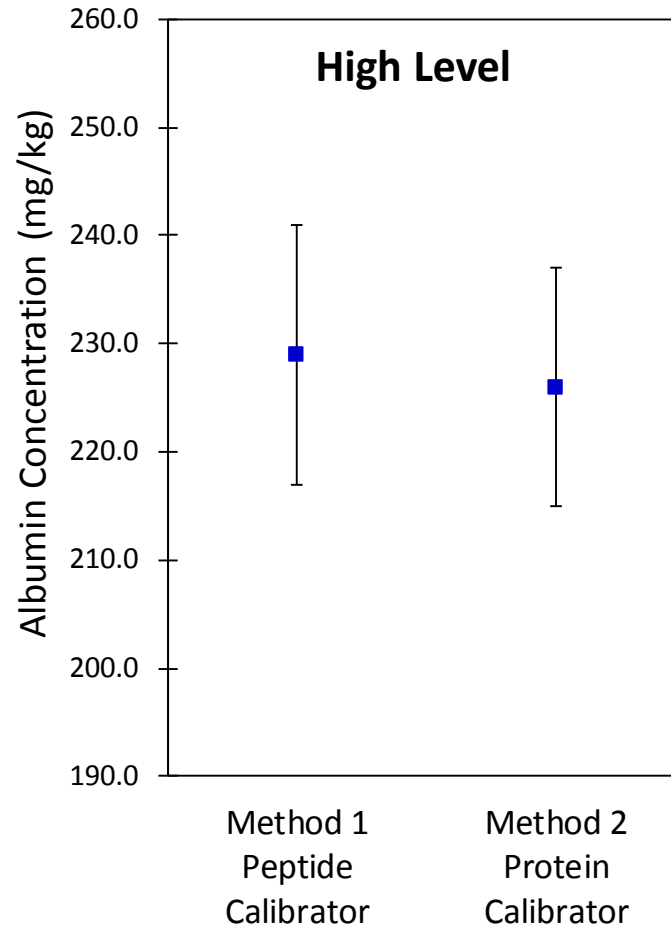
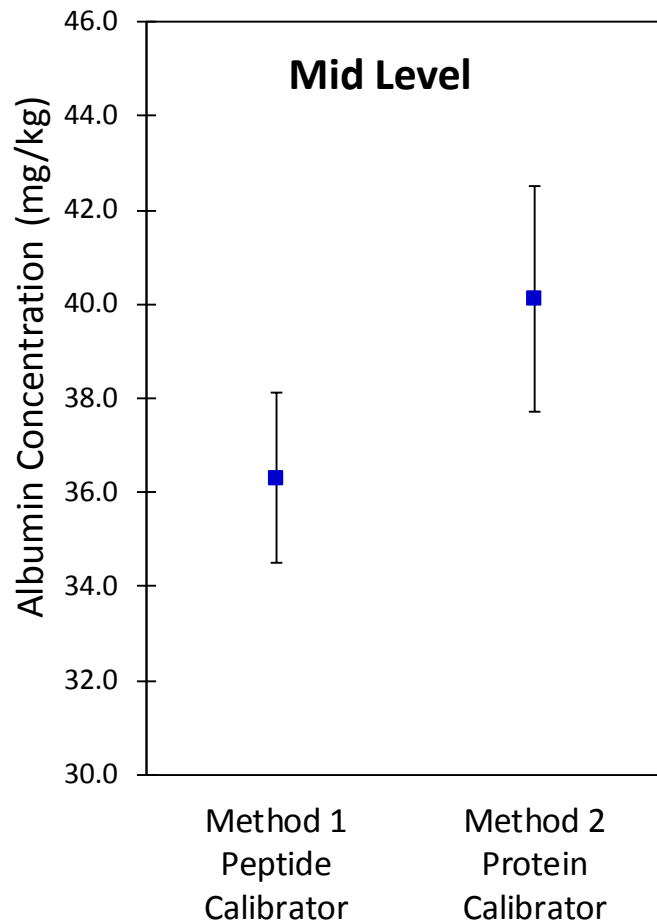
Peptides	% Recovery Low Level (~ 7 mg/kg), n = 6	% CV	% Recovery Mid Level (~ 40 mg/kg), n = 7	% CV
L-K	100.2	2.99	103.1	4.42
A-K	100.5	3.38	100.2	3.55
Y-R	104.1	2.73	103.3	3.34
D-K	104.9	4.52	104.4	2.49
F-R	100.1	2.25	104.2	3.24
T-K	101.1	2.36	105.6	5.63
Q-K	101.4	1.57	101.8	3.19
V-K	103.2	4.19	102.9	3.30

