

# IDMS Method for the Measurement of Urine Albumin and its Application in Accuracy-based EQA Programmes in Singapore

**JCTLM Members & Stakeholders Meeting 2019**

Qinde Liu

Chemical Metrology Division

Applied Sciences Group

Health Sciences Authority

3 December 2019

## Background

- Urine albumin is an important biomarker for assessing the health status of kidneys.
- Urine albumin-to-creatinine ratio (ACR) helps to identify early-stage kidney disease.
- Diabetes and hypertension are the leading causes for kidney disease. Hence, 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 Assay in Urine (in collaboration with NKDEP) with the objectives of developing reference measurement procedures and commutable CRMs, as well as harmonising routine measurement procedures with reference measurement procedures.

IFCC: International Federation of Clinical Chemistry and Laboratory Medicine

NKDEP: National Kidney Disease Education Program (United States)

# Objectives of Our Research

- To develop an isotope dilution mass spectrometric (IDMS) method for the measurement of albumin in urine using peptide and/or protein calibration standards.
- To provide metrologically traceable assigned values in 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 efforts in the standardisation of albumin in urine by collaborating with other metrology institutes and reference laboratories.

# Choice of Calibrators and Internal Standards for the IDMS Method

## ☐ Peptide Calibrator with isotope-labelled Peptide as Internal Standard

### ***Pros:***

- Purity can be determined by “peptide impurity corrected amino acid” (PICAA) method.
- 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 as Internal Standard

### ***Pros:***

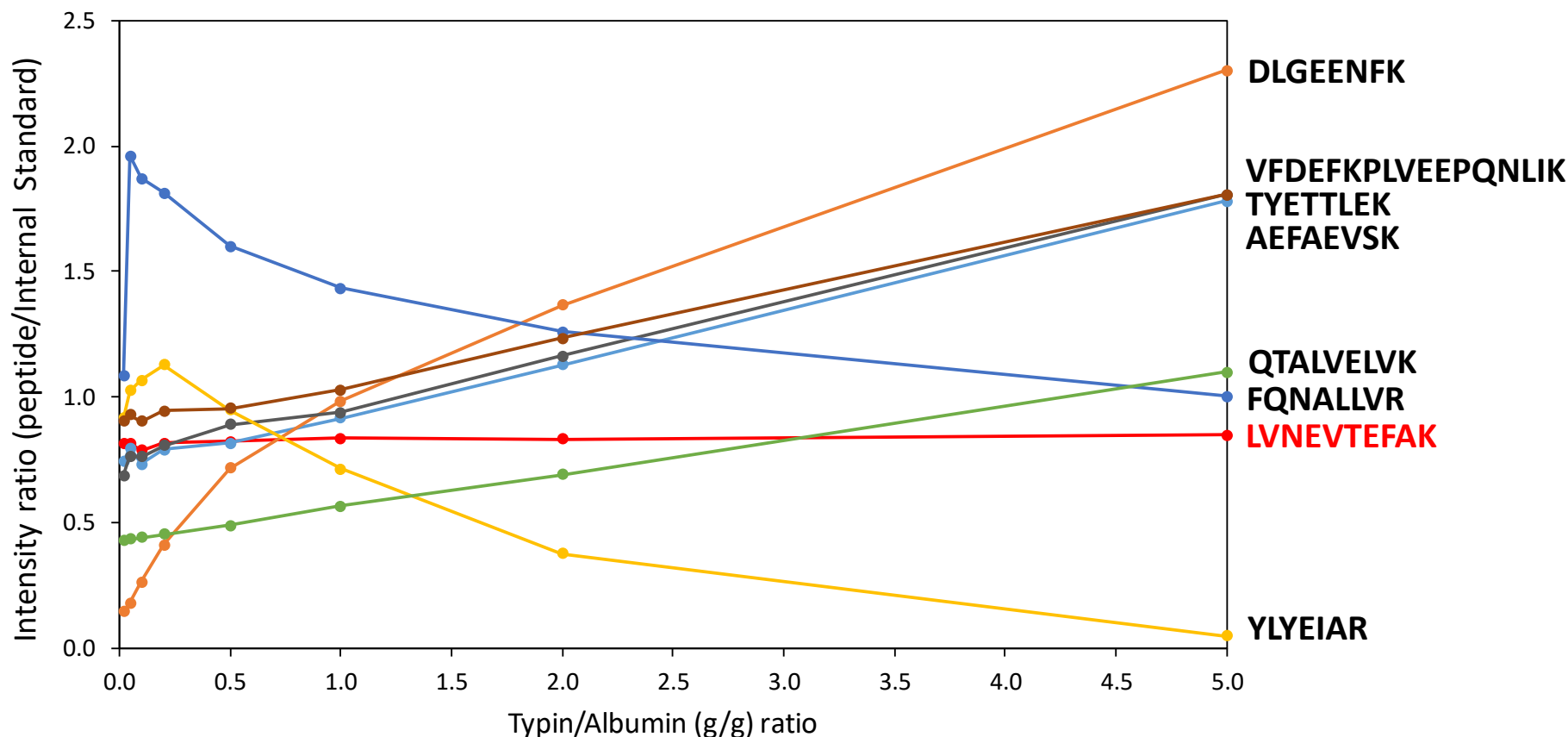
- Less influence from incomplete proteolysis, matrix effect and/or poor stability of the peptides.
- Pure albumin CRMs from metrology institutes are readily available.

