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

- The production of certified reference materials:
  - A certified reference material (CRM) preparation project that was supported by TUBITAK UME titled “25-Hydroxy Vitamin D2 and 25-Hydroxy Vitamin D3 in Lyophilized Serum” was completed and the CRM is on sale. The CRM is listed under JCTLM database for higher-order reference materials.
  
  - 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 32 amino acids in lyophilized plasma and 47 organic acids in lyophilized urine, respectively. These CRMs has been submitted for listing under JCTLM CRM database for higher-order reference materials.
  
  - HbA1c CRM project is expected to be completed in 2020 and the CRM will be on sale in 2021.
  
  - C-Reactive Protein CRM project has just started in 2018 and it is expected to be finished in 2021.
Development of reference measurement methods

- **Mass fraction of amino acids in acidic aqueous solution.**

- **Counting of cells.**

- **Mass fraction of folic acid in high purity material**

- **Digital PCR as a reference measurement procedure for DNA copy number quantification of KRAS mutation.**
Projects in the field of Laboratory Medicine:

- **EMPIR projects:**

  Chemistry Group Laboratories have been collaborating in health related EMPIR projects and contributes to the development of new measurements methods and production of new certified reference materials in Laboratory Medicine. Our laboratories were partners in the following EMPIR projects completed in the period of 2018-2019:

  **Completed projects:**

  - ReMiND - Role of metals and metal containing biomolecules in neurodegenerative diseases such as Alzheimer’s disease, 2016-2019.

  **Ongoing projects:**

  Our laboratories are also collaborating in the following health related EMPIR projects:

  - 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 -

- **Dual Collaboration Project:**

  A dual collaboration project, “Korea-Turkey collaboration project on the development of an international standard system for the measurement of gene methylation” has been completed in 2018. A new measurement method for genomic DNA methylation measurement, which is a critical marker on the diagnosis of several diseases including cancer, will be suggested. The results of the project will be presented by Dr. Sema AKTUREK at JCTLM Members’ and Stakeholders’ meeting, on 2-3 December 2019, BIPM.
The establishment of calibration (reference) measurement services.

The following reference measurement services were offered in 2019 and listed under TUBITAK UME services (Table 1).

Table 1. TUBITAK UME measurement services offered in 2019.