Overall Recovery using Method 2 (Protein Calibrator)

	<u>Low Level (~ 7 mg/kg)</u>	<u>Mid Level (~ 40 mg/kg)</u>
No. of Peptides	8	8
% Recovery	101.9	103.2
% CV	1.85	1.60

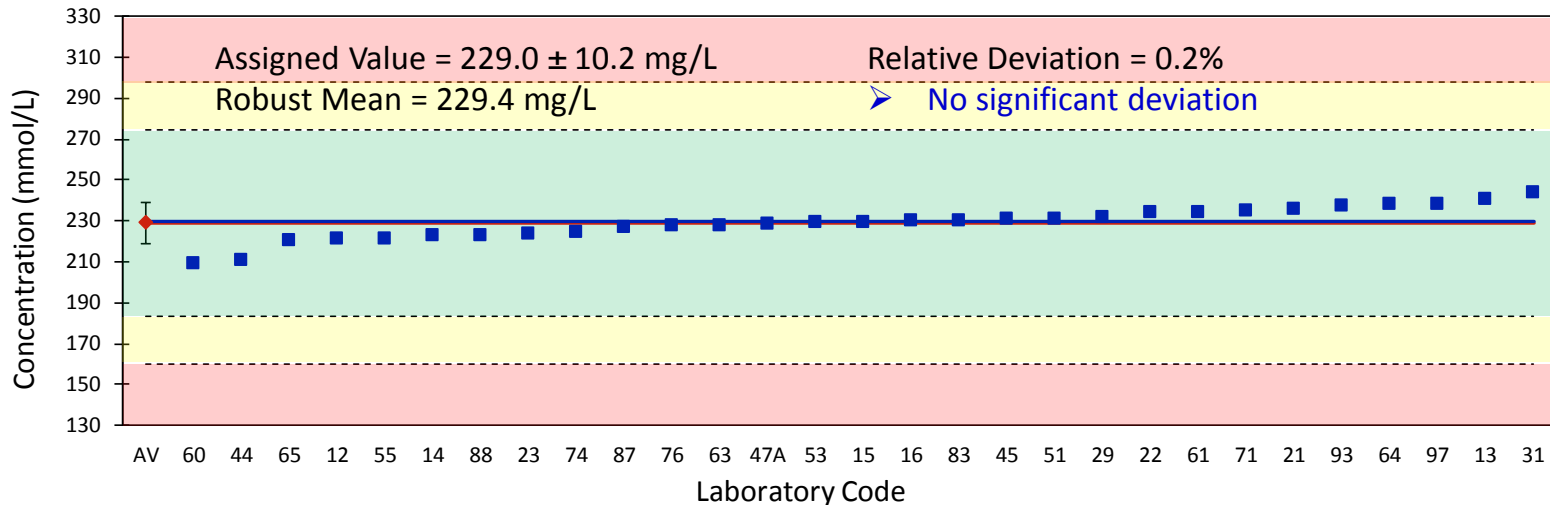
- Good recovery was obtained even for very low concentration of albumin (close to Detection Limit of some clinical analysers).
- Suitable for urine samples with a wide albumin concentration range.