### ***Cons:***

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

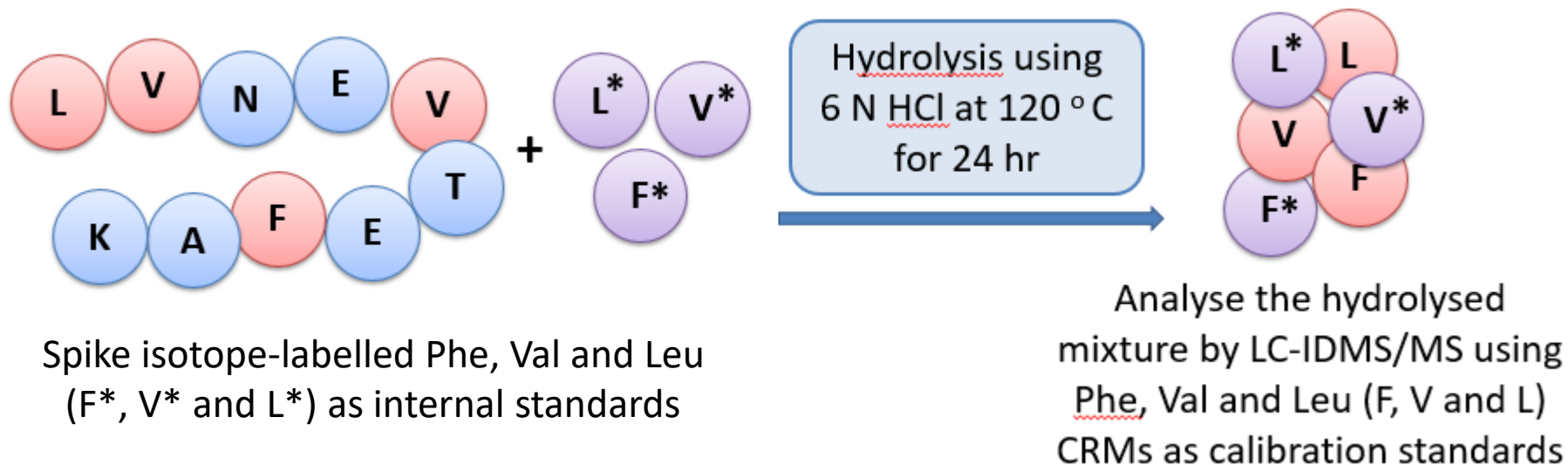
# Procedure for Method 1 (Peptide Calibrator) – Choice of Peptide for Quantification

Eight signature peptides of albumin were measured. Only one peptide, LVNEVTEFAK (L-K), was found to be suitable for quantification.

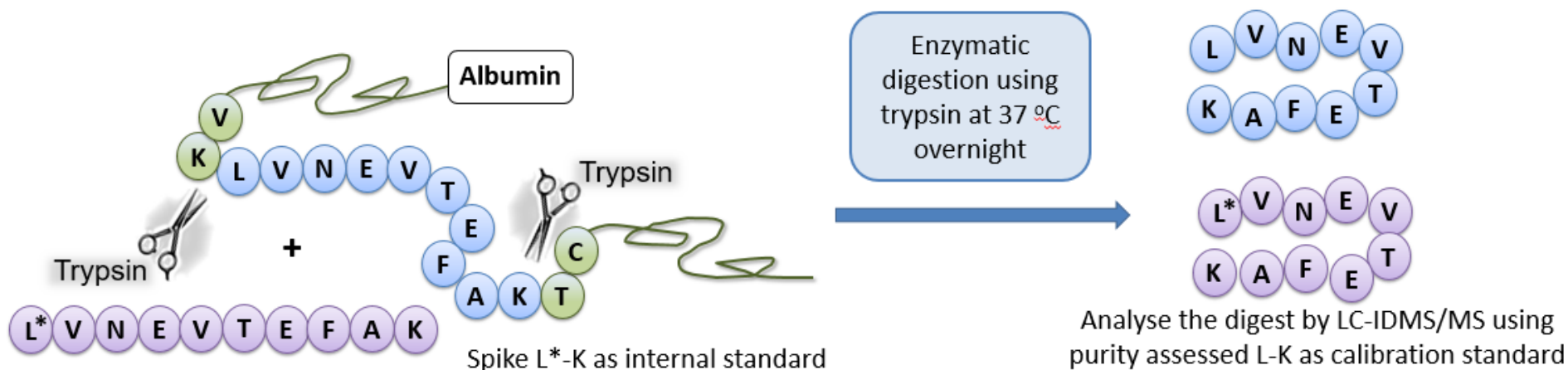


# Procedure for Method 1 (Peptide Calibrator) – Peptide Purity

The purity of L-K was determined by PICA method.



