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STUDY PROTOCOL

CCQM-K139 and CCQM-P173

Elements in Human Serum

Introduction

Elements in serum serve as important biomarkers and reflect the well-being of an individual. For example, electrolytes such as sodium and chloride are commonly measured since they play a central role in maintaining the normal distribution of water, the osmotic pressure and the electrical neutrality in the body. Trace element such as copper is involved in many oxidation-reduction reactions and metalloenzymes. The majority of selenium occurs in the form of selenoproteins. Physiologically, these proteins serve as cofactors in the glutathione peroxidase activity, in which a major role involves protection against free radicals. Phosphorus is the most abundant element in the body after calcium. Both elements are required for strong bones and teeth. Phosphorus, on its own, is indispensable for growth, maintenance and repair of tissues and cells.

At the IAWG meeting in October 2014, the Health Sciences Authority (HSA), Singapore proposed to organise a comparison for the determination of elements in human serum in 2017. In the IAWG's five year plan, a comparison under the Measurement Category 10 (Biological Fluids and Materials) is required in order to best cover current and future CMCs. After conducting a survey and further discussions at the IAWG meeting in November 2015, the elements (Na, Cl, Cu, Se and P) were chosen. These elements were not covered in the last two comparisons in the clinical area [CCQM-K14: Ca in serum and CCQM-K107 & P146: Elements (K, Ca, Mg, Fe) and selenomethionine in human serum] and will offer different analytical challenges.

Objectives

The comparison aims to enable participating NMIs/DIs to demonstrate their competence in the determination of elements (electrolytes and essential elements) in human serum. It will also enable NMIs/DIs with the relevant services to submit CMC claims upon successful completion.

Preparation of the Comparison Material

The comparison material is frozen human serum. An experienced commercial human blood products supplier (Solomon Park Research Laboratories, Kirkland, WA, USA) was engaged by HSA to prepare the material. One pool of human serum material was prepared, and pre-packed in 200 bottles containing 3 mL of serum each.

The homogeneity of the elements in the comparison material was assessed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). A sample size of 0.10 g was used in the assessment of homogeneity. Eleven bottles were randomly and stratifically selected, and two subsamples were taken from each bottle. Using ANOVA at 95 % level of confidence, the material was found to be sufficiently homogeneous. The relative standard uncertainties of inhomogeneity were found to be 0.11%, 0.41%, 0.22%, 0.64% and 0.44% for sodium, chloride, copper, selenium and phosphorus, respectively.

The stability of the elements at -20 °C in the comparison material was assessed by ICP-MS. A short-term stability study using isochronous design was carried out over a period of 8 weeks. Two randomly selected bottles were transferred from the reference temperature of -80 °C to -20 °C on five occasions over the study period. One subsample was then taken from each bottle. Using Student's *t*-test at 95 % level of confidence, no significant instability of the elements in the comparison material was observed.

The long term stability of the elements in the comparison material at -80 °C will be assessed using the ICP-MS. The testing will be carried out on four occasions over a period of about 12 months using classical design. For each occasion of the stability testing, two bottles will be randomly selected, and two subsamples will be taken from each bottle. Student's *t*-test at 95 % level of confidence will be used for the evaluation of instability of the elements in the comparison material.

The Measurands

The expected mass fractions of the measurands in the study material are listed in the Table below. The concentration levels are within the normal biological range and within the range of existing CMC claims in the BIPM KCDB.

Measurand	Expected Mass Fraction (mg/kg)
Sodium	2500 – 4000
Chloride	3000 – 5000
Copper	0.1 – 5.0
Selenium	0.03 – 0.30
Phosphorus	80 – 150

Registration

Interested institutes should complete the Registration Form and return it to Dr Richard Shin (E-mail: HSA_CML@hsa.gov.sg) before the deadline (14 June 2016). An email will be sent to confirm the registration. The institutes may choose to register for the key comparison or the pilot study.

Instructions for Participating Institutes

The material used for this comparison was tested non-reactive/negative for hepatitis B surface antigen (HbsAg), human immunodeficiency (HIV) 1 and 2 antibodies, and hepatitis C virus (HCV) by the supplier before distribution. However, the material should be handled as biohazards material capable of transmitting infectious diseases.

The comparison material will be transported using dry ice. Upon receipt, the material should be immediately stored at below -60 ^{o}C for long-term storage. A freezer temperature of -20 ^{o}C is also acceptable for storage up to 8 weeks. The material should be used as soon as possible after it is thawed.

Each participating NMI/DI will receive between two to six bottles of serum material depending on the number of measurand it registers for, e.g. a NMI/DI will receive a total of 6 bottles if it registers for all 5 measurands. The participating NMIs/DIs may decide on the number of times that each subsample is to be measured. Before sampling, the material should be allowed to thaw and warm to room temperature (18 – 25 ^{o}C), and homogenised

by gentle swirling and inversing the bottle several times. The recommended minimum subsample size is 0.10 g.

The participating NMIs/DIs should use their own methods for the determination. Metrologically traceable certified reference materials (CRMs) should be used as calibration standards.

Reporting of Results

A Report of Results Form will be provided to the participating NMIs/DIs for completion. The participating NMIs/DIs are expected to report their results based on at least five subsamples for each measurand. The results should be reported in the unit of mg/kg, and should include standard and expanded uncertainties (95 % level of confidence) for the mean of the replicate determinations. Information on the measurement procedure, the calibration standard, the internal standard, the quality control material, the calculation of the results, and the estimation of measurement uncertainty should be included. The completed Results Form and the Core Capability Table (please download the appropriate Core Capability Table from the CCQM IAWG website) should be sent to HSA on or before the scheduled deadline. The submitted results will be considered as final.

Evaluation of Results

Results of all participating NMIs/DIs will be evaluated against the key comparison reference value (KCRV). The KCRV and associated uncertainty will be determined from results of NMIs/DIs that participate in the key comparison using methods with demonstrated metrological traceability. Results from NMIs/DIs that participated in the pilot study will not be included in the calculation of the KCRV.

Schedule

Official call for participation:	03 May 2016
Deadline for registration:	14 June 2016
Distribution of comparison samples:	14 October 2016
Deadline for submission of results:	14 February 2017

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