<table>
<thead>
<tr>
<th>Code</th>
<th>Measurement Service Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>G3OK-4600</td>
<td>Determination of Vitamin 3-epi-25-Hydroxyvitamin D3</td>
</tr>
<tr>
<td>G3OK-4610</td>
<td>Determination of Vitamin 25-OH D2</td>
</tr>
<tr>
<td>G3OK-4620</td>
<td>Determination of Vitamin 25-OH D3</td>
</tr>
<tr>
<td>G3OK-5600</td>
<td>Determination of Cholesterol in Serum</td>
</tr>
<tr>
<td>G3OK-5700</td>
<td>Determination of Glucose in Serum</td>
</tr>
<tr>
<td>G3OK-5800</td>
<td>Determination of Creatinine in Serum</td>
</tr>
<tr>
<td>G3OK-6900</td>
<td>Determination of Urea and Uric Acid in Biological Matrix</td>
</tr>
<tr>
<td>G3IK-3301</td>
<td>Determination of As in Biological Materials</td>
</tr>
<tr>
<td>G3IK-3302</td>
<td>Determination of Pb in Biological Materials</td>
</tr>
<tr>
<td>G3IK-3303</td>
<td>Determination of Cr in Biological Materials</td>
</tr>
<tr>
<td>G3IK-3306</td>
<td>Determination of K in Human Serum</td>
</tr>
<tr>
<td>G3IK-3307</td>
<td>Determination of Ca in Human Serum</td>
</tr>
<tr>
<td>G3IK-3308</td>
<td>Determination of Mg in Human Serum</td>
</tr>
<tr>
<td>G3IK-3309</td>
<td>Determination of Fe in Human Serum</td>
</tr>
<tr>
<td>G3IK-3310</td>
<td>Determination of Selenomethionine in Human Serum</td>
</tr>
<tr>
<td>G3IK-3311</td>
<td>Determination of Cl in Serum</td>
</tr>
<tr>
<td>G3IK-3312</td>
<td>Determination of Cu in Serum</td>
</tr>
<tr>
<td>G3IK-3313</td>
<td>Determination of Na in Serum</td>
</tr>
<tr>
<td>G3IK-3314</td>
<td>Determination of P in Serum</td>
</tr>
<tr>
<td>G3IK-3315</td>
<td>Determination of Se in Serum</td>
</tr>
<tr>
<td>G3IK-3320</td>
<td>Determination of Hg in biological materials</td>
</tr>
<tr>
<td>G3IK-3321</td>
<td>Determination of Ni in biological materials</td>
</tr>
<tr>
<td>G3IK-3322</td>
<td>Determination of P in biological materials</td>
</tr>
<tr>
<td>G3IK-3323</td>
<td>Determination of Zn in biological materials</td>
</tr>
<tr>
<td>G3BA-2100</td>
<td>Determination of Relative Gene Expression Levels - Real Time PCR</td>
</tr>
<tr>
<td>G3BA-4100</td>
<td>Absolute quantification of Plasmid DNA - Digital PCR instrument</td>
</tr>
<tr>
<td>G3BA-4200</td>
<td>Relative quantification of oncogenes - Digital PCR Instrument</td>
</tr>
<tr>
<td>G3BA-5100</td>
<td>Amino Acid Analysis For Pure Protein/Peptide - LC-ID-MS</td>
</tr>
<tr>
<td>G3BA-5200</td>
<td>Protein Identification - Peptide Mass Fingerprinting Method, LC-MS/MS</td>
</tr>
<tr>
<td>G3BA-5300</td>
<td>Quantification of C-peptide/angiotensin - LC-ID-MS(n)</td>
</tr>
<tr>
<td>G3BA-5400</td>
<td>Quantification of Protein Concentration - HPLC</td>
</tr>
<tr>
<td>G3BA-5500</td>
<td>Quantification of Protein Concentration - ELISA</td>
</tr>
<tr>
<td>G3BA-5600</td>
<td>Quantification of Protein Concentration - PICAA</td>
</tr>
<tr>
<td>G3BA-5700</td>
<td>Relative Quantification of HbA1c Protein - HPLC</td>
</tr>
<tr>
<td>G3BA-5800</td>
<td>Relative Quantification of HbA1c Protein- LC-ID-MS</td>
</tr>
<tr>
<td>G3BA-5900</td>
<td>Determination of Amylase Enzyme Activity</td>
</tr>
<tr>
<td>G3BA-8100</td>
<td>Cell counting - Flow Cytometer instrument</td>
</tr>
</tbody>
</table>
Outline of the measurement area(s)/measurands covered:

- **CCQM and EURAMET Measurement Comparison Studies**
  
  TUBITAK UME Chemistry Group Laboratories have participated to several international measurement comparison studies in the field of Laboratory Medicine between 2018 and 2019 (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 2018 and 2019.

<table>
<thead>
<tr>
<th>Comparison #</th>
<th>Comparison Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>EURAMET 1185</td>
<td>Determination of Selenomethionine in Human Serum</td>
</tr>
<tr>
<td>CCQM-K107</td>
<td>Total Elements and Selenomethionine in Human Serum</td>
</tr>
<tr>
<td>CCQM-K139*</td>
<td>Elements in Human Serum</td>
</tr>
<tr>
<td>CCQM-P164</td>
<td>Mass fraction of human growth hormone in serum</td>
</tr>
<tr>
<td>CCQM-K151</td>
<td>Determination of the amount content of a purity-assessed recombinant protein in an aqueous calibration solution- Insulin</td>
</tr>
<tr>
<td>CCQM-K115b</td>
<td>Peptide purity - synthetic oxytocin (OXT)</td>
</tr>
<tr>
<td>CCQM-P184</td>
<td>Copy Number Concentration and Fractional Abundance of a Mutation (SNV or INDEL) Mixed with Wild-type DNA</td>
</tr>
<tr>
<td>CCQM-P199</td>
<td>Capability in low level detection/identification of specific (pathogen) sequence: Measurement of HIV-1 RNA copy number concentration</td>
</tr>
<tr>
<td>CCQM-K115c*</td>
<td>Peptide purity - synthetic glycated hexapeptide of HbA1c (GE)</td>
</tr>
<tr>
<td>CCQM-P55.2C*</td>
<td>Peptide purity - synthetic hexapeptide of HbA0 (VE)</td>
</tr>
<tr>
<td>CCQM-K115.2018*</td>
<td></td>
</tr>
<tr>
<td>CCQM-P55.2*</td>
<td></td>
</tr>
</tbody>
</table>