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



**Calibrator:** Custom synthesised peptide, LVNEVTEFAK

**Internal Standard:** isotope-labelled peptide, L<sup>\*</sup>-VNEVTEFAK

# Recovery Test using Method 1 (Peptide Calibrator)

Urine samples with three albumin concentrations were used in recovery test:

- Low level: ~ 7 mg/kg
- Mid level: ~ 40 mg/kg
- High level: ~220 mg/kg

	<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

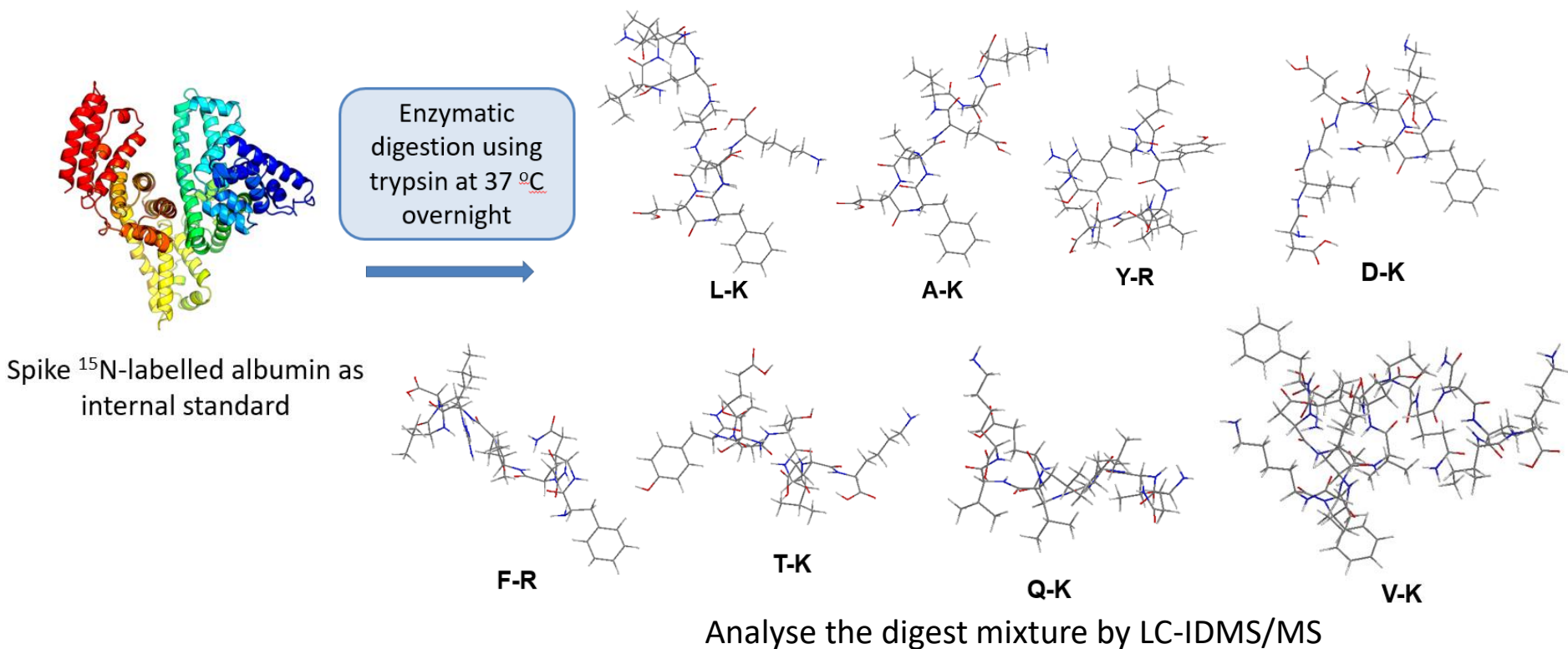
*Recovery = (Obtained Conc. – Intrinsic Conc.)/Spiked Conc.*