Comparison of Two Methods

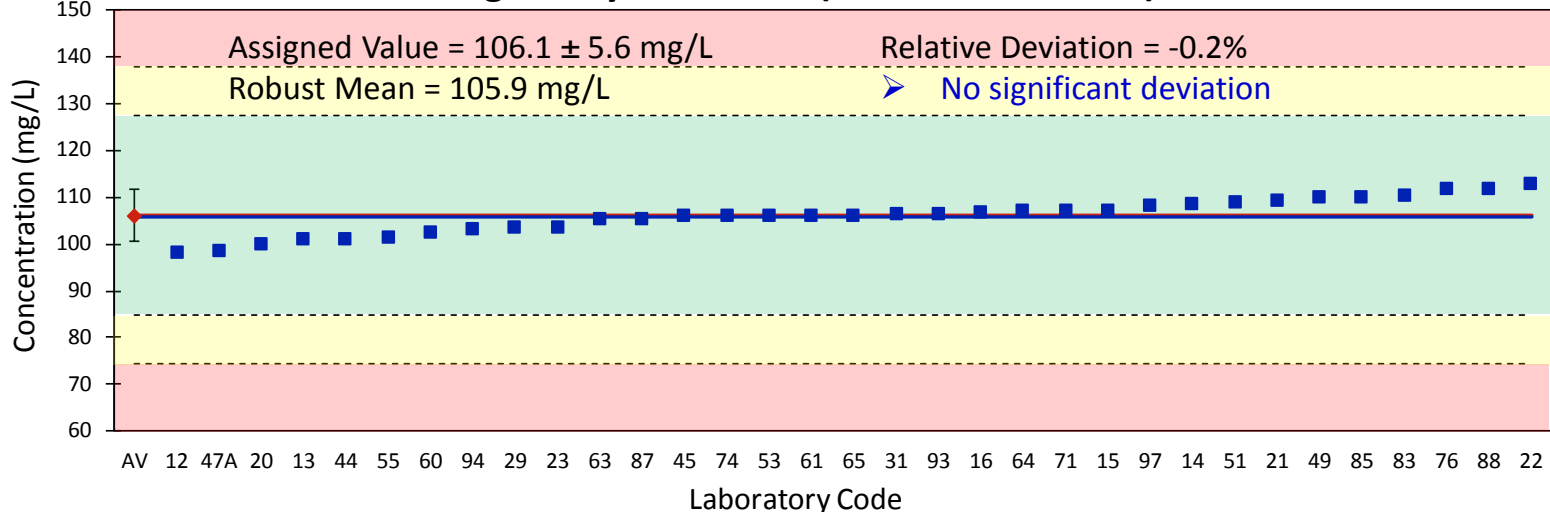




Value Assignments in HSA EQA Programmes




2017 EQA: Value assigned by Method 1 (Peptide Calibrator)



2018 EQA: Value assigned by Method 2 (Protein Calibrator)



 Assigned Value with Measurement Uncertainty
 Robust Mean of Reported Results

 "Acceptable" Range
 "Questionable" Range
 "Unacceptable" Range

Certification of CRMs

- Urine materials in 2017 HSA EQA Programme were developed into Certified Reference Materials.
- Certified values were determined using Method 2 (Protein Calibrator).

Certified Reference Material (HRM-3004A)

Albumin and Creatinine in Human Urine

Certified Values of Albumin (mg/L)*	
STY-0018-053	STY-0018-054
40.1 ± 2.4	226 ± 11



*Converted from mg/kg using urine density

Conclusion

- The newly developed LC-IDMS/MS methods were shown to be accurate and precise.
- Method 1 (peptide calibration) was suitable for urine samples with albumin concentrations close to or higher than 40 mg/kg.
- Method 2 (protein calibration) was found to be suitable for urine samples with a wide albumin concentration range.
- Methods 1 and 2 were successfully applied in the value assignments of albumin in urine in the 2017 & 2018 HSA EQA Programmes, respectively. No significant deviation was found between the assigned values and the results from the participating clinical laboratories.
- CRMs with certified values determined by the developed LC-IDMS/MS method were developed.

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Thank you