*In progress
Calibration and Measurement Capabilities:
By using its expertise and providing services in clinical measurements, TUBITAK UME Chemistry Group Laboratories applied for calibration and measurement capabilities (CMC) and qualified for several measurement capabilities (Table 3).

Table 3. TUBITAK UME Calibration and Measurement Capabilities gained between 2018 and 2019.

<table>
<thead>
<tr>
<th>Measurement Service Sub-Category</th>
<th>Matrix</th>
<th>Analyte or Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biological fluids and materials</td>
<td>Blood serum</td>
<td>Sodium</td>
</tr>
<tr>
<td>Biological fluids and materials</td>
<td>Blood serum</td>
<td>Chlorine</td>
</tr>
<tr>
<td>Biological fluids and materials</td>
<td>Blood serum</td>
<td>Copper</td>
</tr>
<tr>
<td>Biological fluids and materials</td>
<td>Blood serum</td>
<td>Selenium</td>
</tr>
<tr>
<td>Biological fluids and materials</td>
<td>Blood serum</td>
<td>Phosphorus</td>
</tr>
<tr>
<td>Biological fluids and materials</td>
<td>Blood serum</td>
<td>Transferrin</td>
</tr>
<tr>
<td>Other</td>
<td>Pure DNA</td>
<td>Linearized plasmid DNA between 3 kbp and 6 kbp in buffered matrix</td>
</tr>
</tbody>
</table>
Listing of the relevant technical/scientific publications:


to investigate the performance of HbA1c assays in 2166 laboratories across 17 countries and 24 manufacturers using the IFFC Model for Quality Targets. Clinical Chemistry, 2018, 64 (8)


- Kirmizibekmez, H., Inan, Y., Reis, R., Sipahi, H., Goren, A.C., Yesilada, E. Phenolic Compounds from the Aerial Parts of Clematis Viticella L. and Their in Vitro Anti-Inflammatory Activities, Natural Product Research, 2018, 1-4

- Atay Balkan, I., Dogan, H., Zengin, G., Colak, N., Ayaz, F., Goren, A.C., Kirmizibekmez, H., Yesilada, E. Enzyme Inhibitory and Antioxidant Activities of Nerium Oleander L. Flower Extracts and Activity Guided Isolation of the Active Components. Industrial Crops and Products, 112, 2018, 24-31
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 of 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. Different genomic mutations will be measured and new measurement methods will be developed.

  For measurement of DNA methylation, a new CCQM measurement study will be offered in collaboration with KRISS.

  New peptide/proteins based CRM projects, HbA1c and CRP will be finalized.

  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 started and it will be extended to other analytes in the field of Laboratory Medicine.

  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 4).

<table>
<thead>
<tr>
<th>Date of Release</th>
<th>Description of CRM or RM</th>
</tr>
</thead>
<tbody>
<tr>
<td>On sale</td>
<td>25-OH Vitamin D2 and D3 in serum (2012-2014)</td>
</tr>
<tr>
<td>On sale</td>
<td>Amino acids for neonatal screening (2014-2018)</td>
</tr>
<tr>
<td>Will be on sale 2021</td>
<td>HbA1c CRM (2016-2020)</td>
</tr>
<tr>
<td>Will be on sale 2021</td>
<td>CRP CRM (2019-2021)</td>
</tr>
<tr>
<td></td>
<td>Elements in serum (2020-2022)</td>
</tr>
<tr>
<td></td>
<td>Elements in whole blood (2023-2026)</td>
</tr>
<tr>
<td></td>
<td>Elements in urine (2023-2026)</td>
</tr>
</tbody>
</table>
EMPIR projects:
The following EMPIR projects will be completed or continued as outlined in the project proposals and new measurement methods and 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 -
- 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

New EMPIR health call will be advertised in 2021, Chemistry Group Laboratories plan to be involved in these health related projects.