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



# Procedure for 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

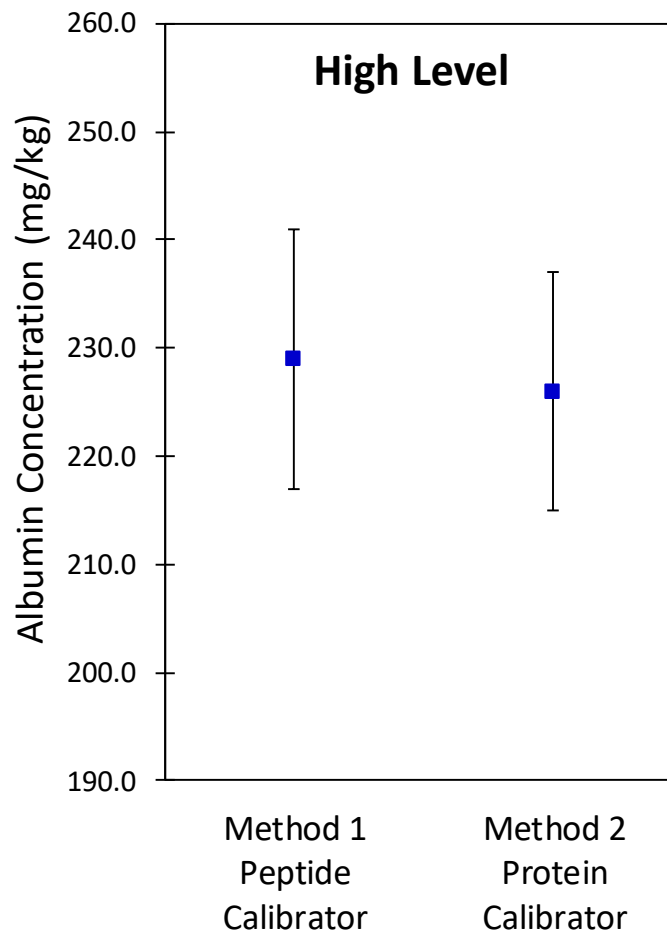
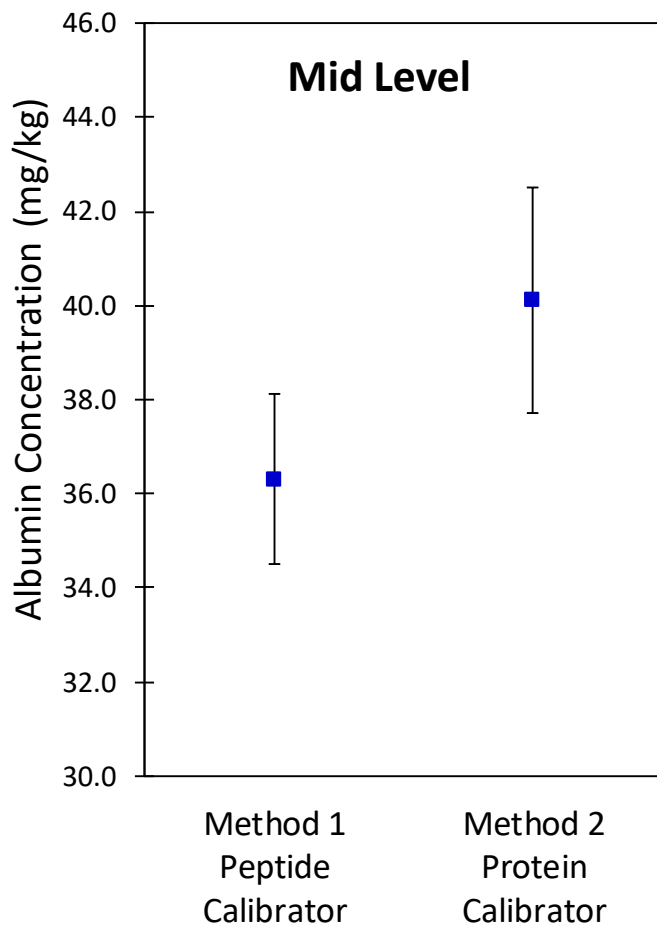
Peptides	% Recovery Low Level (~ 7 mg/kg), n = 6	% CV	% Recovery Mid Level (~ 40 mg/kg), n = 7	% CV
L-K	100.2	2.99	103.1	4.42
A-K	100.5	3.38	100.2	3.55
Y-R	104.1	2.73	103.3	3.34
D-K	104.9	4.52	104.4	2.49
F-R	100.1	2.25	104.2	3.24
T-K	101.1	2.36	105.6	5.63
Q-K	101.4	1.57	101.8	3.19
V-K	103.2	4.19	102.9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

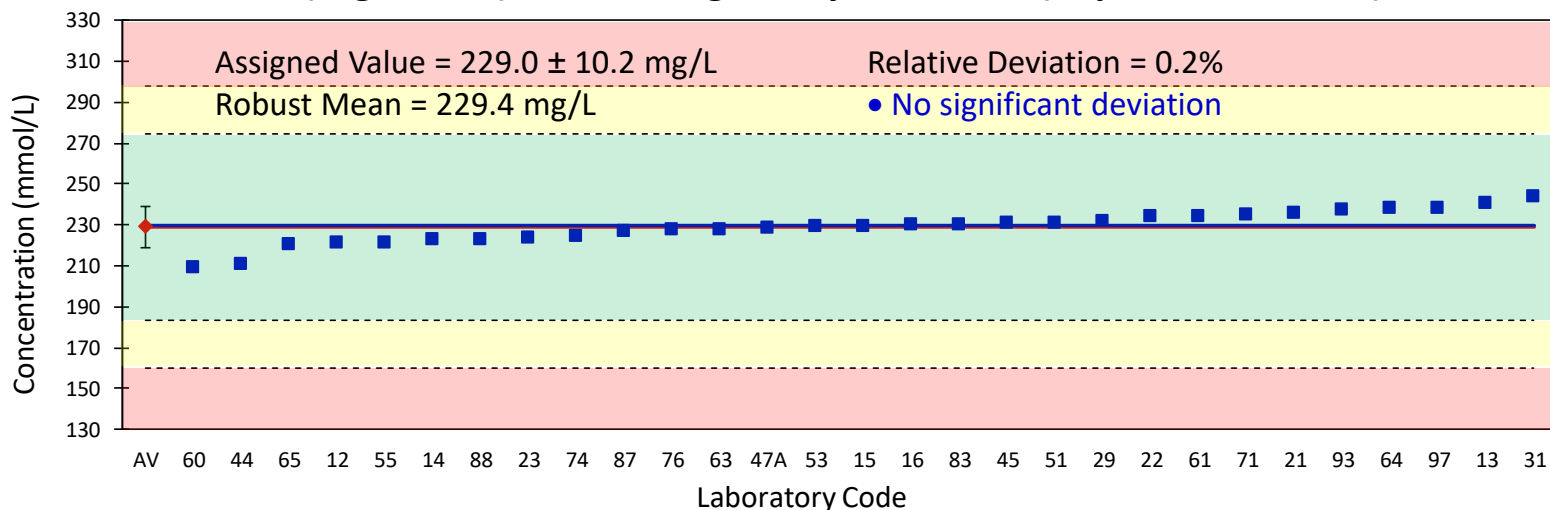
- Good recovery was obtained even for very low concentration of albumin (close to detection limit of some clinical analysers).
- Suitable for urine samples with a wide albumin concentration range.

