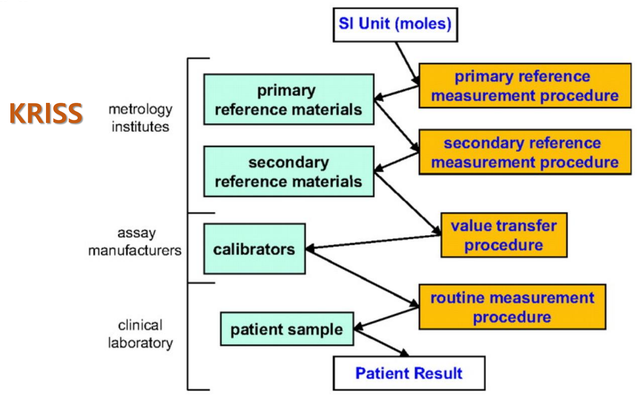
Development of SI-traceable certified reference materials for clinical applications by KRISS

Ji-Seon Jeong, Ha-Jeong Kwon, and Ji-Youn Lee

Center for Bioanalysis, Division of Chemical and Medical Metrology, Korea Research Institute of Standards and Science (KRISS)

Metrological traceability in Laboratory medicine (LM)

As a national metrology institute in Korea, KRISS has provided the foundation for the national standards and international equivalencies through the development of RM and participation into international key comparisons based on the CIPM-MRA.



Clin Chem, 58(1)165;2012

Human Serum for Lipids

Human Serum for Glucose

CRMs by KRISS for LM and its applications

Numerous CRMs for clinical applications have developed and disseminated to establish metrological traceability based on clinical matrices such as serum, plasma, hemolysate.
Target compounds were aligned to target disease and applications in related fields.
CRMs have been applied to accuracy-based proficiency testing, and to product quality assessment in IVD devices development.
The “Korean Health Index” for blood glucose and cholesterol were established by National Standard Reference Data based on KRISS CRMs.

Current available CRM/RM list for clinical applications produced by KRISS

CRM No.	Name	JCTLM DB
111-01-007	Human Serum for Glucose (3 Levels)	-
111-01-010	Human Serum for Lipids (2 Levels)	Under review
111-01-019	Human Plasma for Amino Acid Analysis (2 Levels)	Under review
111-01-005	Human Serum for Creatinine (2 Levels)	Under review
111-01-012	Glycated hemoglobin (2 Levles)	- (new batch coming in 2021)

Korea Health Index
https://nhiss.nhis.or.kr/

Human Plasma For Amino Acid Analysis (KRISS CRM 111-01-019)

Now Available!

Five amino acids as markers for inherited metabolic diseases (Phenylketonuria (PKU), and Maple Syrup Urine Disease (MSUD)) were certified with two different concentrations using isotope-dilution HPLC-MS/MS in human plasma.
Five amino acids; phenylalanine, tyrosine, valine, isoleucine, and leucine were certified, and results of 19 amino acids by conventional AAA method were produced as additional information.

KRISS Korea Research Institute of Standards and Science 267 Gajeong-ro, Yuseong-gu, Daejeon 34113, Republic of Korea Phone. +82-(0)42-868-5555, Fax. +82-(0)42-868-5556

Certificate No.: Page 1 / 5 Pages

CERTIFICATE OF REFERENCE MATERIAL

- CRM Description: Frozen Human Plasma for Amino Acid Analysis
- CRM No.: 111-01-019 · Serial No.: 171025
- Specification: 0.5 mL/bottle
- Producer: *Korea Research Institute of Standards and Science, 267 Gajeong-ro, Yuseong-gu, Daejeon 34113, Republic of Korea*

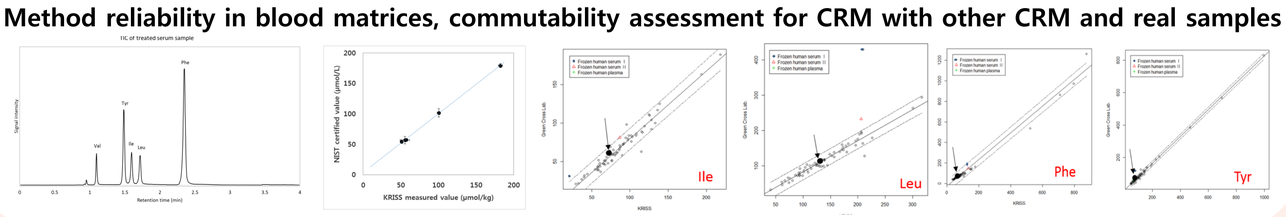
The KRISS CRM 111-01-019 is frozen human plasma for determination of free amino acids. Two concentration levels of valine, isoleucine, leucine, and phenylalanine are prepared as level I and II. A single set of CRM 111-01-019 consists of four vials as two vials for each of concentration levels.