Measurement Comparison Studies:

TUBITAK UME Chemistry Group Laboratories have been planning to attend to the following measurement comparison studies (Table 5).

Table 5. CCQM international measurement comparison studies planned between 2020 and 2021.

<table>
<thead>
<tr>
<th>Comparison #</th>
<th>Comparison Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCQM-K162</td>
<td>Selenoproteins in serum</td>
</tr>
<tr>
<td>CCQM-K159</td>
<td>Free Amino Acids in Plasma</td>
</tr>
<tr>
<td>CCQM-XX</td>
<td>Quantification of DNA Methylation (Planning to organize collaboration with KRISS)</td>
</tr>
<tr>
<td>CCQM-XX</td>
<td>HER2 Genetic Mutation Quantification</td>
</tr>
<tr>
<td>CCQM-XX</td>
<td>ProCalcitonin in Serum</td>
</tr>
<tr>
<td>CCQM-XX</td>
<td>Brain Natriuretic Peptide (BNP)</td>
</tr>
</tbody>
</table>
Calibration and Measurement Capabilities:

Participating to the new measurement comparisons in clinical area, it is aimed to develop new measurement methods and new reference measurement materials, to broaden our services in the field of Laboratory Medicine (Table 6) leading to new CMC entries in the area.

Table 6. New Calibration and Measurement Capabilities applications planned between 2020 and 2021.

<table>
<thead>
<tr>
<th>Measurement Service Sub-Category</th>
<th>Matrix</th>
<th>Analyte or Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptides</td>
<td>Lyophilized or mQ-H2O 20 mM&lt;br&gt;Phosphate or other buffers with similar level of complexity</td>
<td>pure peptide primary reference material</td>
</tr>
<tr>
<td>Biological Fluids and materials</td>
<td>Blood Serum, Plasma</td>
<td>Amino Acids</td>
</tr>
</tbody>
</table>
3. Promoting traceability in laboratory medicine

- **EMN: TraceLabMed - European Metrology Network - Traceability in Laboratory Medicine**, Under this EMN, a workshop has been organized titled “Elements of Metrological Traceability for Laboratory Medicine” on 27th Balkan Clinical Laboratory Federation Meeting and 30th National Turkish Biochemical Society Meeting on 27-31 October 2019 in Antalya, Turkey. The Congress was supported by mother organizations, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM).

During the workshop, the following oral presentations were given on 29th October 2019.

- Traceability in laboratory medicine and IVD directives
  Tomris Ozben, Akdeniz University, Turkey

- Introduction of the European Metrology Network on Traceability in Laboratory Medicine
  Muslum Akgoz, TUBITAK, Turkey

- Amino acid and organic acid CRMs for newborn screening
  Dr. Simay Gunduz, TUBITAK, Turkey

- ID-MS based reference measurement method for small analytes: vitamin D, creatinine, glucose, cholesterol, amino acids
  Mine Bilsel, TUBITAK, Turkey

- Reference methods for quantification of peptides & proteins: β-amyloid in CSF (ReMIND Project), human C-peptide, oxytocin, HbA1c, insulin, human growth hormone
  Merve Öztug Kılınç, TUBITAK, Turkey

- Latest developments on NMR; reference method for purity determination of small analytes and peptides: 17β-estradiol, folic acid, human C-peptide, oxytocin, HbA1c
  Dr. Ilker Un, TUBITAK, Turkey

- Development of a reference method for transferrin quantification in serum
  Dr. Gonca Coskun, TUBITAK, Turkey

- A Reference method for genetic mutation quantification of KRAS
  Dr. Muslum Akgoz, TUBITAK, Turkey

The following poster presentations were also submitted.