# Comparison of Two Methods

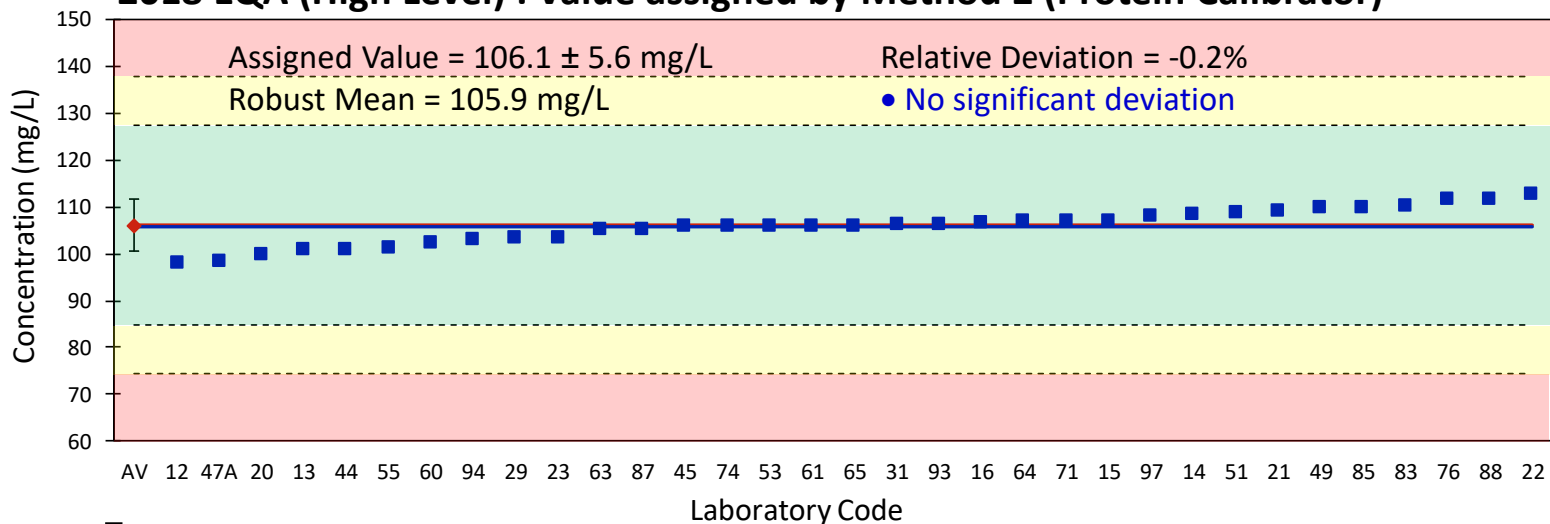


# Value Assignments in HSA EQA Programmes

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



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



Assigned Value with Measurement Uncertainty



Robust Mean of Reported Results



"Satisfactory" Range



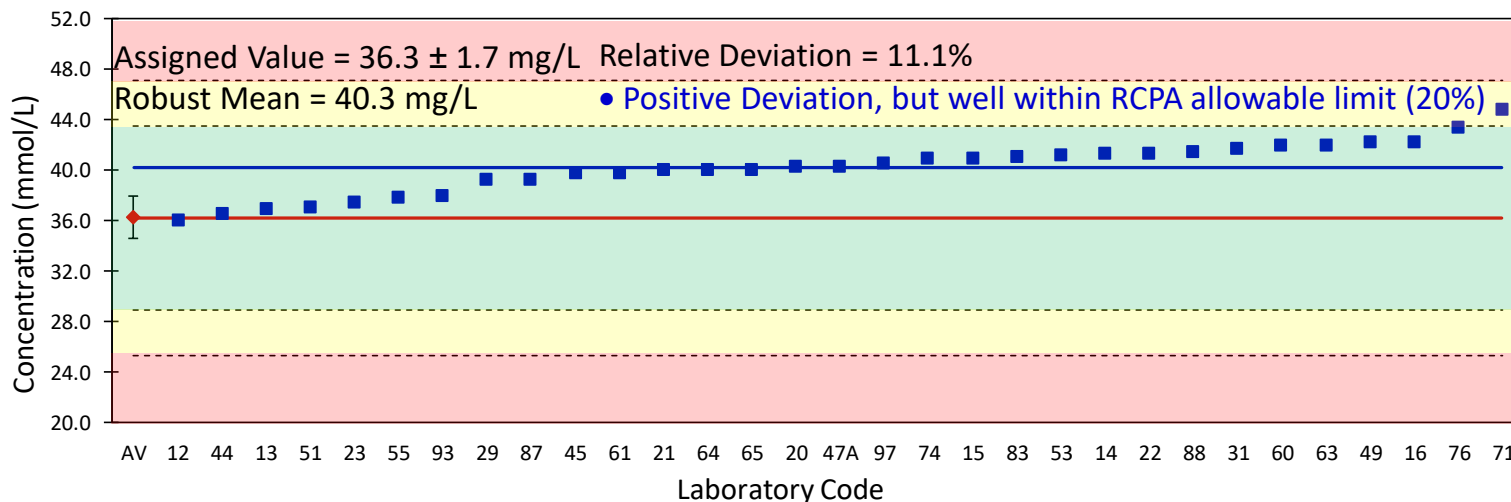
"Questionable" Range