Description of Certification

- Intended Use: This CRM is intended primarily for validation of methods for determining amino acids in human plasma and similar materials. This CRM can also be used for comparison of measurement technologies used in amino acid analysis and for quality assurance when assigning values to in-house reference materials. This CRM is intended to represent "normal" human plasma as level I, and "abnormal" range of valine, isoleucine, leucine, and phenylalanine as level II.
- Instructions and Notices: Refer to the "Certification Results" on the next page.
- Methods of Certification: Isotope-dilution HPLC-tandem mass spectrometry
- Certified Values and Measurement Uncertainty: Refer to the "Certification Results" on the next page.

Level I	Certified value ±*U*exp.	*k*
L-valine	[21.5 ± 1.0] mg/kg	2.11
L-isoleucine	[9.9 ± 0.5] mg/kg	2.10
L-leucine	[16.3 ± 0.7] mg/kg	2.12
L-phenylalanine	[9.5 ± 0.3] mg/kg	2.57
L-tyrosine	[9.2 ± 0.5] mg/kg	2.23
Level II		
L-valine	[63.9 ± 3.5] mg/kg	2.13
L-isoleucine	[55.9 ± 2.3] mg/kg	2.16
L-leucine	[173.7 ± 7.1] mg/kg	2.26
L-phenylalanine	[156.0 ± 6.2] mg/kg	2.57
L-tyrosine	[9.1 ± 0.5] mg/kg	2.31

Method reliability in blood matrices, commutability assessment for CRM with other CRM and real samples

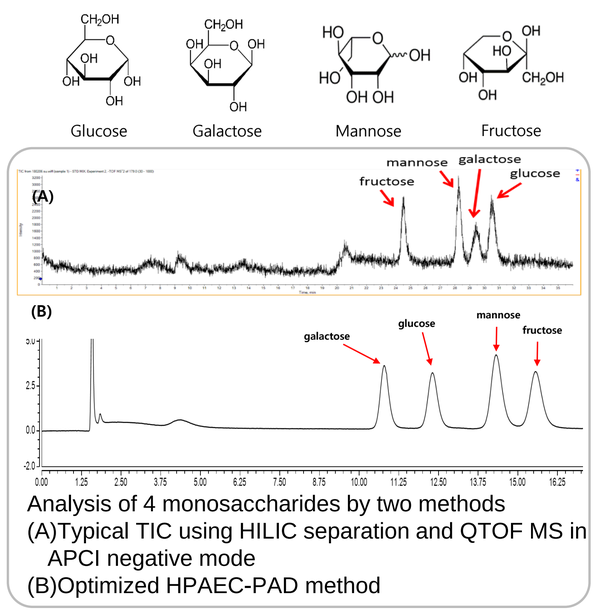


Human Serum For Monosaccharide Analysis

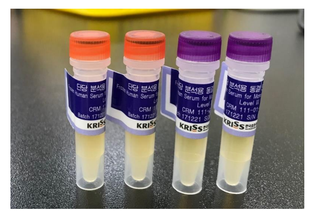
Will be released in 2020!

(KRISS CRM 111-01-020)

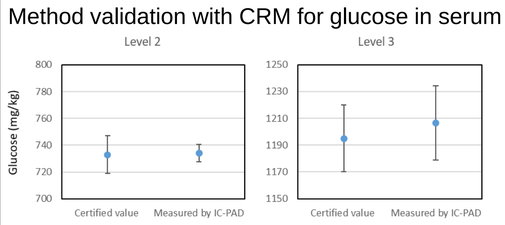
Four monosaccharides; glucose, galactose, mannose, and fructose, will be certified with two different concentrations for diagnosis diabetes mellitus, and galactosemia.
For certification HPAEC-PAD* method was adopted for clear separation and sensitivity.



