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Certificate of Analysis

CERTIFIED REFERENCE MATERIAL
HRM-3005A

Cortisol in Human Serum

Batch Number
STY-0063-001

Foreword
A unit of the certified reference material (CRM) HRM-3005A consists of 1 vial of 1 ml of frozen human serum sample. The serum material appears 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 of cortisol in HRM-3005A is provided in Table 1. The certified value of cortisol with the unit of nmol/L was calculated from the concentration value of µg/g, the measured serum density at 23 °C (1.02611 g/ml), and the relative molecular mass of cortisol (362.46 g/mol).

Table 1. Certified Value of Cortisol in HRM-3005A

	STY-0063-001
Cortisol (nmol/L)	112.2 ± 3.1

The certified value is the mean of measurements of at least six samples taken from a minimum of three bottles. The certified concentration for HRM-3005A was determined using isotope dilution mass spectrometry (IDMS). A four-point calibration curve was used in the measurements.

The associated measurement uncertainty of each certified value was estimated in accordance with ISO/IEC Guide 98-3:2008 [4]. The expanded uncertainty (coverage factor of 2) corresponded to a level of confidence of about 95%.

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

Homogeneity
Homogeneity testing on cortisol was performed on two subsamples taken from six vials using liquid

chromatography-isotope dilution tandem mass spectrometric (LC-IDMS/MS) method. The sample size taken for homogeneity testing was 330 µL. No significant difference in the between and within-vial variances was 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 cortisol in HRM-3005A 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 value of HRM-3005A is valid within the specified measurement uncertainty until **12 Mar 2027**. The validity of HRM-3005A will be extended if it is tested to be sufficiently stable for continuous use. The certified value of HRM-3005A is invalid when the serum material has deteriorated or is mishandled.

Analytical Methods

A fully validated LC-IDMS/MS method was used. The method involved spiking with isotope-labeled cortisol, separation using an Agilent Zorbax Eclipse Plus C18 column, followed by tandem mass spectrometric measurement.

Metrological Traceability

The certified concentration value is traceable to the International System of Units (SI) through the use of cortisol CRM (SRM 921) from the National Institute of Standards and Technology (NIST, USA).

Intended Use

HRM-3005A is intended for use in the validation of methods or as quality control materials for the determination of cortisol 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-3005A 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-3005A should be treated the same as patient specimens. Accordingly, the material 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 material should be mixed well by gentle swirling before withdrawing any aliquots. The certified value may not be valid for re-thawed and opened bottles as the stability of cortisol subjected to such conditions has not been investigated.

The recommended minimum sample size of HRM-3005A is 330 µL. The certified value may not be valid if smaller amount is taken.

Transport and Storage

The CRM is transported in frozen state (in dry ice). Upon receipt, it should be stored at below – 60 °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 value.

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 – General and statistical principles for certification.
[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-3005A/02 replaces Certificate of Analysis CML-HRM-3005A/01 issued on 12 Mar 2019.

Certificate of Analysis CML-HRM-3005A/03 replaces Certificate of Analysis CML-HRM-3005A/02 issued on 4 Mar 2020.

Certificate of Analysis CML-HRM-3005A/04 replaces Certificate of Analysis CML-HRM-3005A/03 issued on 15 Jan 2021.

Certificate of Analysis CML-HRM-3005A/05 replaces Certificate of Analysis CML-HRM-3005A/04 issued on 16 Feb 2022.

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