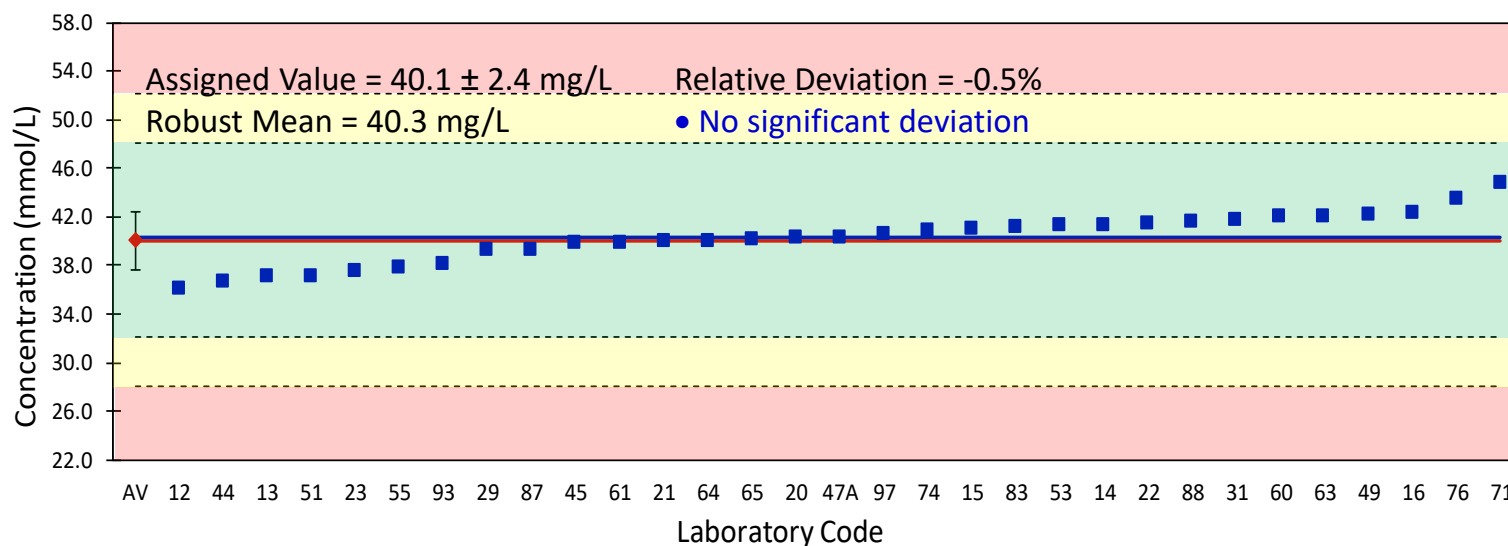
"Unsatisfactory" Range

# Value Assignments in HSA EQA Programmes

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

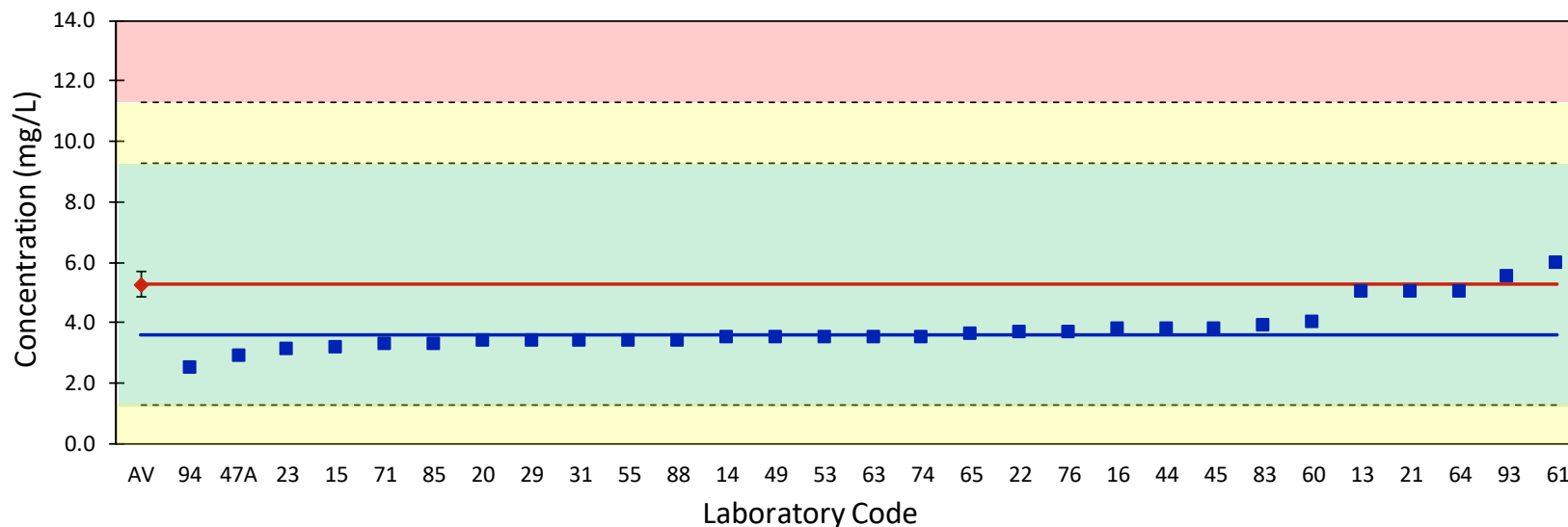


## 2017 EQA (Mid Level) : Value assigned by Method 2 (Protein Calibrator)



# Value Assignments in HSA EQA Programmes

## 2018 EQA (Low Level, close to LOD of clinical analyzers) : Value assigned by Method 