Organization Name: TUBITAK National Metrology Institute, TUBITAK UME

JCTLM Member status: National and Regional Member

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Period covered: 2020 – 2021

1. Major achievement(s) in support of standardization in laboratory medicine

➢ The production of reference materials:

- With the emergence of COVID-19 disease, SARS-CoV-2 reference materials were developed in a very short time.

- Project titled "Development and Production of Certified Reference Materials and Quality Control Materials for Newborn Screening and Measurements Routinely Performed by Clinical Biochemistry Labs for Amino Acids and Organic Acids" was financially supported by TUBITAK 1003 program. Zivak Technologies, a LC-MS based test and instrument producer company, was also an industrial partner in the project. The project has been completed and two different CRMs are on sale: UME CRM 1314 and UME CRM 1315 were certified for 29 amino acids in lyophilized plasma and 37 organic acids in lyophilized urine, respectively. These CRMs have been submitted for listing under JCTLM CRM database for higher-order reference materials and they are under review process.

- HbA1c CRM & C-Reactive Protein CRM production projects are expected to be completed in 2021 and both CRMs will be on sale in 2022.

➢ Development of reference measurement methods

- Voltammetric sensor & device production for COVID-19 disease detection
  In this project, it is aimed to develop/produce a voltammetric method, sensor and prototype device for the determination of specific proteins of the SARS-CoV-2 virus that causes COVID-19 disease. Developments related to the project continue.

- Primary reference measurement methods were developed for synthetic glycated hexapeptide of HbA1c (GE) and synthetic hexapeptide of HbA0 (VE).

- A primary measurement method was developed for quantification of SARS-CoV-2 monoclonal antibody in solution.

- Primary measurement methods were developed for quantification of HIV-1 RNA and SARS-CoV-2 RNA.

- **Projects in the field of Laboratory Medicine:**

EMPIR projects:

Chemistry Group Laboratories have been collaborating in health related EMPIR projects and contribute to the development of new measurements methods and production of new certified reference materials in Laboratory Medicine. TUBITAK UME laboratories actively participated in the following EMPIR projects in the period of 2020-2021:

- CardioMet - Improvement and metrological underpinning of quantitative diagnostic methods for biomarkers of coronary heart diseases, 2019 - 2022.

- JNP: TraceLabMed - Support for a European Metrology Network on Traceability in Laboratory Medicine, 2019 - 2024.

- EMN: TraceLabMed - European Metrology Network on Traceability in Laboratory Medicine, 2019 -

- ALCOREF - Development and certification of forensic alcohol reference materials for the law enforcement of drinking/driving regulation, 2017 - 2020.

- QUIERO - Quantitative MR-based imaging of physical biomarkers, 2019 - 2022.

- **The establishment of calibration (reference) measurement services.**
  The following reference measurement services were offered in 2021 and listed under TUBITAK UME measurement services (Table 1).

Table 1. TUBITAK UME primary level measurement services offered in 2021.