Analysis of 4 monosaccharides by two methods
(A)Typical TIC using HILIC separation and QTOF MS in APCI negative mode
(B)Optimized HPAEC-PAD method



Method validation with CRM for glucose in serum



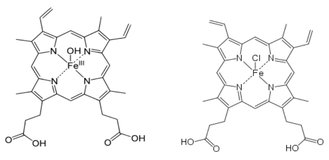
- Certification method was fully validated with a wide ranges of concentration; 1 – 3000 mg/kg.
- Purity assessment for pure material should be progressed for SI-traceability.

**HPAEC-PAD: High performance anion-exchange chromatography with pulsed amperometric detection*

CRM For Total Hemoglobin (KRISS CRM 111-01-021)

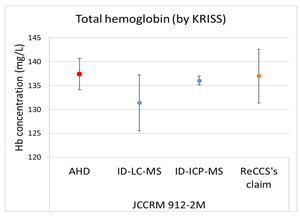
Will be released in 2020!

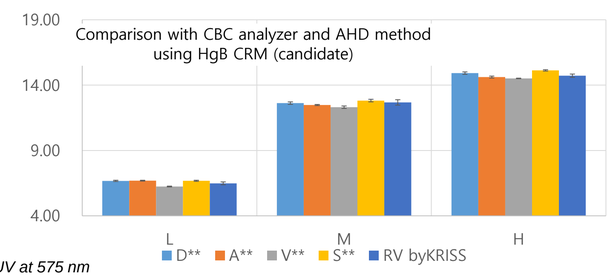
Another hemolysate CRM for total hemoglobin quantification were produced with 3 different concentrations using purified hemolysate. Concentrated erythrocytes were diluted and purified.
Instead of traditional primary method of protein (ID-MS) or reference method (HiCN), AHD* method was adopted as certification method, and the certified value will be SI-traceable via purity assessed hemin chloride.



Hematin (oxidized form of heme)

Hemin (hemin chloride)





**AHD method: Alkaline hematin detergent method using UV at 575 nm*

Dried Blood Spot CRM For Metabolites (KRISS CRM 111-01-022)

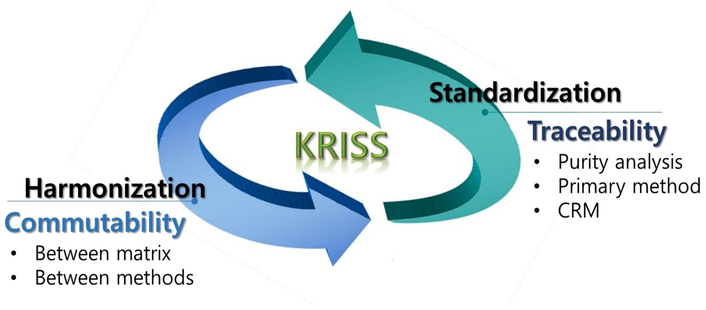
Will be released in 2020!

A new type of matrix, dried blood spot will be released for newborn screening.
Various amino acids and acylcarnitines, and monosaccharides will be certified stepwise from start with amino acid in 2020.
For SI-traceability, whole spot (50 μL) is used and the result by punch sampling will be given as additional information.

Disease	Diagnostic markers	Certification
PKU, Tyrosinemia	Phenylalanine, Tyrosine	Certified
MSUD	Valine, Leucine, Isoleucine	Certified
Homocysteinuria	Methionine	Planned (2020)
Hyperprolinemia Ornithinemia	Proline, Hydroxyproline, Ornithine	Planned (2020)
Citrullinemia	Citrulline	Planned (2021)
Galactosemia	Galactose	Planned (2021)
Diabetes	Glucose, Mannose, Fructose	Planned (2022)
Acidemia, FAOD (Fatty acid oxidation disorders)	Acylcarnitines	Planned (2021~)

Spotting onto filter paper

Summary and Future Plans



- The new line-up CRMs for clinical application were introduced.
- For reference material of clinical applications, both standardization and harmonization should be achieved in terms of measurement traceability and commutability.
- New methodologies with SI-traceability were tried in certification; AHD for Hgb, and HPAEC-PAD for monosaccharides, and both can overcome weak points of traditional reference procedures in terms of improvement of selectivity and sensitivity (HPAEC-PAD), or cost-effective and safety (AHD).
- Sufficient commutability assessment should be added in all new CRMs for ready-to-use standards in clinical applications

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