2 (Protein Calibrator)



Assigned Value =  $5.27 \pm 0.42$  mg/L

Robust Mean = 3.57 mg/L

Deviation = - 1.7 mg/L

• Negative Deviation, but well within RCPA allowable limit at low concentration level ( $\pm 4$  mg/L when concentration is below 20 mg/g)

# 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

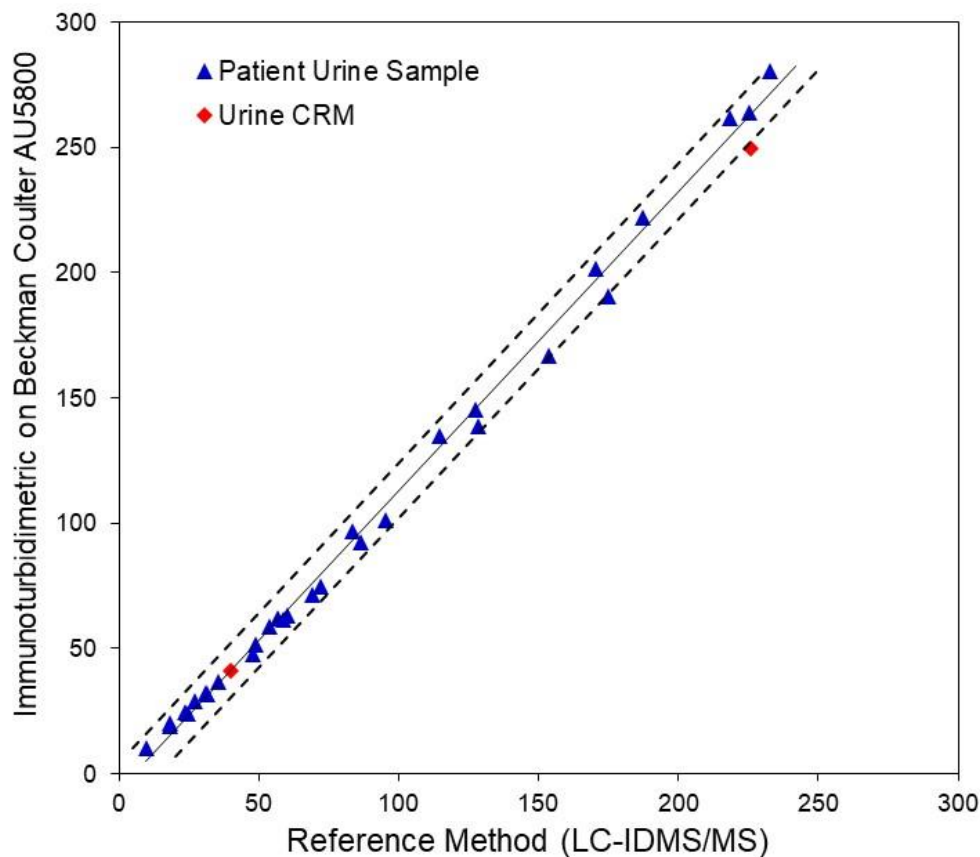
\*Converted from mg/kg using urine density