Code	Measurement Service Name
G3OK-4600	Determination of Vitamin 3-epi-25-Hydroxyvitamin D3
G3OK-4610	Determination of Vitamin 25-OH D2
G3OK-4620	Determination of Vitamin 25-OH D3
G3OK-5600	Determination of Cholesterol in Serum
G3OK-5700	Determination of Glucose in Serum
G3OK-5800	Determination of Creatinine in Serum
G3OK-6900	Determination of Urea and Uric Acid in Biological Matrix
G3IK-3301	Determination of As in Biological Materials
G3IK-3302	Determination of Pb in Biological Materials
G3IK-3303	Determination of Cr in Biological Materials
G3IK-3306	Determination of K in Human Serum
G3IK-3307	Determination of Ca in Human Serum
G3IK-3308	Determination of Mg in Human Serum
G3IK-3309	Determination of Fe in Human Serum
G3IK-3310	Determination of Selenomethionine in Human Serum
G3IK-3311	Determination of Cl in Serum
G3IK-3312	Determination of Cu in Serum
G3IK-3313	Determination of Na in Serum
G3IK-3314	Determination of P in Serum
G3IK-3315	Determination of Se in Serum
G3IK-3320	Determination of Hg in Biological Materials
G3IK-3321	Determination of Ni in Biological Materials
G3IK-3322	Determination of P in Biological Materials
G3IK-3323	Determination of Zn in Biological Materials
G3BA-2100	Determination of Relative Gene Expression Levels - Real Time PCR
G3BA-4100	Absolute quantification of Plasmid DNA - Digital PCR instrument
G3BA-4200	Relative quantification of oncogenes - Digital PCR Instrument
G3BA-5100	Amino Acid Analysis For Pure Protein/Peptide - LC-ID-MS
G3BA-5200	Protein Identification - Peptide Mass Fingerprinting Method, LC-MS/MS
G3BA-5300	Quantification of C-peptide/angiotensin - LC-ID-MS(n)
G3BA-5400	Quantification of Protein Concentration - HPLC
G3BA-5500	Quantification of Protein Concentration - ELISA
G3BA-5600	Quantification of Protein Concentration - PICAA
G3BA-5700	Relative Quantification of HbA1c Protein - HPLC
G3BA-5800	Relative Quantification of HbA1c Protein- LC-ID-MS
G3BA-5900	Determination of Amylase Enzyme Activity
G3BA-6000	Complex Proteome Analysis - Relative Quantification (LFQ-TMT)
G3BA-6100	Identification of Post Translational Modifications
G3BA-6200	Peptide Fragmentation (HCD)
G3BA-6300	LC-MS/MS Method based protein identification

- **Outline of the measurement area(s)/measurands covered:**

CCQM Measurement Comparison Studies

TUBITAK UME Chemistry Group Laboratories have participated to several international measurement comparison studies related to the field of Laboratory Medicine between 2020 and 2021 (Table 2). More resources will be devoted to the development of new reference measurement methods in this field.

Table 2. CCQM and EURAMET international measurement comparison studies participated by TUBITAK UME between 2020 and 2021.

Comparison #	Comparison Name
CCQM-K115b	Peptide Purity - Synthetic Oxytocin (OXT)
CCQM-K115c	Peptide Purity - Synthetic Glycated Hexapeptide of HbA1c (GE)
CCQM-K115.2018	Peptide Purity - Synthetic Hexapeptide of HbA0 (VE)
CCQM-P216	Quantification of SARS-CoV-2 Monoclonal Antibody in Solution
CCQM-P219	Determination of the Amount-of-Substance Fraction of [HbA1c/(HbA1c+HbA0)] in Human Hemolysate*
CCQM-P184	Copy Number Concentration and Fractional Abundance of a Mutation (SNV or INDEL) Mixed with Wild-type DNA
CCQM-P199	Measurement of HIV-1 RNA Copy Number Concentration
CCQM-P199.b	SARS-CoV-2 RNA Copy Number Quantification
CCQM-K176	Breast Cancer Biomarker HER2 Copy Number Variation (CNV) Measurement

*In progress

- **Listing of the relevant technical/scientific publications:**

- Liv L, Çoban G, Nakiboğlu N, Kocagöz T. A rapid, ultrasensitive voltammetric biosensor for determining SARS-CoV-2 spike protein in real samples. Biosens Bioelectron. (2021), 192:113497. https://doi.org/10.1016/j.bios.2021.113497.

- Liv L. Electrochemical immunosensor platform based on gold-clusters, cysteamine and glutaraldehyde modified electrode for diagnosing COVID-19. Microchem J. (2021), 168:106445. https://doi.org/10.1016/j.microc.2021.106445.

- Karakuş E, Erdemir E, Demirbilek N, Liv L. Colorimetric and electrochemical detection of SARS-CoV-2 spike antigen with a gold nanoparticle-based biosensor. Anal Chim Acta. (2021). 1182:338939. https://doi.org/10.1016/j.aca.2021.338939.

- Liv, L., Yener, M., Çoban, G. et al. Electrochemical biosensing platform based on hydrogen bonding for detection of the SARS-CoV-2 spike antibody. Anal Bioanal Chem (2021). https://doi.org/10.1007/s00216-021-03752-3.

