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Certificate of Analysis

CERTIFIED REFERENCE MATERIAL
HRM-3006A

Testosterone in Human Serum

Batch Number
STY-0087-001
STY-0087-002

Foreword
A unit of the certified reference material (CRM) HRM-3006A consists of two vials of frozen human serum sample with different testosterone concentration levels. Each vial contains about 1 mL of frozen human serum. The serum materials appear as a transparent (or slightly cloudy) brownish yellow liquid after thawing.

The CRM is produced with reference to the requirements set out in ISO/IEC 17025:2017 [1], ISO 17034:2016 [2] and ISO Guide 35:2017 [3].

Certified Concentration Values
The certified concentration values of testosterone in HRM-3006A are provided in Table 1. The certified values of testosterone with the unit of nmol/L were calculated from the concentration value of µg/g, the measured serum density at 23 °C (1.0241 g/mL and 1.0250 g/mL for STY-0087-001 and STY-0087-002, respectively), and the relative molecular mass of testosterone (288.42 g/mol).

Table 1. Certified Values of Testosterone in HRM-3006A

	STY-0087-001	STY-0087-002
Analyte	**(nmol/L)**	**(nmol/L)**
Testosterone	19.28 ± 0.65	6.15 ±0.16

Each certified value is the mean of measurements of at least six samples taken from a minimum of three vials. The certified concentration values for HRM-3006A were determined using isotope dilution mass spectrometry (IDMS). A four-point calibration curve was used in the measurements.

The associated measurement uncertainty (including contribution from characterisation, homogeneity and stability) of each certified value was evaluated in accordance with ISO/IEC Guide 98-3:2008 [4]. The expanded uncertainty (coverage factor of 2, for both levels) corresponded to a level of confidence of about 95%.

Source of Materials
The serum materials were prepared by Solomon Park Research Laboratories (Kirkland, WA, USA).

Homogeneity
Homogeneity testing on testosterone was performed on two subsamples taken from six vials using liquid chromatography-isotope dilution tandem mass spectrometry (LC-IDMS/MS) method. The sample size taken for homogeneity testing was 350 µL for STY-0087-001 and 400 µL for STY-0087-002. No significant differences in the between and within-vial variances were found using *F*-test (ANOVA) at 95 % confidence level. The u_{bb} was evaluated from the uncertainty due to between-vial inhomogeneity.

Stability
The stability of testosterone in HRM-3006A stored at a temperature of below – 60 °C was evaluated on at least three occasions over a period of up to six months. The results showed that the analyte was stable when stored at below – 60 °C over the study period [3]. The u_{stab} was estimated from the standard error of the slope.

Validity
The certified values of HRM-3006A are valid within the specified measurement uncertainty until **06 Apr 2027**. The validity of HRM-3006A will be extended if it is tested to be sufficiently stable for continuous use. The certified values of HRM-3006A are invalid when the serum materials have deteriorated or are mishandled.

Analytical Methods

A fully validated LC-IDMS/MS method was used. The method involved spiking with isotope-labeled testosterone, liquid–liquid extraction with tert-butyl methyl ether and measurement by LC-MS/MS.

Metrological Traceability
The certified concentration values are traceable to the International System of Units (SI) through the use of testosterone CRM (NMIJ 6002-a) from the National Metrology Institute of Japan (NMIJ).

Intended Use
HRM-3006A is intended for use in the validation of methods or as quality control materials for the determination of testosterone in human serum. Users may refer to ISO Guide 33:2015 [5] for the recommended statistical treatment of the certified reference value and the associated uncertainty of the CRM as control materials.

Warning and Safety Precautions for Users
HRM-3006A is intended for in-vitro use only and shall be handled as a biohazardous material with the potential of transmitting infectious disease. Hence, this material shall be handled using biosafety level 2 (or higher) practices, equipment, and facility [6].

Instructions for Use
HRM-3006A should be treated the same as patient specimens. Accordingly, these materials should be handled and disposed according to associated regional, national and local legislation and regulations for any potentially infectious human specimen.

Prior to use, the CRM should be thawed at room temperature (between 18 °C to 25 °C), then analysed immediately. The materials should be mixed well by gentle swirling before withdrawing any aliquots. The certified values may not be valid for re-thawed and opened vials as the stability of testosterone subjected to such conditions has not been investigated.

The recommended minimum sample sizes of HRM-3006A are 350 µL for STY-0087-001 and 400 µL

for STY-0087-002. The certified values may not be valid if smaller amounts are taken.

Transport and Storage

The CRM is transported in frozen state (in dry ice). Upon receipt, it should be stored at below – 60 ^{o}C. The CRM should not be exposed to sunlight or ultraviolet radiation. Storage of the thawed material at room temperature or in the refrigerator may result in changes in the certified values.

Further Information

Please direct all enquiries regarding this CRM to the contact in this Certificate.

References

[1] ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories.
[2] ISO 17034:2016 General requirements for the competence of reference material producers.
[3] ISO Guide 35:2017 Reference materials – Guidance for characterisation and assessment for homogeneity and stability.
[4] ISO/IEC Guide 98-3:2008 Uncertainty of measurement – Part 3: Guide to the expression of uncertainty in measurement (GUM: 1995).
[5] ISO Guide 33:2015 Reference materials – Good practice in using reference materials.
[6] U.S Department of Health and Human Services; Biosafety in Microbiological and Biomedical Laboratories, 5th ed.; HHS Publication No. (CDC) 21-1112.

Note

HSA does not assume any liability with respect to any loss caused by improper use and/or storage of the CRM by the customer.

Certificate Revision Records

Certificate of Analysis CML-HRM-3006A/02 replaces Certificate of Analysis CML-HRM-3006A/01 issued on 06 Apr 2020.

Certificate of Analysis CML-HRM-3006A/03 replaces Certificate of Analysis CML-HRM-3006A/02 issued on 16 Mar 2021.

Certificate of Analysis CML-HRM-3006A/04 replaces Certificate of Analysis CML-HRM-3006A/03 issued on 10 Mar 2022.

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