# CRM Commutability Study

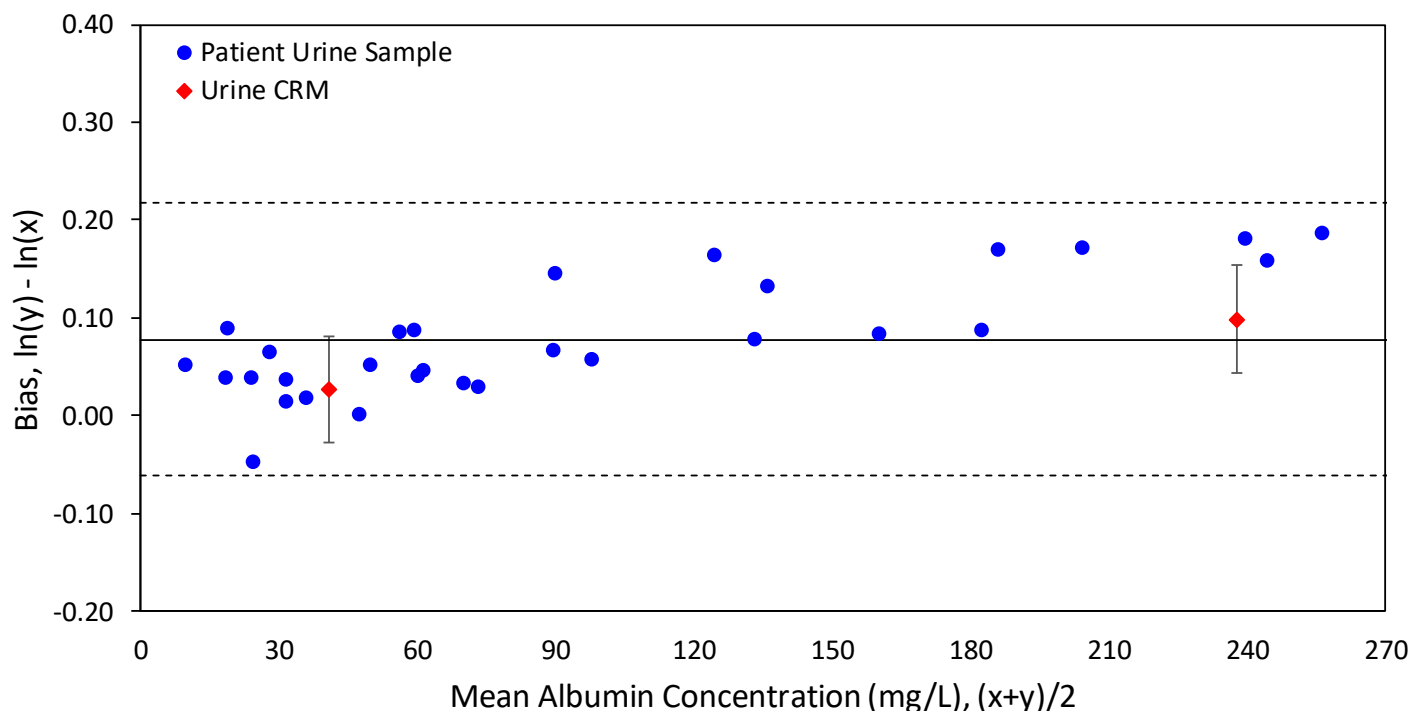
Model suggested by CLSI EP30-A Guideline



- 30 Patient urine samples were analysed for commutability study.
- Both patient urine samples and the CRMs were measured by IDMS method and routine method (Immunoturbidimetric method on a Beckman AU5800 Chemistry System)

# CRM Commutability Study

Log-transformed model recommended by IFCC



- Achievable performance of routine method is 20% based on RCPA allowable limit.
- Slightly more stringent commutability criteria (15%) was used, considering the relatively good precision of the difference between IDMS and routine method in this commutability study.

## Conclusion

- The newly developed LC-IDMS/MS methods were shown to be accurate and precise.
- Summary of suitability

Concentration	Method 1 (Peptide Calibration)	Method 2 (Protein Calibration)
Low level	X	√
Mid Level	√ ?	√
High level	√	√

- Both methods were successfully applied in the value assignments of albumin in urine in the 2017 & 2018 HSA EQA Programmes, respectively.
- Urine CRMs with certified values determined by the developed LC-IDMS/MS method were developed.
- Good commutability of the CRMs were demonstrated using immunoturbidimetric method on a Beckman AU5800 Chemistry System

## **HSA Colleagues**

Dr Yizhao Chen

Ms Hong Liu

Dr Tang Lin Teo

Dr Tong Kooi Lee

## **National University Hospital Singapore**

Dr Tze Ping Loh

A/Prof Sunil Kumar Sethi

# Thank you