- Karakus, E., Erdemir, E., Garen, S., Liv, L., Gunduz, S., Can, S.A. Fluorescein Based Three-channel Probe for the Selective and Sensitive Detection of CO_3^{2-} Ions in an Aqueous Environment and Real Water Samples. Journal of Fluorescence. (2021), 31:6: 1617 – 1625.

- Yalcinkaya, B., Guzel Tanoglu, E., Tastekin, D., Pence, S. "Role of mir-33a, mir-203b, mir361-3p, and mir-424 in Hepatocellular Carcinoma", Turkish Journal of Medical Sciences, 51:2 (2021), 638-643.

- Akyurek, S., Sanal Demirci, S.N., Bayrak, Z., Isleyen, A., Akgoz, M. "The Production and Characterization of SARS-CoV-2 RNA Reference Material", Analytical and Bioanalytical Chemistry, 413:13 (2021) : 3411-3419.

- Yalcinkaya, B., Coskun, K., Akgoz, M., Pence, S. "A Simple Silica Based DNA Isolation Method for Cell-Free DNA Analysis from Liquid Biopsy", Turkish Journal of Biochemistry, 45:6 (2020) : 701-705.

- Guzel, E., Okyay, T.M., Yalcinkaya, B., Karacaoglu, S., Gocmen, M., Akcakuyu, M.H. "Tumor Suppressor and Oncogenic Role of Long Non-Coding RNAs in Cancer", Northern Clinics of Istanbul, 7:1 (2020) : 81-86.

2. Planned activity(ies) in support of standardization in laboratory medicine

- **R&D project(s) and/or programme(s)**

TUBITAK UME Chemistry Group Laboratories collaborate with public and private institutions to develop measurement procedures in the field scientific metrology.

In Bioanalysis Laboratory, the levels of biological molecules such as bacterial or eukaryotic genomic DNA and genomic mutations are measured with digital PCR systems, considered as reference measurement system. In case of new infectious diseases or other variants of SAR-CoV-2 emerges, new measurement methods will be developed for measuring these viruses.
For measurement of DNA methylation, a new CCQM measurement study will be offered in collaboration with KRISS.

The measurements of small biological molecules such as amino acids, organic acids, hormones, lipids, antibiotics, pesticides and other metabolites are carried out by Organic Chemistry Laboratory which utilizes the quantification power of qNMR as a new reference measurement system in addition to the classical primary reference measurement methods.

Inorganic Chemistry Laboratory develops new measurement methods with the use of a new clean laboratory and new state-of-the-art instruments.

HbA1c proficiency testing (EurA1c) has been carried out by IFCC since 2016 and we will continue to contribute to this study in the following years.

After establishing measurement capabilities further, priority will be given to the preparation of more certified reference materials according to the needs of clinical laboratories in Turkey (Table 3).

Table 3. Certified Reference Materials Preparation Planning at TUBITAK UME, 2022-2023.

Date of Release	**Description of CRM or RM**
On sale	25-OH Vitamin D2 and D3 in serum (2012-2014)
On sale	Amino acids for neonatal screening (2014-2018)
On sale	Organic acids for neonatal screening (2014-2018)
Will be on sale in 2022	HbA1c CRM (2016-2021)
Will be on sale in 2022	C-Reactive Protein CRM (2019-2021)
Planning	DNA methylation CRMs
Planning	Elements in serum (2024-2025)
Planning	Elements in whole blood (2024-2025)
Planning	Elements in urine (2024-2025)

➢ **EMPIR projects:**

The following EMPIR projects will be completed or continued as outlined in the project proposals and new measurement methods and/or new CRMs/RMs will be produced.

- CardioMet - Improvement and metrological underpinning of quantitative diagnostic methods for biomarkers of coronary heart diseases, 2019 - 2022
- JNP: TraceLabMed - Support for a European Metrology Network on Traceability in Laboratory Medicine, 2019 - 2024
- EMN: TraceLabMed - European Metrology Network - Traceability in Laboratory Medicine, 2019 - 2024
- ALCOREF - Development and certification of forensic alcohol reference materials for the law enforcement of drinking/driving regulation, 2017 – 2020
- QUIERO - Quantitative MR-based imaging of physical biomarkers, 2019 – 2022

Chemistry Group Laboratories plan to participate to the EMPIR 2022 health call projects.