- 25-OH Vitamin D2, D3 in Lyophilized Serum
  Gökhan Bilsel, TUBITAK, Turkey

- HbA1c CRM project
  Dr. Gonca Altın Yılmazer, TUBITAK, Turkey
A workshop titled “1st Biometrology and Molecular Biology Workshop” has been organized by TUBITAK Chemistry Group personnel at TUBITAK UME on 16 Jan 2018.

During the workshop, the following oral presentations were given.

- Metrology in Biological Measurements
  Assoc.Prof. Dr. Müslüm AKGÖZ, TÜBİTAK UME

- Turkey-South Korea Bilateral Cooperation Project: Development of Gene Methylation Measurement Method
  Sema AKYÜREK, TÜBİTAK UME

- DNA Defective RASSF1A Gene Methylation Detection in Plasma
  Prof. Dr. Hülya YAZICI, Istanbul University

- Effect of Circadian Clock Disruption on DNA Methylation
  Meltem AŞIOĞLU, Gebze Technical University - TÜBİTAK UME

- Antibiotic War Microorganisms or People Will Win?
  Prof. Dr. Tanıl KOCAGÖZ, Acıbadem University

- Aging Without Getting Old
  Prof. Dr. Ali Demir TİRYAKİ, İstanbul Yeni Yüzyıl University

- Importance of Internal Quality Control in Clinical Biochemical Measurements
  Prof. Dr. Abdurrahman COŞKUN, Acıbadem University

- From Peptides to Proteins: Traceability to SI
  Merve ÖZTUĞ KILINÇ, TÜBİTAK UME
Congress Posters:

- Mine Bilsel, Hasibe Yılmaz, Gökhan Bilsel, Ahmet Ceyhan Gören, Determination of Vitamin D Metabolites in Human Serum by LC-MS/MS, Medical Measurement Application, 26-28 Jun 2019, Istanbul, Turkey

- Sema AKYUREK, Development of dPCR Method for Rapid Screening of Carbapenem-Resistant Enterobacteriaceae, 13-16 Apr 2019, Amsterdam

- Gunduz, S., Yilmaz, H., Bilsel, G., Goren, A.C. Determination of Urea and Uric Acid in Human Serum (İnsan Serumunda Üre ve Ürik asidin LC-MS Yöntemi ile Belirlenmesi), 7th International Drug Chemistry Conference, Antalya, 14-17 Mar 2019, 1

- Yalçinkaya B., Çevik M., Aydin E., Comparison of miRNA Extraction Method From Blood Plasma, 7th Drug Chemistry Conference, March 2019


- Burhanettin Yalcinkaya, Sadrettin Pence. Optimization of primers and probes that can be used in the diagnosis of mitochondrial diseases. International IVEK Biotechnology Congress, 26-28 Nov 2018 Istanbul
Congress Oral Presentations:


- Un, I. Purity determination of Oxytosin Hormone by qNMR and TFA impurity (Oksitosin Hormonunun qNMR ile Saflık Tayini ve TFA Safsızlığının Belirlenmesi), 7th International Drug Chemistry Conference, Antalya, 14-17 Mar 2019, 1 p


- Evren Kilinç, Merve Oztug, Emel Timuçin. Modeling and dynamics of the full-length structure of the factor XII protein: Insights into the mechanism of activation through Zinc binding, 30th Biophysics Congress, Bodrum, Turkey, 10-13 Oct 2018
4. Reference laboratory networks /collaborations focusing on developing /implementing reference measurement systems

- **JCTLM Activities**
  TUBITAK UME become a member of JCTLM in 2012 and has actively participated to the JCTLM Members’ and Stakeholders’ Meetings since then. Biennial report was also submitted to JCTLM Executive Committee in 2018.

- **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 2018 and 2019.

  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 - 2019 several EQA organizers decided to participate in the “EurA1c” projects which are organized by The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Committee Education in the Use of Biomarkers in Diabetes (C-EUBD). TUBITAK UME was also involved in these trials as a partner and submitted the measurement results of Turkish clinical laboratories in 2016, 2017 and 2018. In 2019, TUBITAK UME also participated to the new study and new samples are being also distributed to the clinical laboratories.

5. Open questions and suggestions to be addressed by JCTLM