



### Certified reference materials for ensuring traceability: From experience with steroids and peptide

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## Outline

- 1. Introduction of our institute and production of CRMs
- 2. Our experience in standardization of clinical measurements with CRM technology
  - cortisol and aldosterone
- 3. Toward the standardization of peptide measurements



### National Institute of Advanced Industrial Science and Technology (AIST)

 established by the integration of 15 national research institutes under the supervision by Ministry of Economy, Trade and Industry (METI) in 2001





#### **Organization of NMIJ/AIST**





## Mission of NMIJ

Development of new national measurement standards and dissemination of measurement standards to industrial and social users

Improvement on basic national measurement standards

Contribution to the global metrology system

In physics, engineering, and chemistry (and bio) field





# Production & Supply of NMIJ CRMs

## Total 289 CRMs & RMs



https://www.nmij.jp/english/service/C/



#### Metrological Traceability Calibration Hierarchy : ISO 17511





### NMIJ CRMs for Clinical Chemistry (1)

Pure substance Metabolites & Lipids Cholesterol, Creatinine, Urea, Uric acid, Triolein

#### **Amino Acids**

- L-Alanine, L-Leucine, L-Isoleucine, L-Phenylalanine, L-Valine, L-Proline, L-Arginine,
- L-Lysine monohydrochloride, L-Tyrosine, L-Threonine, L-Serine, Glycine, L-Methionine, L-Histidine, L-Cystine L-Glutamic acid, L-Aspartic acid

#### **Steroids**

Testosterone, Progesterone, 17β-Estradiol, Hydrocortisone (Cortisol)



### NMIJ CRMs for Clinical Chemistry (2)

#### **Serum Material**

Cortisol in human serum (4 concentration levels) Aldosterone in human serum (3 concentration levels)

#### **Protein & peptide standard solution**

- C-reactive protein solution
- C-peptide
- Human serum albumin
- Human insulin solution

#### Nucleic acid (DNA & RNA)

- RNA solution for quantitative analysis (Synthetic RNA)\*
- DNA solution for quantitative analysis (Synthetic DNA)\*

\*) Collaboration with Biomedical Research Institute, AIST



#### Network in Japan for Standardization in Laboratory Medicine







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## Process of Standardization Study

Medical Needs (from diagnostics, etc) for standardization.

Request for cooperation: Clinical personnel, Manufacturers, Reference material producers etc.

Establishment of high order measurement method (such as isotope dilution mass spectrometry: IDMS) and material preparation method

#### **Collaboration study**

Understanding of the difference between current measurement kits. Confirmation of calibration effect by reference materials

Production of Serum based certified reference materials

Establishment of traceability by manufacturer

Maintenance and monitoring of standardization by EQA



#### **Reference Materials for Serum Cortisol Measurement**





### Serum Cortisol Measurement: Preparation of materials IDMS vs. routine method





## Standardization of aldosterone

Collaboration study for standardization of low concentration serum aldosterone measurement for development of reference materials

Aldosterone: the main mineralocorticoid hormone



- Participants: manufacturers of clinical test reagent kits (commercial radioimmunoassay kit, 3 kits under development (CLEIA, ELISA))
   & testing laboratory (LC/MS/MS).
- We distributed serum samples (candidate RMs, and patient samples), and evaluated the degree of agreement between the measured value and the reference value.
- For candidate RMs reference values were determined using isotope dilution mass spectrometry at NMIJ.
- For patient samples LC/MS/MS values were used as (tentative) reference values.

Nishikawa et. al; *Endocr J.* 2016, 63(12):1065-1080.



#### Measurement of candidate reference materials





### Is calibration with CRM effective?

Calibration curve was made from results of candidate CRMs.

Results of 5 patient samples were re-calculated with calibration curve.



Fig. Relative difference between obtained value and reference value.

Calibration is effective in some of the commercial kits.



#### Serum Reference Material for Aldosterone Measurement







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#### **Traceability in Protein Quantification**

#### Amino Acid CRMs (Pure)

Amino Acid CRMs Alanine: CRM 6011-a Leucine: CRM 6012-a Isoleucine: CRM 6013-a Phenylalanine: CRM 6014-a

Valine: CRM 6015-a Proline: CRM 6016-a Arginine: CRM 6017-a Lysine(HCI) : CRM 6018-a



Tyrosine: CRM 6019-a Threonine : CRM 6020-a Serine : CRM 6021-a Glycine: CRM 6022-a Histidine : CRM 6024-a Glutamic acid: CRM 6026-a Aspartic acid: CRM 6027-a Methionine : CRM 6023-a Cystine : CRM 6025-a

#### **Amino Acid Analysis**



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### Profile of Insulin related peptides

Insulin: Insulin is a peptide hormone, produced by beta cells of the pancreatic islets. It is responsible for regulating the body's glucose levels.
C-peptide: A byproduct of insulin production, usually by the pancreas.
C-peptide is useful as a marker of insulin production, since the pancreas typically releases C-peptide and insulin in about equal amounts.

#### Insulin

two polypeptide chains are linked together by disulfide bonds. A-chain: 21 amino acid B-chain: 30 amino acid

#### **C-peptide** 31 amino acid peptide





#### **Single Peptide Solution CRMs**

### NMIJ CRM 6901-b C-peptide

Lyophilized material of C-peptide in phosphate buffer solution.

Material: synthetic peptide having human C-peptide sequence

Certified Concentration (for prepared solution) C-peptide:  $100 \pm 5 \text{ (mg/L)}$ Total C-peptide (Mixture of C-peptide, deamidated C-peptide, and pyroglutamylated C-peptide):  $102 \pm 5 \text{ (mg/L)}$ 



#### NMIJ CRM 6209-a Human Insulin Solution

Purified recombinant human insulin in solution.

Certified Mass Concentration (77.9± 2.3) mg/L





#### **Traceability in Protein Quantification**





### **Development of IDMS for serum insulin**



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#### IDMS for serum insulin IDMS vs immunoassay



## Further research will be necessary to establish as a higher order reference method.



## Summary

- NMIJ/AIST is producing and maintaining primary level reference materials.
- CRM is not a goal for standardization.
- There are many steps for ensuring traceability to clinical lab.
- Cooperation among clinical communities, NMIs, manufactures, etc. is very important.





## Thank you for your attention!



#### http://www.nmij.jp/english/