➢ **CCQM Measurement Comparison Studies:**

TUBITAK UME Chemistry Group Laboratories have been planning to attend to the following CCQM measurement comparison studies (Table 4).

Table 4. CCQM international measurement comparison studies planned between 2022 and 2023.

Comparison #	**Comparison Name**
CCQM-K162	Selenoproteins in Serum
CCQM-K159	Free Amino Acids in Plasma
CCQM-XX	Quantification of DNA Methylation (Planning to organize collaboration with KRISS)
CCQM-K/P	Peptide purity - Parthyroid Hormone
CCQM-K/P	Triskelion Peptide
CCQM-KC/P	Quantification of HbA1c in Human Hemolysate
CCQM-KC	Follow-up of P164 Mass Fraction of Human Growth Hormone in Serum
CCQM-KC	Follow-up of P201 Total Haemoglobin Concentration in Human Whole Blood

3. Promoting traceability in laboratory medicine

➢ **Technical/scientific publications:**

- Jeong, J., Yim, Y., Liu, Q., Yong, S., Liu, H., Beltrão, P., Scapin, S., Sade, Y., Garrido, B., Boeuf, A., Delatour, V., Kilpatrick, E.L., Lowenthal, M.S., Toman, B., Mi, W., Wu, L., Wang, J., Kinumu, T., Henrion, A., Ohlendorf, R., Oztug Kilinc, M., Akgoz, M. "Key Comparison Study on Protein Quantification Purity-Assessed Recombinant Protein Contents in Buffer Solution Using Insulin Analogue", Metrologia, 58:1A (2021) : 08007.

- Josephs, R., Li, M., Daireaux, A., Choteau, T., Martos, G., Westwood, S., Wielgosz, R., H., L., X, L., Shi, N., Wu, P., Feng, L., Huang, T., Zhang, T., Li, S., Beltrão, P., Saraiva, A., Garrido, B., Scapin, S., Wollinger, W., Sade, Y., Thibeault, M., Stocks, B., Oztug Kilinc, M., Akgoz, M. "Key Comparison Study on Peptide Purity-Synthetic Oxytocin", Metrologia, 57:1A (2020) : Article number 08014.

➢ **Congress Oral Presentations:**

- Meltem Asicioglu-Kucuk, Merve Oztug, Evren Saban, Nevin Gül Karagüler. Measurement of Cardiac Troponin I in Human Serum. 32nd National Biochemistry Congress, 27-30 October 2021, Gaziantep, Turkey

- Evren Saban, Meltem Asicioglu Kucuk, Aybaran Olca Kebapci, Gonca Altin1, Merve Oztug. Novel Method Development and Validation of HbA1c Measurement in Human Plasma By Liquid Chromatography- Mass Spectrometry. 32nd National Biochemistry Congress, 27-30 October 2021, Gaziantep, Turkey

4. Reference laboratory networks /collaborations focusing on developing /implementing reference measurement systems

➢ **JCTLM Activities**

TUBITAK UME become a member to JCTLM in 2012 and has actively participated to the JCTLM Members' and Stakeholders' Meetings since 2012. Biennial report was also submitted to JCTLM Executive Committee in 2019.

➢ **CCQM Activities**

As a member of CCQM, TUBITAK UME Chemistry Group scientists attended and actively contributed to OAWG, IAWG, NAWG, PAWG, CAWG, EAWG and SAWG meetings in 2020 and 2021.

Merve OZTUĞ is also a member of PAWG Focus Group 1 (SI Value Assignment of Purity) and PAWG Focus Group 2 (SI-traceable determination of peptides and proteins in complex matrices).

➢ **EurA1c: The European HbA1c Trial "EQA Program"**

In 2016, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Committee Education in the Use of Biomarkers in Diabetes (C-EUBD) started a proficiency testing program for standardization of HbA1c measurements (EurA1c) throughout Europe. TUBITAK UME has also attended to these proficiency tests in 2020 and 2021.